Spiroalkaloids and Coumarins from the Stem Bark of *Pauridiantha callicarpoides*

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A new spiroalkaloid, 7-*epi*-javaniside (**1**), was isolated from the Cameroonian plant *Pauridiantha callicarpoides*, along with seven known compounds *viz*., javaniside (**2**), sweroside (**3**), hymexelsin (**4**), scopoletin (**5**), 7,7′-dihydroxy-6,6′-dimethoxy-8,8′-biscoumarin (**6**), 7,7′-dihydroxy-6,6′-dimethoxy-3,3′-biscoumarin (**7**), and 4-hydroxy-3-methoxybenzoic acid (**8**). The structures of the new alkaloid, as well as those of the known compounds, were elucidated by detailed spectroscopic analysis including 1D and 2D NMR and by comparison with the literature data for related compounds.

Key words: *Pauridiantha callicarpoides*, Rubiaceae, Coumarins, Javaniside, *epi*-Javaniside, Spiroalkaloids

Introduction

Natural products are known to be more efficient and inherently better tolerated in the body as compared to synthetic compounds [1]. In the literature, several reviews have described the importance of genetic resources in drug discovery, and some of them described efficacy of natural and synthetic products [1 – 4]. Although natural and synthetic compounds usually have similarities in their chemical structures, the natural compounds significantly outperform their synthetic counterparts for several reasons. For example, synthetic vitamin E has a lower biological activity than natural vitamin E since it is a racemic mixture [5]. Javaniside isolated from *Alangium javanicum*, can better mediate DNA strand scission than the synthetic one [6]. It is thus far more effective and important to use chirally pure natural compounds for the treatment of human ailments. Consequently, a comprehensive knowledge of the available genetic re-

sources, capable of producing bioactive compounds is both important and vital. *Pauridiantha callicarpoides* of the Rubiaceae family is a small tree native to Africa [7, 8]. Previous phytochemical studies on this plant led to the isolation of two new alkaloids [9]. As part of our continuing search for compounds from Cameroonian medicinal plants [10, 11], a dichloromethane/methanol extract of the stem bark of *P. callicarpoides* was investigated, and herewith we describe the isolation, structure elucidation of the new spiroalkaloid.

Results and Discussion

The HPLC profile of a fraction obtained after silica gel flash column chromatography (see Experimental Section) showed what turned out to be two isomers in the ratios shown in Fig. 1. These were successfully separated by using a semi-preparative HPLC to afford *epi*-javaniside (**1**) and javaniside (**2**) (Fig. 2).

Compound **1**, named *epi*-javaniside was obtained as an optically active colorless powder ($[\alpha]_D^{23} = -115.9°$). Its molecular formula $C_{26}H_{30}N_2O_9$ was determined from its HRESI-MS which showed a quasi-molecular ion peak $[M+H]^+$ at $m/z = 515.2029$ (calcd. 515.2024), requiring 13 degrees of unsaturation. UV absorption bands were observed at 206 and 248 nm characteristic of an aromatic ring. IR absorptions at 3184, 3086, 1711, and 1658 cm^{-1} revealed the presence of hydroxyl, spiroamide and α,β-unsaturated amide groups, respectively, which was consistent with NMR observations at $\delta_C = 166.1$ (C-21) and 179.1 (C-2) ppm for two amide carbonyl groups [12]. In the ^1H NMR spectrum, signals for a 1,2-disubstituted benzene ring were observed at $\delta_H = 6.89$ (d, $J = 7.6$ Hz, H-9), 6.96 (d, $J = 7.6$ Hz, H-12), 7.01 (td, $J = 7.6$, 1.0 Hz, H-10), and 7.25 (td, $J = 7.6$, 1.2 Hz, H-11) ppm. In addition, a set of signals due to a sugar moiety were observed at $\delta_H = 3.15$ (dd, $J = 9.2$, 8.0 Hz, H-2′), 3.25 (m, H-4′), 3.27 (m, H-5′), 3.35 (m, H-3′), 4.63

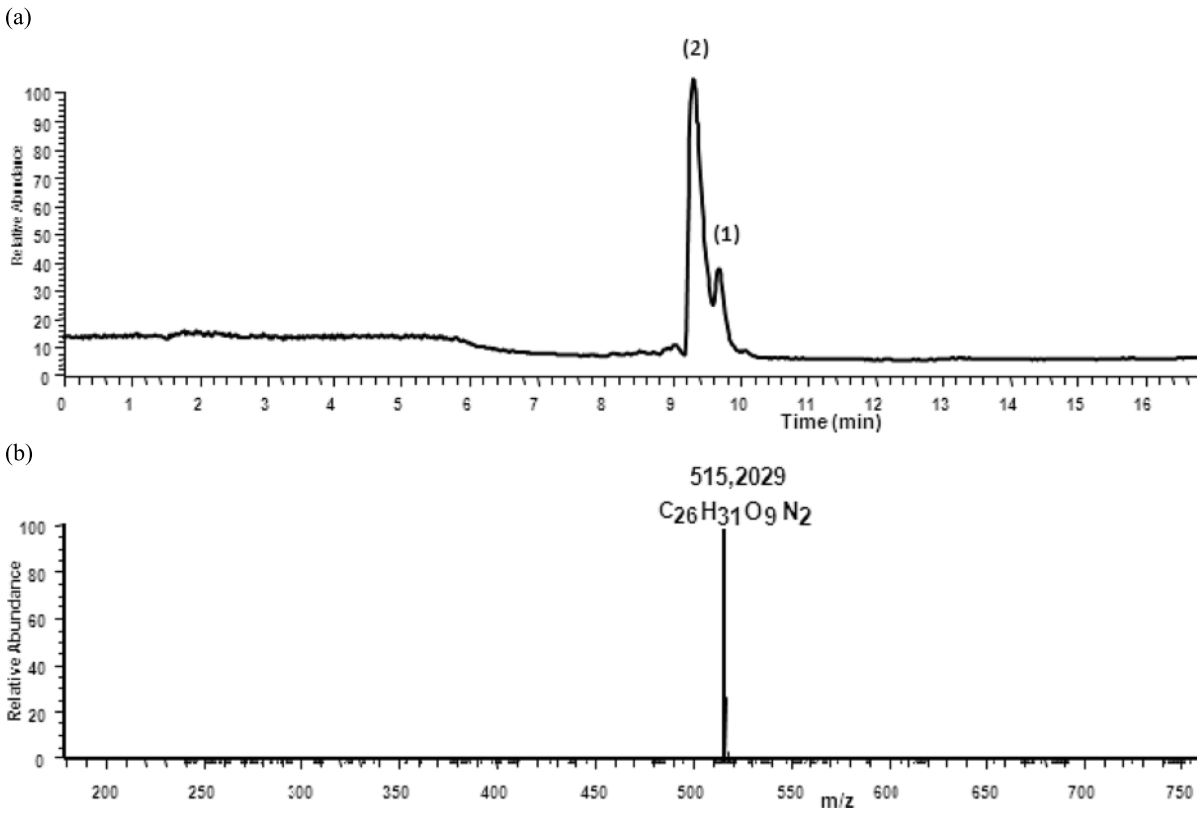

Fig. 1. HPLC chromatogram (a) and high-resolution mass spectrum (b) of the two isomers **1** and **2**.

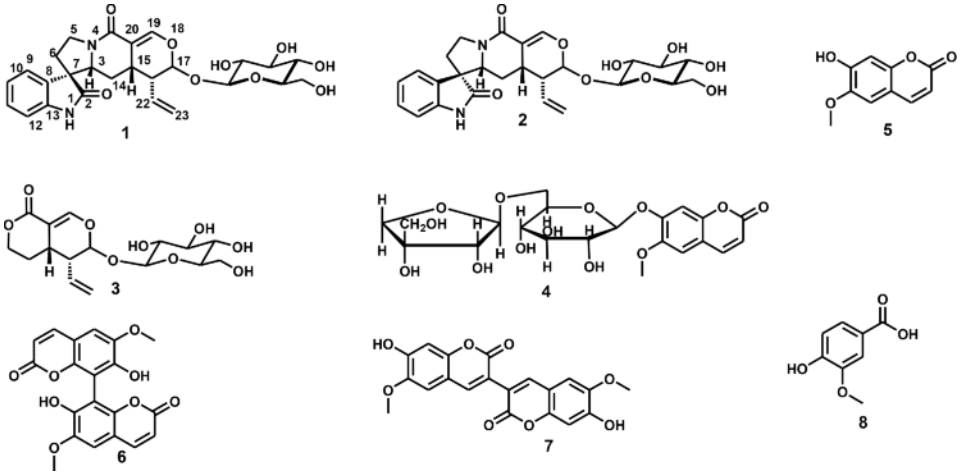

Fig. 2. Chemical structures of compounds **1**–**8**.

(d, $J = 8.0$ Hz, H-1′), 3.63 (dd, $J = 12.0$, 5.6 Hz, H-6′), and 3.86 (dd, $J = 12.0$, 1.6 Hz, H-6′) ppm. The presence of the sugar unit was further supported by the ^{13}C NMR spectrum with characteristic signals for a glucose moiety. The anomeric carbon was observed at $\delta_C = 99.5$ (C-1′) ppm. The large coupling constant of the anomeric proton at $\delta_H = 4.63$ (d, $J = 8.0$ Hz) ppm indicated its β configuration. Additionnally, the ^{13}C NMR and DEPT spectra showed signals for 20 carbons including four methylenes, ten methines (two olefinic and four aromatic ones) and six quaternary carbons, of which two are amide carbonyl groups and two are aromatic carbons at $\delta_C = 131.2$ (C-8) and 142.5 (C-13) ppm. Analysis of the 2D ^{1}H-^{1}H COSY and HMQC spectra of compound **1** further indicated the presence of the fragments CH(3)–CH$_2$(14)–CH(15)–CH(16)–CH(22)–CH$_2$(23) and CH(16)–CH(17) which typically resembled the systems found in sweroside and secologanin [13, 14]. Comparison of the NMR data of compound **1** and sweroside (**3**) indicated the presence of a partial sub-structure of **1** [13]. In the HMBC spectrum, a signal characteristic of a hemiacetal group at $\delta_H = 5.41$ (d, $J = 1.4$ Hz, H-17) ppm showed a strong correlation with the anomeric carbon at $\delta_C = 99.5$ ppm (Fig. 3). Compound **1** showed UV, IR, MS, and NMR spectral features closely resembling those of **2** (see Table 1). A small difference between the two compounds was observed, and the signals of the atoms around the asymmetric C-7 suggest that **1** is the C-7 epimer of javaniside (**2**), which was synthesized in 2005 [6]. Thus, compound **1**, based also on the HPLC and the above spectral evidences, was identified to be isomeric with javaniside (**2**) isolated from *Alangium javanicum* in 2004 [15], and the trivial name 7-*epi*-javaniside was assigned.

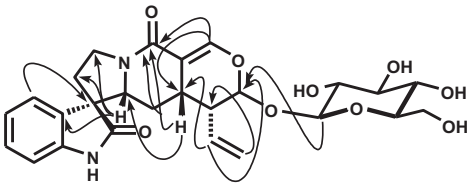

Fig. 3. Key HMBC correlations for compound **1**.

Seven known compounds (Fig. 2) were also isolated from the plant extract and were identified as javaniside (**2**) [6], sweroside (**3**) [13], hymexelsin (**4**) [16], scopoletin (**5**) [17], 7,7′-dihydroxy-6,6′-dimethoxy-8,8′-biscoumarin (**6**) [17], 7,7′-dihydroxy-6,6′-dimethoxy-3,3′-biscoumarin (**7**) [17], and 4-hydroxy-3-methoxybenzoic acid (**8**) [18], by comparison of their spectroscopic data with those reported.

Experimental Section

General experimental procedures

The ^{1}H and ^{13}C NMR spectra were recorded on Avance AV-500 and AV-600 spectrometers at $\nu = 500.130$ and 600.233 MHz (^{1}H); 125.757 and 150.927 MHz (^{13}C), respectively. Chemical shifts (δ) are given in parts per million (ppm) with tetramethylsilane (TMS) as internal standard. UV spectra were recorded in methanol on a Thermo

Table 1. ^{1}H and ^{13}C NMR spectroscopic data for compounds **1** and **2** (CD_3OD, 500/125.75 MHz)[a].

Position	**1**		**2**	
	δ_H	δ_C	δ_H	δ_C
1-NH	–		10.19	
2	–	179.1	–	180.9
3	4.07 (dd, 3.2, 11.2)	64.8	4.05 (dd, 3.2, 11.2)	65.4
5	3.82 (brd, 12.4)	45.5	3.75 (brt, 10.0, 11.6)	45.6
	3.93 (td, 7.3, 12.4)		4.00 (dd, 8.0, 11.6)	
6	2.02 (dd, 7.3, 12.8)	34.3	2.21 (dd, 8.0, 13.2)	33.4
	2.47 (dd, 10.4, 12.8)		2.40 (ddd, 10.0, 12.8, 13.2)	
7	–	58.8	–	58.0
8	–	131.2	–	129.5
9	6.89 (d, 7.6)	124.8	7.30 (d, 7.6)	124.0
10	7.01 (td, 1.0, 7.6)	123.7	7.07 td, 0.8, 7.6)	123.7
11	7.25 (td, 1.0, 7.6)	129.8	7.25 (td, 0.8, 7.6)	130.1
12	6.96 (d, 7.6)	111.5	6.92 (d, 7.6)	111.0
13		142.5		143.5
14	0.87 (q, 12.6)	27.2	1.24 (q, 12.0)	27.0
	1.38 (dt, 3.6, 12.6)		1.33 (dt, 4.0, 12.0)	
15	3.05 (m)	28.2	2.96 (m)	28.7
16	2.53 (ddd, 1.4, 6.8, 9.6)	44.5	2.50 (ddd, 1.4, 6.8, 8.4)	44.6
17	5.41 (d, 1.4)	97.2	5.41 (d, 1.4)	97.3
19	7.40 (d, 2.4)	148.5	7.38 (d, 2.0)	148.3
20	–	108.7	–	108.9
21	–	166.1	–	165.8
22	5.28 (dt, 10.2, 17.2)	133.4	5.43 (dt, 10.0, 17.2)	133.8
23	4.98 (dd, 1.8, 10.2)	120.4	5.13 (dd, 1.8, 10.0)	120.6
	5.06 (dd, 1.8, 17.2)		5.15 (dd, 1.8, 17.2)	
1′	4.63 (d, 8.0)	99.5	4.65 (d, 7.8)	99.5
2′	3.15 (dd, 8.0, 9.2)	74.7	3.17 (dd, 7.8, 8.8)	74.8
3′	3.35 (m)	77.8	3.38 (t, 8.8)	77.9
4′	3.25 (m)	71.5	3.26 (t, 9.6)	71.6
5′	3.27 (m)	78.3	3.29 (m)	78.3
6′	3.63 (dd, 5.6, 12.0)	62.6	3.64 (dd, 9.6, 12.0)	62.6
	3.86 (dd, 1.6, 12.0)		3.86 (dd, 1.6, 12.0)	

[a] The coupling constants (J) are in parentheses and reported in Hz; chemical shifts (δ) are given in ppm.

Electron, VISION PRO software V4.10, and IR spectra on a VECTOR22 instrument. For optical rotation measurements, a Polarimeter P-2000 was used. EI-MS was performed at 70 eV, and the data were recorded on a Jeol JMS 600-H Instrument. The high-resolution mass spectra were reordered with an LTQ Orbitrap spectrometer (Thermo Fisher, USA) equipped with an HESI-II source. The mass spectrometer was coupled to an Agilent 1200 HPLC system using a Macherey-Nagel Nucleodur Gravity C18 column (50×2 mm, 1.9 μm particle size) and an acetonitrile/H_2O gradient. Normal-phase TLC and reverse-phase TLC were carried out using pre-coated aluminum-backed supported silica gel 60 F_{254} (Merck, 0.2 mm thickness) and RP C_{18} silica gel (0.25 mm thickness), respectively. Spots for compounds on TLC were detected using UV light (254 and 366 nm) and/or by spraying with a 50% aqueous solution of H_2SO_4, (or ninhydrin), followed by heating. Silica gel (Merck, 0.040 – 0.063 mm) was used for flash chromatography, and column chromatography was carried out over silica gel 60 (Merck, 70 – 230 mesh). Reverse-phase C_{18} HPLC and Sephadex LH-20 were also used for purification.

Plant material

The stem bark of *P. callicarpoides* was collected from the Dja rain forest in the eastern region of Cameroon in December of 2010. The plant was identified by Mr. Victor Nana, botanist at the National Herbarium of Cameroon in Yaoundé, where a voucher specimen (no. 39807/SPDK) was deposited.

Extraction and isolation

The air-dried stem bark of *P. callicarpoides* (2.8 kg) was powdered and extracted with CH_2Cl_2-MeOH (1 : 1, 10 L) twice at room temperature for 48 h and 12 h, respectively. The solvent was evaporated under reduced pressure to afford a crude extract (345.4 g) which was subjected to flash silica gel column chromatography with CH_2Cl_2-MeOH using a gradual increase of solvent polarity to

give 5 fractions: A (CH_2Cl_2, 9.3 g); B [CH_2Cl_2-MeOH (2.5 – 12.5 %), 17.97 g]; C [CH_2Cl_2-MeOH (12.5 – 22.5 %), 52.2 g]; D [CH_2Cl_2-MeOH (22.5 – 30 %), 50.92 g], and E [CH_2Cl_2-MeOH (30 – 50 %), 45.5 g]. Repeated silica gel column chromatography of fraction A eluted with a gradient of hexane-EtOAc yielded scopoletin (**5**) (2.0 g) and a mixture of sterols (60 mg). Column chromatography of fraction B over silica gel followed by Sephadex LH-20 and eluting with a gradient of CH_2Cl_2-MeOH and then pure MeOH afforded again compound **5** and 4-hydroxy-3-methoxybenzoic acid (**8**) (5.92 mg). Compounds **7** (15.0 mg), **6** (10.0 mg), glycoside of β-sitosterol (25.0 mg), hymexelsin (**4**) (1.8 g) and a mixture of two other compounds named PC-A were obtained after further purification of fraction C over a silica gel column eluting with CH_2Cl_2-MeOH (1.5 – 30 %). The mixture of PC-A was subjected to RP C_{18} HPLC eluting with MeOH-H_2O (1 : 1), UV lamp 254 nm, flow rate 5 mL min^{-1} to yield compound **2** (46.4 mg) with RT of 20 min and compound **1** (4.8 mg) with RT of 22 min. Column chromatography of fraction D over silica gel, eluting with an EtOAc-MeOH gradient, afforded six sub-fractions D1–D6. D4 was again subjected separately to column chromatography over silica gel and then to the semi-preparative HPLC at wavelength 254 nm with the gradient solvent system H_2O (B)-MeOH (A). Sub-fractions 6 – 8 were resubmitted to semi-preparative HPLC with the isocratic solvent system MeOH-H_2O (4 : 6) to yield sweroside (**3**) (17.74 mg) with RT of 25 min.

*7-Epi-javaniside (**1**)*, colorless solid. – $[\alpha]_D^{23} = -115.9°$ ($c = 0.001$, MeOH). – HRMS ((+)-ESI): $m/z = 515.2029$ (calcd. 515.2024 for $C_{26}H_{31}N_2O_9$, $[M+H]^+$). – UV (MeOH): λ_{max} = 196, 206 and 248 nm. – IR (CH_3OH): ν = 3184, 3086, 1711, 1658, 1587, 1472, 1336, 1175, 1073 cm^{-1}. – ^{1}H NMR (500.13 MHz, CD_3OD, 25 °C, TMS): see Table 1. – ^{13}C NMR (125.75 MHz, CD_3OD, 25 °C, TMS): see Table 1.

*Javaniside (**2**)*, colorless amorphous powder. – $[\alpha]_D^{23} = -141.1°$ ($c = 0.0014$, MeOH). – HRMS ((+)-ESI): $m/z = 515.2029$ (calcd. 515.2024 for $C_{26}H_{31}N_2O_9$, $[M+H]^+$). – IR (CH_3OH): ν = 3409, 1705, 1656, 1619, 1578, 1467, 1069 cm^{-1}. – UV (MeOH): λ_{max} = 196, 238, 258 nm. – ^{1}H NMR (500.13 MHz, CD_3OD, 25 °C, TMS): see Table 1. – ^{13}C NMR (125.75 MHz, CD_3OD, 25 °C, TMS): see Table 1.

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