Beilschmiedic Acids F and G, Further Endiandric Acid Derivatives from *Beilschmiedia anacardioides*

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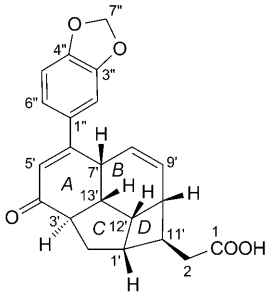
Two new endiandric acid derivatives, beilschmiedic acid F (**1**) and beilschmiedic acid G (**2**), together with three known constituents, beilschmiedic acid A, beilschmiedic acid C, and sitosterol 3-β-D-glucopyranoside, were isolated from the stem bark of *Beilschmiedia anacardioides*. Their structures were elucidated mainly by using a combination of 1D- and 2D-NMR techniques. The structure and relative configuration of beilschmiedic acid G (**2**) was also confirmed by X-ray crystallographic analysis.

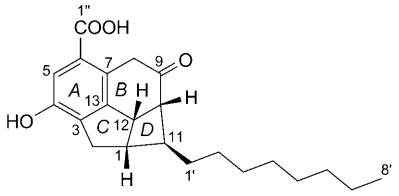
Introduction. – The genus *Beilschmiedia* (Lauraceae) comprises *ca.* 200 species widely distributed in the intertropical region [1]. The Lauraceae family is known as a source of lignans and neolignans according to the huge amount of the arylpropanoid derivatives isolated from this family [2–4]. *B. anacardioides* stem bark is used in the Western Region of Cameroon to cure uterine tumors, rubella, female genital infections, and rheumatisms [5]. The previous investigation led to the isolation of six endiandric acid derivatives from the CH_2Cl_2 extract of the stem bark of *B. anacardioides*, with potent antibacterial activity [6][7]. We report here the isolation and structure elucidation of the new compounds **1** and **2**.

Results and Discussion. – The AcOEt-soluble part of the MeOH extract of the stem bark of *B. anacardioides* was fractionated by column chromatography (silica gel). Successive purifications by column chromatography and prep. TLC afforded the two new endiandric acid derivatives **1**[1]) and **2**[1]), along with the known constituents beilschmiedic acid A, beilschmiedic acid C [6], and sitosterol 3-β-D-glucopyranoside [8].

[1]) Arbitrary atom numbering; for systematic names, see *Exper. Part*.



1[1])

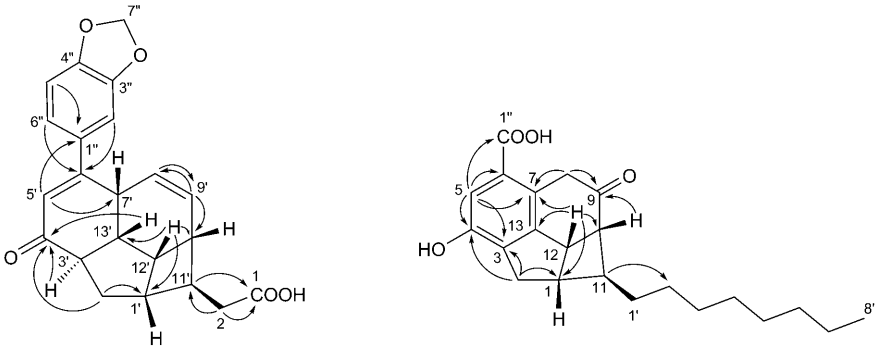


2[1])

Beilschmiedic acid F (**1**) was obtained as a yellow powder. The positive-mode HR-ESI-MS exhibited a quasimolecular-ion peak at *m/z* 365.13793 ($[M+H]^+$), which corroborated the molecular formula $C_{22}H_{20}O_5$ and indicated 12 degrees of unsaturation. Its IR spectrum displayed bands at 3444 (OH), 1665 (C=O), and 1593 (C=C) cm^{-1}. The ^{13}C-NMR spectrum of compound **1** (*Table 1*) contained signals of 22 C-atoms. DEPT and HMQC data allowed to assign them to six quaternary C-atoms and 13 CH and 3 CH_2 groups. The resonances of the CH groups at δ(C) 39.5 (C(1′)), 47.5 (C(3′)), 35.7 (C(7′)), 33.8 (C(10′)), 41.5 (C(11′)), 33.5 (C(12′)), and 42.4 (C(13′)) and of a CH_2 group at δ(C) 29.8 (C(2′)) in the DEPT spectrum were in part characteristics for the tetracyclic endiandric acid skeleton [6][9–13] (endiandric acid = *rel*-(1*R*,1a*R*,2a*R*,5*S*,5a*S*,7a*S*,7b*R*,7c*R*)-1a,2,2a,5,5a,7a,7b,7c-octahydro-5-phenyl-1*H*-cyclobut[*bc*]acenaphthylene-1-acetic acid). The ^{13}C-NMR spectrum of **1** exhibited, among other peaks, signals of two C=O groups at δ(C) 173.4 (C(1)) and 199.5 (C(4′)), of three olefinic CH groups at δ(C) 124.4 (C(5′)), 122.2 (C(8′)), and 130.3 (C(9′)), of six aromatic C-atoms at δ(C) 130.6 (C(1″)), 107.3 (C(2″)), 148.9 (C(3″)), 148.0 (C(4″)), 108.4 (C(5″)), and 122.0 (C(6″)), and of an OCH_2O moiety at δ(C) 101.6 (C(7″)). The latter, together with the aromatic signals, suggested the presence of the (methylenedioxy)phenyl (=1,3-benzodioxolyl) moiety [11]. The ^{1}H-NMR spectrum of **1** (*Table 1*) showed signals of three olefinic H-atoms at δ(H) 6.26 (br. *s*, H–C(5′)), 5.18 (*dd*, J = 10.7, 8.8 Hz, H–C(8′)), and 5.82 (*dt*, J = 10.0, 3.7 Hz, H–C(9′)). In addition, an *ABX* system at δ(H) 7.30 (br. *s*, H–C(2″)), 7.21 (*dd*, J = 8.1, 1.9 Hz, H–C(6″)), and 6.99 (*d*, J = 8.1 Hz, H–C(5″)), and OCH_2O H-atoms at δ(H) 6.08 (br. *s*, CH_2(7″)) confirmed the presence of the (methylenedioxy)phenyl moiety. The signal of the remaining low-field CH_2 group at δ(H) 2.44–2.47 (CH_2(2)) suggested the fragment CH_2CO_2H, which was confirmed by the HMBC spectrum, where the correlations (*Fig. 1*) CH_2(2)/C(1), CH_2(2)/C(11′), and H–C(11′)/C(1) were observed. These correlations also supported the position of this fragment at C(11). The second C=O group was located at C(4′) (δ(C) 199.5) according to HMBCs from CH_2(2′) (δ(H) 1.49–1.65), H–C(3′) (δ(H) 2.88–2.94), and H–C(13′) (δ(C) 2.33–2.38) to C(4′). The attachment of the (methylenedioxy)phenyl moiety at C(6′) was supported by the correlations from H–C(2″), H–C(6″), and H–C(5′) to C(6′). Some COSY cross-peaks (CH_2(2′)/H–C(3′), H–C(1′)/CH_2(2′), H–C(1′)/H–C(12′), H–C(10′)/H–C(12′), H–C(1′)/H–C(11′)) supported the proposed structure. The relative configuration of the stereogenic centers was

Table 1. *^{13}C- and ^{1}H-NMR Data* ((D_6)DMSO) *of Compound* **1**[1]). δ in ppm, J in Hz.

	δ(C)	δ(H)		δ(C)	δ(H)
C(1)	173.4	–	H–C(10′)	33.8	2.50–2.53 (*m*)
CH_2(2)	40.3	2.44–2.47 (*dd*, *J* = 15.7, 6.9)	H–C(11′)	41.5	1.72–1.76 (*m*)
H–C(1′)	39.5	2.36–2.42 (*m*)	H–C(12′)	33.5	2.28–2.32 (*m*)
CH_2(2′)	29.8	1.49 (*ddd*, *J* = 12.5, 11.9, 5.6), 1.65 (*dd*, *J* = 12.5, 5.6)	H–C(13′)	42.4	2.33–2.38 (*m*)
H–C(3′)	47.5	2.88–2.94 (*ddd*, *J* = 12.5, 6.9, 5.6)	C(1″)	130.6	–
C(4′)	199.5	–	H–C(2″)	107.3	7.30 (br. *s*)
H–C(5′)	124.4	6.26 (*s*)	C(3″)	148.9	–
C(6′)	159.5	–	C(4″)	148.0	–
H–C(7′)	35.7	3.81 (br. *s*)	H–C(5″)	108.4	6.99 (*d*, *J* = 8.1)
H–C(8′)	122.2	5.18 (*dd*, *J* = 10.7, 8.8)	H–C(6″)	122.0	7.21 (*dd*, *J* = 8.1, 1.9)
H–C(9′)	130.3	5.82 (*dt*, *J* = 10.0, 3.7)	CH_2(7″)	101.6	6.08 (br. *s*)



1[1]) **2**[1])

Fig. 1. *Selected HMBCs of compounds* **1** *and* **2**

attributed according to biogenetic considerations [9–11]. Therefore, compound **1** was elucidated as 2-{6′-[3″,4″-(methylenedioxy)phenyl]-4-oxotetracyclo[5.4.2.0$^{3',13'}$.0$^{10',12'}$]-trideca-5′,8′-dien-11′-yl}acetic acid[1]), for which the trivial name beilschmiedic acid F was proposed.

Beilschmiedic acid G (**2**) was obtained as colorless crystals. The positive-mode HR-ESI-MS exhibited a quasimolecular-ion peak at *m*/*z* 379.18805 ($[M + Na]^+$), which corroborated the molecular formula $C_{22}H_{28}O_4$ and was consistent with nine degrees of unsaturation. Its IR spectrum showed strong bands at 3409 (OH), 1681 (C=O), and 1638 (C=C) cm^{-1}. The ^{13}C-NMR spectrum of **2** (*Table 2*) contained signals of 22 C-atoms, which were sorted by DEPT and HMQC into 7 quaternary C-atoms, and 5 CH, 9 CH_2, and 1 Me group. Among them, two C=O groups at δ(C) 211.5 (C(9)) and 168.2 (C(1″)), five aromatic quaternary C-atoms at δ(C) 153.5 (C(4)), 149.8 (C(13)), 133.6 (C(3)), 128.5 (C(6)), and 123.9 (C(7)), and one aromatic CH group at δ(C) 118.4 (C(5)) were attributed. The number of aromatic C-atoms and the number of unsaturations suggested the presence of a pentasubstituted aromatic ring bearing one

OH group. Furthermore, the ^{13}C-NMR spectrum of **2** exhibited CH signals at δ(C) 42.7 (C(12)), 44.0 (C(1)), 47.4 (C(10)), and 48.5 (C(11)). The seven CH_2 groups at δ(C) 22.4–35.9 and the terminal Me group at δ(C) 14.3 suggested the presence of an octyl fragment. These data were in part comparable to those of some beilschmiedic acids [6] and suggested that compound **2** has the tetracyclic endiandric acid skeleton, with ring *A* aromatic. In fact, the ^{1}H-NMR spectrum of **2** (*Table 2*) confirmed the presence of the aromatic H-atom at δ(H) 7.28 (*s*, H–C(5)) and the octyl fragment at δ(H) 1.10–1.43 (7 CH_2) and 0.83 (*t*, J = 6.2 Hz, Me(8′)). The COOH group C(1″) (δ(C) 168.2) was located at C(6) (δ(C) 128.5) following the HMBCs (*Fig. 1*) from H–C(5) to C(6) and C(1″). The position of the second (C)=O group at C(9) (δ(C) 211.5) was supported by the HMBCs from CH_2(8) (δ(H) 3.65–4.01), H–C(10) (δ(H) 2.62–2.66), and H–C(11) (δ(H) 1.59–1.64) to C(9). The OH group at C(4) (δ(C) 153.5) was suggested by correlations from CH_2(2) (δ(H) 2.52–3.10) and H–C(5) to C(4). The attachment of the octyl fragment at C(11) (δ(C) 48.5) was established by the HMBC from H–C(11) to C(1′) (δ(C) 35.9). Significant COSY cross-peaks (H–C(1)/CH_2(2), H–C(1)/H–C(12), and H–C(10)/H–C(12)) also supported the proposed structure. The relative configuration of the stereogenic centers was attributed according to biogenetic considerations [9]. The structure and the relative configuration of beilschmiedic acid G (**2**) were also confirmed by the single-crystal X-ray analysis (*Fig. 2*).

Table 2. *^{13}C- and ^{1}H-NMR Data* ((D_6)DMSO) *of Compound* **2**[1]). δ in ppm, J in Hz.

	δ(C)	δ(H)		δ(C)	δ(H)
H–C(1)	44.0	2.62–2.66 (*m*)	H–C(12)	42.7	3.77 (*dd*, *J* = 13.1, 6.9)
CH_2(2)	35.6	2.52 (*d*, *J* = 16.9), 3.10 (*dd*, *J* = 16.9, 5.6)	C(13)	149.8	–
C(3)	133.6	–	CH_2(1′)	35.9	1.24–1.43 (*m*)
C(4)	153.5	–	CH_2(2′)	26.6	1.10–1.24 (*m*)
H–C(5)	118.4	7.28 (*s*)	CH_2(3′)	29.2	1.10–1.24 (*m*)
C(6)	128.5	–	CH_2(4′)	29.3	1.10–1.24 (*m*)
C(7)	123.9	–	CH_2(5′)	28.9	1.10–1.24 (*m*)
CH_2(8)	44.8	3.65 (*d*, *J* = 14.4), 4.01 (*d*, *J* = 13.8)	CH_2(6′)	31.6	1.10–1.24 (*m*)
C(9)	211.5	–	CH_2(7′)	22.4	1.10–1.24 (*m*)
H–C(10)	47.4	2.62–2.66 (*m*)	Me(8′)	14.3 (Me)	0.83 (*t*, *J* = 6.2)
H–C(11)	48.5	1.59–1.64 (*m*)	C(1″)	168.2	–

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Experimental Part

General. CC = Column chromatography. M.p.: *Büchi-B-540* melting-point apparatus; uncorrected. Optical rotations: *Jasco-DIP-360* digital polarimeter. IR Spectra (KBr, neat): *Jasco-FT/IR-410* spectrometer; $\tilde{\nu}$ in cm^{-1}. NMR Spectra: *Bruker-AM-Advance-DRX-500* spectrometer; at 500 (^{1}H) and 125 MHz (^{13}C); δ in ppm rel. to Me_4Si as internal standard, J in Hz. EI-MS: *VG Autospec X* (*Micromass*

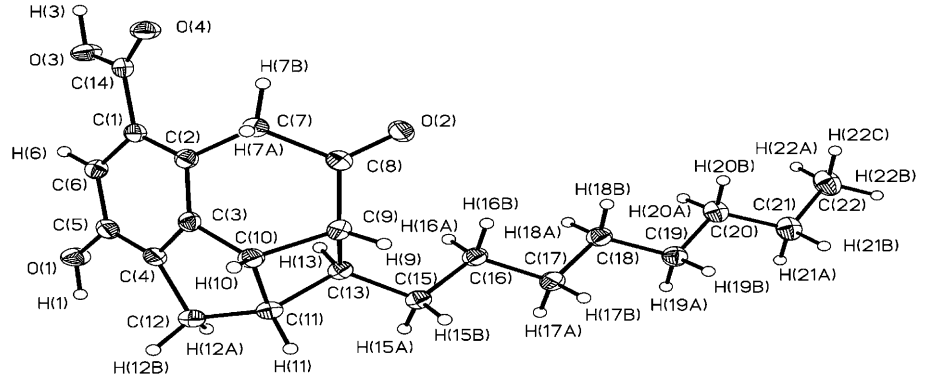

Fig. 2. *ORTEP Diagram of compound* **2**. Arbitrary atom numbering.

Co.) spectrometer; at 70 eV; in *m*/*z* (rel. %). HR-ESI-MS: FT-ICR-MS *Apex-III-Bruker* spectrometer (*Daltonik Co.*); in *m*/*z*.

Plant Material. The stem bark of *Beilschmiedia anacardioides* (Lauraceae) was collected in January 2007 at Foumban, Noun Division of the West Province, Cameroon. The identity of the plant was confirmed by Mr. *Paul Mezili*, botanist at the National Herbarium, Yaoundé, Cameroon (No. 1005 HNC).

Extraction and Isolation. Dried powdered stem bark of *B. anacardioides* (4.0 kg) was macerated for 72 h at r.t. with MeOH (15 l), and then the MeOH soln. was concentrated to afford 275.0 g of a crude extract. This extract was re-extracted with CH_2Cl_2 (17 g), and AcOEt (34.0 g), resp. The AcOEt-soluble part (34 g) was subjected to CC (silica gel (0.063–0.200 mm), gradient hexane/AcOEt of increasing polarity): *Fractions 1–94* (*ca.* 200 ml each; combined by TLC). *Frs. 21–31* (3.6 g; with hexane/AcOEt 7.2:2.5) were concentrated and (silica gel, isocratic hexane/AcOEt 4:1): beilschmiedic acid A (15 mg) and beilschmiedic acid C (25 mg). *Frs. 33–34* (400 mg; with hexane/AcOEt 7:3) afforded **2** (50 mg), which gave a single crystal after recristallization from MeOH. *Frs. 38–39* (300 mg; with hexane/AcOEt 7:3) afforded **1** (35 mg) after prep. TLC (hexane/AcOEt 7:3). *Frs. 57–60* (1.2 g; with hexane/AcOEt 1:1) were resubjected to CC (silica gel, isocratic hexane/AcOEt 5.5:4.5): sitosterol 3-*β*-D-glucopyranoside (85 mg).

Beilschmiedic Acid F (= rel-*(1*R*,1a*R*,2a*R*,5a*S*,7a*S*,7b*R*,7c*R*)-5-(1,3-Benzodioxol-5-yl)-1a,2,2a,3,5a,7a,7b,7c-octahydro-3-oxo-1*H*-cyclobut[*bc*]acenaphthylene-1-acetic Acid*; **1**): Yellow powder. $[\alpha]_D^{20} = -43.7$ ($c = 0.185$, MeOH). IR (neat): 3444 (OH), 1665 (C=O), 1593 (C=C). ^{1}H- and ^{13}C-NMR ((D$_6$)DMSO): *Table 1*. EI-MS: 364 (100, M^+), 265 (94), 235 (36), 207 (21), 165 (27), 147 (43). HR-ESI-MS: 365.13793 ($[M+H]^+$, $C_{29}H_{37}O_5^+$; calc. 365.13835).

Beilschmiedic Acid G (= rel-*(1*R*,1a*R*,7a*S*,7b*R*)-1a,2,6,7,7a,7b-Hexahydro-3-hydroxy-1-octyl-7-oxo-1*H*-cyclobut[*bc*]acenaphthylene-5-carboxylic Acid*; **2**): Colorless crystals. M.p. 217–218°. $[\alpha]_D^{20} = -64$ ($c = 0.2$, MeOH). IR (KBr): 3409 (OH), 1681 (C=O), 1638 (C=C). ^{1}H- and ^{13}C-NMR ((D$_6$)DMSO): *Table 2*. EI-MS: 356 (6, M^+), 189 (30), 172 (100), 167 (24). HR-ESI-MS: 379.18805 ($[M+Na]^+$, $C_{22}H_{28}NaO_4^+$; calc. 379.18983).

X-Ray Crystallographic Data of Beilschmiedic Acid G (**2**). A colorless crystal was obtained from MeOH. The data were collected with a *Bruker-Nonius-Kappa-CCD* instrument. Cell parameters: $a = 7.9900(2)$ Å; $b = 21.9056(6)$ Å; $c = 10.8257(3)$ Å; $V = 1834.58(8)$ Å^3, space group monoclinic $P2_1/c$, $Z = 4$, $D_{calc.} = 1.291$ Mg/m^3, λ 0.71073 Å, $\mu(MoK_\alpha) = 0.087$ mm^{-1}, T 100 K. CCDC-732394 contains the complete crystallographic data for this paper. These data can be obtained free of charge *via* www.ccdc.cam.ac.uk/data_request/cif.

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