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Chemical constituents of the leaves of *Campylospermum elongatum*

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Abstract: The leaves of *Campylospermum elongatum* have furnished the cyano-glycoside (lithospermoside), nine isomeric biflavonoid derivatives among which five are I_3–II_6 linked (robustaflavone; 4′-*O*-methyl robustaflavone; 4′,4‴-di-*O*-methyl robustaflavone; 7,4′,4″-tri-*O*-methyl robustaflavone; 4′,7″-di-*O*-methyl robustaflavone) and four I_3–II_8 linked (amentoflavone; 7-*O*-methyl amentoflavone; 7,7″-di-*O*-methyl amentoflavone; 7, 4′,7″-tri-*O*-methyl amentoflavone) and a flavone glycoside, 4″-*O*-methyl-7-*O*-β-D-galactosylapigenin. All structures were established from a complete spectroscopic analysis (MS, IR, 1D, and 2D NMR, including HSQC, HMBC, and NOESY) as well as by comparing the obtained spectroscopic data with literature. This is the first report on the characterization of 4′-*O*-methyl-7-*O*-β-D-galactosylapigenin from the genus *campylospermum* and thus has important chemotaxonomic implications.

Keywords: *Campylospermum elongatum*; chemotaxonomy; flavone galactoside; Ochnaceae.

1 Introduction

Campylospermum elongatum (Oliv.) Tiegh, a plant of the Ochnaceae family, is represented as shrubs growing up to 2.5 m high under dense secondary forests, well exposed to sunlight [1]. In Cameroun, this plant is found in the eastern and southern regions where natives of the Baka pigmy tribe use the leaves and alcohol to prepare medicinal portions destined to remedy many health problems such as palpitations, heart pain, and stomach disorders. According to our knowledge, no previous phytochemical work has been reported on this species.

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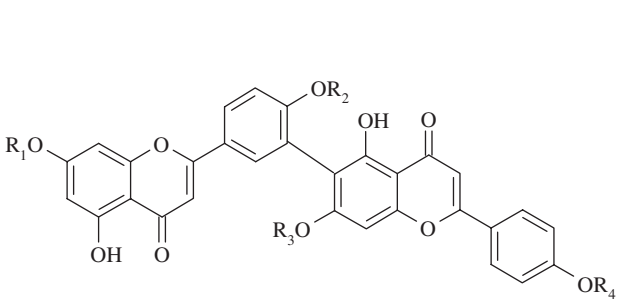
2 Results and discussion

In the continuation of the phytochemical investigations of our local plants used in folk medicine, we have isolated and characterized from the leaves of *C. elongatum* 11 secondary metabolites (Figure 1). The structures of these compounds were established from a complete spectroscopic study (IR, UV, RMN, and MS), as well as by comparing the obtained data with literature. These include the biflavonoids: robustaflavone **1**; 4′-*O*-methyl robustaflavone **2**; 4′,4‴-di-*O*-methyl robustaflavone **3**; 7,4′,4″-tri-*O*-methyl robustaflavone **4**; 4′,7″-di-*O*-methyl robustaflavone **5**; amentoflavone **6**; 7-*O*-methyl amentoflavone **7**; 7,7″-di-*O*-methyl amentoflavone **8**; 7,4′,7″-tri-*O*-methyl amentoflavone **9**; and a cyano-glycoside, lithospermoside **10** [2–9]. All were earlier reported as secondary metabolites from other sources with important biological activities [10–15].

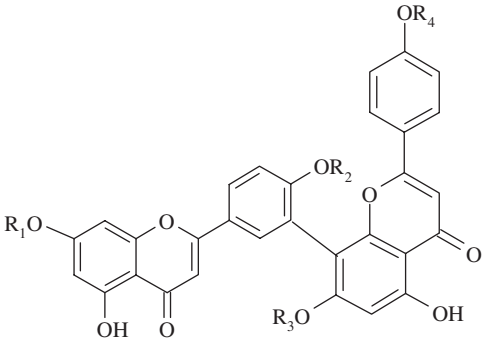
Equally was obtained compound **11** as an amorphous pale yellow solid for which the molecular formula $C_{22}H_{22}O_{10}$ was assigned in conformity with its high-resolution mass spectrum in which the $[M+H]^+$ peak appeared at *m*/*z* 447.1282. This compound is a flavonoid glycoside since it gave a dark coloration with aqueous $FeCl_3$ solution, a brick red coloration with Mg turnings in the presence of concentrated HCl [16] and a violet coloration with Molish reagent [17].

The IR spectrum was consistent with that of a flavonoid glycoside as it displayed important absorption bands for hydroxyl and phenol functions (3318 and 3120 cm^{-1}), the chromanone carbonyl (1642 cm^{-1}), the conjugated double bond (1622 cm^{-1}), and aromatic rings (1601 and 1596 cm^{-1}) [18].

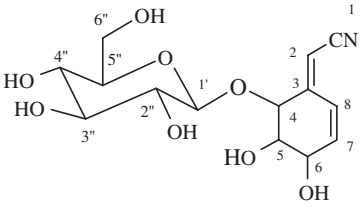
The UV spectrum of compound **11** confirmed the implication of the flavone motif as it displayed two intense bands at 257 and 349 nm attributed to the benzoyl and the cinnamoyl chromophores, respectively [19]. Evidence of the absence of free OH groups at positions 4′ and 7 was obtained when the benzoyl band was stable with methanol



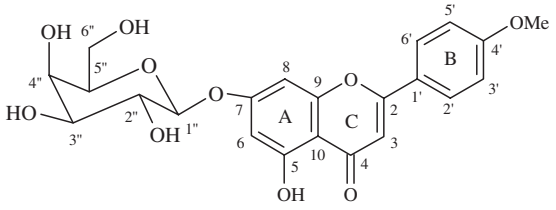
1 R = H
2 $R_2 = CH_3$, $R_1 = R_3 = R_4 = H$
3 $R_2 = R_4 = CH_3$, $R_1 = R_3H$
4 $R_1 = R_2 = R_4\ CH_3$, $R_3 = H$,
5 $R_2 = R_3 = CH_3$, $R_1 = R_4 = H$



6 $R_1 = R_2 = R_3 = R_4 = H$
7 $R_1 = CH_3$, $R_2 = R_3 = R_4 = H$
8 $R_1 = R_3 = CH_3$, $R_2 = R_4 = H$
9 $R_1 = R_2 = R_3 = CH_3$, $R_4 = H$



10



11

Figure 1: Structures of compounds **1–11**.

as solvent and the cinnamoyl band varied very little with addition of NaOAc. On the other hand, the presence of a free hydroxyl group at the position 5 of the flavone moiety was confirmed by the bathochromic shift of 16 nm of the benzoyl band with the addition of HCl.

The ^{1}H NMR (1D and 2D) spectra of compound **11** confirmed the presence of the flavone motif as the characteristic signals of protons corresponding to the four different proton systems on the flavone nucleus were identified (Table 1). These included a proton singlet at δ_H 6.76 (1H, s, H-3); an AB system of two *meta* coupled protons on ring A at δ_H 6.44 (1H, d, 2.1 Hz, H-6) and at δ_H 6.82 (1H, d, 2.1 Hz, H-8); an AA′BB′ system of four protons on a *para* disubstituted benzene ring B at δ_H 7.90 (2H, d, 9.0 Hz, H-2′/H-6′) and at δ_H 6.90 (2H, d, 9.0 Hz, H-3′/H-5′). In addition, two singlet signals observed at δ_H 3.86 ppm (3H, s, CH_3-O-4′) and at δ_H 12.96 (1H, s, OH-5) were accounted for by an *O*-methyl substituent on ring B and a much deshielded phenol proton *peri* to the chromanone carbonyl function. The connections displayed in the NOESY spectrum between the protons of methoxy group at δ_H 3.86 and the two aromatic protons at δ_H 6.90 (H-3′/H-5′, ring B), suggested the implication of 4′-*O*-methylapigenin as aglycone in the structure of **11**.

In addition, the signals of a system of seven sugar protons observed at δ_H 5.34 (1H, d, 7.8 Hz, H-1″), 3.58 (1H, dd, 7.8 and 9.1 Hz, H-2″), 3.34 (1H, dd, 3.0 and 9.1 Hz, H-3″), 3.64 (1H, dd, 3.0 and 3.3 Hz, H-4″), 3.31 (1H, ddd, 3.3, 5.6 and 6.1 Hz, H-5″), 3.37 (1H, dd, 6.1 and 10.3 Hz, H-6″a) and 3.45 (1H, dd, 5.6 and 10.3 Hz, H-6″b) correspond to those reported for galactose [21].

These structural elements were confirmed when after the acid hydrolysis of **11**, the spectral data of the aglycone was very similar to those described for 4′-*O*-methylapigenin [20] while those of the sugar were identical to the values obtained for a commercial sample of D-galactose.

The ^{13}C NMR spectrum of **11** (Table 1) displayed signals indicating the presence of all the 20 two carbon atoms given in the molecular formula among which were identified seven quaternary carbon atoms, 10 methines, a methyl, and a methylene groups. In its HSQC spectrum, the signal of the anomeric carbon C-1″ of the sugar appeared at δ_C 102.2, that of the methylene carbon at δ_C 60.1, while those of the other sugar carbons appear at δ_C 71.4, 77.6, 68.4, and δ_C 76.2. All these values correspond to those of the carbon atoms in galactose [20]. Signals of protonated carbon atoms on the aglycone include that of the methoxy carbon at δ_C 56.6, the carbons C-3, C-6, and

Table 1: NMR (^{1}H 500 MHz) and (^{13}C 125 MHz) data for compounds **11** and **12** in acetone d_6.

11					**12 4′-*O*-methylapigenin [20]**	
Position	**δ_C ppm**	**DEPT**	**δ_H ppm (J, Hz)**	**δ_C HMBC correlations**	**δ_C ppm**	**δ_H ppm (J, Hz)**
2	163.2	C	–	–	164.4	–
3	102.6	CH	6.76 (1H, s)	182.2, 163.2, 121.4, 103.1	106.6	6.58 (1H, s)
4	182.2	C	–	–	180.5	–
5	161.3	C	12.96 (1H, s, OH)	161.3, 103.1, 98.7	164.9	13.11 (1H, s, OH)
6	98.7	CH	6.44 (1H, d, 2.1 Hz)	161.8, 161.3, 103.1, 94.1	104.8	6.83 (1H, d, 2.1 Hz)
7	163.8	C	–	–	160.2	–
8	94.1	CH	6.82 (1H, d, 2.1 Hz)	163.8, 157.6, 98.7, 103.1	99.3	6.71 (1H, d, 2.1 Hz)
9	157.6	C	–	–	160.7	–
10	103.1	C	–	–	109.4	–
1′	121.4	C	–	–	123.1	–
2′/6′	128.2	CH	7.90 (2H, d, 9.0 Hz)	163.2, 161.5, 121.4, 116.1	129.3	7.83 (2H, d, 8.8 Hz)
3′/5′	116.1	CH	6.90 (2H, d, 9.0 Hz)	161.5, 128.2, 121.4	117.1	6.92 (2H, d, 8.8 Hz)
4′	161.5	C	–	–	162.6	–
CH_3-O-4′	56.6	CH_3	3.86 (3H, s)	–	56.4	3.86 (3H, s)
1″	102.2	CH	5.34 (1H, d, 7.8 Hz)	163.8, 77.6, 71.4		
2″	71.4	CH	3.58 (1H, dd, 7.8 and 9.1 Hz)	102.2, 77.6, 68.4		
3″	77.6	CH	3.34 (1H, dd, 3.0 and 9.1 Hz)	102.2, 71.4, 76.2, 68.4		
4″	68.4	CH	3.64 (1H, dd, 3.0 and 3.3 Hz)	77.6, 76.2, 71.4, 60.1		
5″	76.2	CH	3.31 (1H, ddd, 3.3, 5.6, and 6.1 Hz)	77.6, 68.4, 60.1,		
6″	60.1	CH_2	3.37 (1H, dd, 6.1 and 10.3 Hz)	76.2, 68.4		
			3.45 (1H, dd, 5.6 and 10.3 Hz)	76.2, 68.4		

C-8 of the chromanone moiety that appear, respectively, at δ_C 102.6, 98.7, and 94.1. HMBC correlations confirm that the signals at δ_C 182.2, 163.8 and at 161.5 are those of the carbonyl C-4, and the carbon atoms C-7 and C-4′, respectively.

The position of the glycosidic bond was deduced from the connections observed in the HMBC spectrum of **11** which included a correlation between the anomeric proton of the sugar H-1″ (δ_H 5.34) and the carbon C-7 of the aglycone at δ_C 163.8. This suggests that the glycosidic bond is between the anomeric carbon C-1′ (δ_C 102.2) and the oxygen on the aglycone carbon C-7 (δ_C 163.8). This was confirmed by the NOESY spectrum of **11** that displayed cross peaks between the anomeric proton (δ_H 5.34) and the aglycone protons H-6 (δ_H 6.44) and H-8 (δ_H 6.82), thus establishing its structure as 4′-*O*-methyl-7-*O*-β-D-galactosylapigenin. This glycoside had earlier been characterized as an active anti-HIV agent in *Chrysanthemum morifolium* [22], but the given spectroscopic information is incomplete with several overlapping peaks and missing coupling constants. Our report gives both the complete assignments of all the chemical shifts of the protons and carbon atoms in the molecule of 4′-*O*-methyl-7-*O*-β-D-galactosylapigenin and all the proton–proton coupling constants thus completing this earlier reported data.

2.1 Chemotaxonomic significance

Flavonoids, biflavonoids, and cyanoglycosides have been reported as regular constituents of many species of *Campylospermum* [23–25]. To our knowledge, this is the first report of the characterization of a flavone galactoside from this genus placing it as a potential source of this bioactive galactoside.

3 Materials and methods

3.1 General experimental procedures

Ethanolic solutions of compounds were used to record UV spectra on a Krontron-Uvikon 930 spectrometer (San Diego, CA, USA), whereas transparent KBr pellets were used on a Jasco FTIR-3000E spectrometer (Tokyo, Japan) to obtain IR spectra. Optical rotations were measured on a PERKIN Elma polarimeter (Überlingen, Germany). HR-CIMS was recorded on a Riber Nermag $V_{3.0}$ spectrometer (Rueil-Malmaison, France) and using NH_3 as ionizing gaz. Solutions of compounds in either CD_3COCD_3 or DMSO-d_6 were used to record the 500 MHz ^{1}H and the 125 MHz ^{13}C spectra on

a Bruker WM500 spectrometer (Rheinstettten, Germany). Sephadex LH20 (Pharmacia Fine Chemicals, Uppsala, Sweden) and kieselgel 60 (mesh 0.063, 0.200 mm, Merck, Darmstadt, Germany) were used for column chromatography. Precoated fluorescent silica gel 60 F_{254} aluminum sheets (Merck, Germany) used for thin layer chromatography (TLC) were developed in the eluent mixture CH_2Cl_2/MeOH (1:1, v/v). TLC chromatograms were visualized by spraying plates with 3% H_2SO_4 followed by heating in an oven at 60 °C for 10 min. Preparative TLC plates on glass support were prepared using fluorescent silica gel 60 F_{254} and developed in the same solvent system as above and visualized with a UV lamp of wavelength 254 nm. Bands resulting from the separation were isolated, scraped off, and recovered with methanol and evaporated to get pure compounds.

3.2 Plant material

The leaves of *C. elongatum* were harvested in Massok (Cameroon) in August 2009 and identified by Mr. Paul Mezili, botanist in the National Herbarium, Yaounde, Cameroon where a voucher specimen (#18360) was deposited.

3.3 Extraction and purification

Air-dried ground leaves of *C. elongatum* reduced into fine powder (1.5 kg) and exhaustively extracted with cold methanol in a percolator gave a gum (54 g) after removal of solvent. Warm EtOAc was used to wash the gum leading to an EtOAc soluble fraction further concentrated to give a crude extract (32 g), which was then fractionated by gel exclusion chromatography on a Sephadex LH-20 column, eluted with MeOH to give eight fractions: F_1 20.5 g, F_2 6.5 g, F_3 2.8 g, F_4 0.6 g, F_5 0.8 g, F_6 0.5 g, F_7 0.2 g, and F_8 0.1 g. The purification of F_8 by preparative TLC on silica gel plates, eluted with the mixture CH_2Cl_2/MeOH (10:1, v/v) and using the technique of multiple migrations gave five major bands which were scraped off, recovered with MeOH, and finally concentrated to give five compounds: **1** (22 mg), **2** (8 mg), **3** (16 mg), **4** (12 mg), and **5** (10 mg). The purification of F_7 following the same procedure as above led to the isolation of four other compounds **6** (8 mg), **7** (4 mg), **8** (10 mg), and **9** (9 mg).

Fraction F_6 was subjected to repeated LH-20 separation to give three main fractions: F6a (214 mg), F6b (188 mg), and F6c (98 mg). The last fraction was purified by repeated TLC on silica gel plates developed with the eluent CH_2Cl_2-MeOH (5:1, v/v) to give compounds **10** (23 mg) and **11** (18 mg).

3.4 Hydrolysis of compound 11

Compound **11** (8 mg) was placed in a 10-ml flask and dilute HCl (10%, 3 ml) was added and the set was put under reflux for 1 h after which it was allowed to cool, and water (2 ml) was added. A yellow deposit formed was filtered and washed with water and finally placed in a dessicator under vacuum for 24 h to give the aglycone as yellow powder. Acetone (3 ml) was added to the combined aqueous filtrate and a white precipitate of the sugar deposited was filtered and dried under vacuum for 24 h to give a white amorphous powder.

4′-*O*-methyl-7-O-β-D-galactopyranosylapigenin **11**: amorphous light yellow powder.

– $[\alpha]_D$ – 148 (c 0.1 MeOH) – UV: λ_{max} (nm) log ε: 257 (4,3); 349 (3,8); + NaOAc: 257 (4,3); 348 (3,8) + HCl: 273 (4,3); 352 (3,4) – IR (KBr disc) ν_{max} cm^{-1}: 3318, 3120, 3048, 1642, 1622, 1601, 1596. – ESI-HRMS: *m/z* (%): $[M+H]^+$ *m/z* 447.1282 (calculated for $C_{22}H_{23}O_{10}$ 447.1291). – NMR (^1H 500 MHz and ^{13}C 125 MHz), see Table 1.

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