A New Flavone from the Roots of *Milicia excelsa* (Moraceae)

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A new flavonoid identified as 2-(2,4-dihydroxyphenyl)-5-hydroxy-8,8-dimethyl-4*H*,8*H*-pyrano[2,3-*f*]chromen-4-one (2'-hydroxyatalantoflavone) (**1**) was obtained from the roots of *Milicia excelsa* along with five known compounds including atalantoflavone (**2**), neocyclomorusin (**3**), 6-geranylnorartocarpetin (**4**), cudraxanthone I (**5**), and betulinic acid (**6**). The structures of the isolates were established on the basis of their spectral data and by comparison with those reported in the literature.

Key words: Milicia excelsa, Flavonoid, Structure Elucidation

Introduction

Milicia excelsa Welw. C. C. Berg, a plant belonging to the Moraceae family, is an important commercially grown timber species of Western, Central, and Eastern Africa where it is sold under the trade name Iroko (Ouinsavi and Sokpon, 2010). Locally called "abang" or "momangi" in Cameroon (Ndenecho, 2009), this large tree can reach 50 m in height and about 2 m in diameter (Ouinsavi and Sokpon, 2010). In the Nigerian traditional pharmacopeia, the powder of its stem bark mixed with other ingredients is extracted in hot water and administered to patients, suffering from mental illness, as a calmative (Ibrahim *et al.*, 2007). Herbalists use *M. excelsa* in Buea, a southwest locality of Cameroon, to treat backache, toothache, stomach problems, cough, and heart palpitation (Ndenecho, 2009). In order to identify secondary metabolites which could be responsible for the observed biological activities, *M. excelsa* has been investigated chemically. In the course of this study, a new flavone was isolated, along with five known compounds, and identified by spectroscopic methods. We herein report on the structure elucidation of this new flavonoid.

Results and Discussion

The crude dichloromethane/methanol extract of *M. excelsa* was subjected to repeated silica gel column chromatography yielding six compounds including one new metabolite.

Compound **1** was obtained as a reddish gum from the sub-fractions eluted with a mixture of *n*-hexane/ethyl acetate (65:35, v/v). Its molecular formula $C_{20}H_{16}O_6$ was determined based on the NMR data in conjunction with HR-ESI-MS which revealed a *pseudo*-molecular ion peak at *m/z* 353.1024 (calcd. for $[M + H]^+$, 353.1025). This compound gave green and red colours for the $FeCl_3$ and the Shinoda test, respectively, indicating a flavonoid bearing free phenolic hydroxy groups. Its 1D-NMR spectra (Table I) displayed signals of an ABX spin system [δ 6.96 (dd, J = 2.3, 8.7 Hz)/109.5 ppm, 8.17 (d, J = 8.7 Hz)/131.0 ppm, and 6.97 (d, J = 2.3 Hz)/104.9 ppm], two aromatic singlets (δ 6.58/100.3 ppm and 7.88/108.7 ppm), two olefinic protons [δ 5.71 (d, J = 9.9 Hz)/127.9 ppm and 7.03 (d, J = 9.9 Hz)/115.9 ppm], and two isochronous gem methyl groups at δ 1.48 (s)/28.5 ppm. The latter signal showed HMBC correlations (Fig. 1)

Table I. NMR (^{1}H, 600 MHz; ^{13}C, 150 MHz) data of 2'-hydroxyatalantoflavone (**1**) and atalantoflavone (**2**) in C_5D_5N (*J* in Hz).

Position	**1**		**2**	
	^{1}H	^{13}C	^{1}H	^{13}C
1	–	–	–	–
2	–	163.2	–	164.9
3	7.88 (1H, s)	108.7	6.98 (1H, s)	104.4
4	–	183.9	–	183.3
4a	–	106.1	–	106.1
5	–	163.0	–	162.9
6	6.58 (1H, s)	100.3	6.58 (1H, s)	100.7
7	–	159.9	–	160.0
8	–	102.1	–	102.2
8a	–	152.8	–	152.6
1'	–	110.7	–	122.6
2'	–	161.3	8.03 (1H, d, 8.7)	129.4
3'	6.97 (1H, d, 2.3)	104.9	7.31 (1H, d, 8.7)	117.4
4'	–	164.1	–	163.4
5'	6.96 (1H, dd, 2.3, 8.7)	109.5	7.31 (1H, d, 8.7)	117.4
6'	8.17 (1H, d, 8.7)	131.0	8.03 (1H, d, 8.7)	129.4
1"	7.03 (1H, d, 9.9)	115.9	6.97 (1H, d, 9.9)	115.7
2"	5.71 (1H, d, 9.9)	127.9	5.74 (1H, d, 9.9)	128.2
3"	–	78.6	–	78.7
4" and 5"	1.48 (6H, s)	28.5	1.49 (6H, s)	28.5
5-OH	14.1 (1H, s)	–	13.8 (1H, s)	–

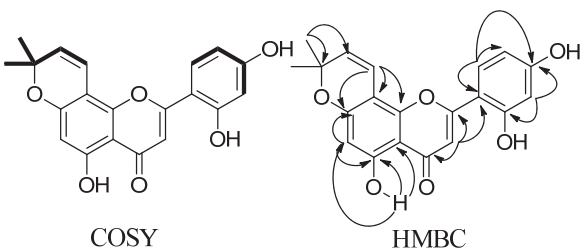

Fig. 1. COSY and HMBC correlations of compound **1**.

with a quaternary carbon atom at δ_C 78.6 ppm and an olefinic carbon atom at δ_C 127.9 ppm; in addition, HMBC correlations were found between the olefinic proton at δ_H 7.03 ppm and the carbon signals at δ_C 127.9, 102.1, and 159.9 ppm suggesting the presence of a 2,2-dimethyl-2*H*-pyran ring in **1**. This assumption was supported by comparison of these data with those reported for atalantoflavone (**2**) (Bacher *et al.*, 2010). We have recorded the NMR data of the latter compound in pyridine-d_5 to allow a better comparison (Table I). Moreover, carbon resonances exhibited in the ^{13}C NMR spectrum of **1** at δ_C 163.2 (C-2), 108.7 (C-3), 183.9 (C-4), 106.1 (C-4a), 163.0 (C-5), 100.3 (C-6), 159.9 (C-7), 102.1 (C-8), and 152.8 ppm (C-8a) were similar to those of rings A and C as well as of the pyran moiety of atalantoflavone. Further correlations were observed in the HMBC spectrum between the proton at δ_H 7.03 ppm and the carbon atom at δ_C 152.8 ppm while the chelated proton correlated with the methine carbon atom C-6 and the quaternary carbon atoms C-4a and C-5. The latter interactions suggested the pyran ring to be fused to carbon atoms C-7 and C-8 in the flavonoid backbone as reported for atalantoflavone (Bacher *et al.*, 2010). The ring C of the flavonoid was established from HMBC correlations observed between the proton at δ_H 7.88 ppm (H-3) and carbon signals at δ_C 163.2 (C-2), 183.9 (C-4), 106.1 (C-4a), and 110.7 ppm (C-1'). The ABX spin system and three quaternary carbon atoms, two of which being oxygenated, constituted ring B, differing from the *para*-disubstituted moiety found in compound **2**. This observation was supported by HMBC correlations (Fig. 1) between one of the protons in the ABX system at δ_H 8.17 ppm with carbon resonances at δ_C 110.7 (C-1'), 161.3 (C-2'), 163.2 (C-2), and 164.1 ppm (C-4'). These data in conjunction with those previously reported allow to establish the structure of compound **1** as 2-(2,4-dihydroxyphenyl)-5-hydroxy-8,8-dimethyl-4*H*,8*H*-pyrano[2,3-*f*]chromen-4-one, trivially named 2'-hydroxyatalantoflavone. The significant downfield shift observed for H-3 in compound **1** of about 0.9 ppm compared to compound **2** is caused by the proximity of the additional phenolic hydroxy group. This effect is also known from other flavones (Park *et al.*, 2007).

The remaining isolated compounds were identified on the basis of their spectral data and by comparison with those reported in the literature (Fig. 2). Thus, the five other compounds were identified as atalantoflavone (**2**) (Bacher *et al.*, 2010), neocyclomorusin (**3**) (Jeong *et al.*, 2009), 6-geranylnorartocarpetin (**4**) (Fukai and Nomura, 1991), cudraxanthone I (**5**) (Hano *et al.*, 1990), and betulinic acid (**6**) (Yili *et al.*, 2009). Atalantoflavone (**2**) was isolated for the first time from *Citrus limona*, a plant belonging to Rutaceae (Chang, 1990), while neocyclomorusin (**3**) obtained from *Morus alba*, a Moraceae species, was reported in 1976 by Nomura *et al.* Nomura together with Fukai reported 6-geranylnorartocarpetin (**4**) isolated from *Morus alba* (Fukai and Nomura, 1991) while cudraxanthone I (**5**) was previously isolated from

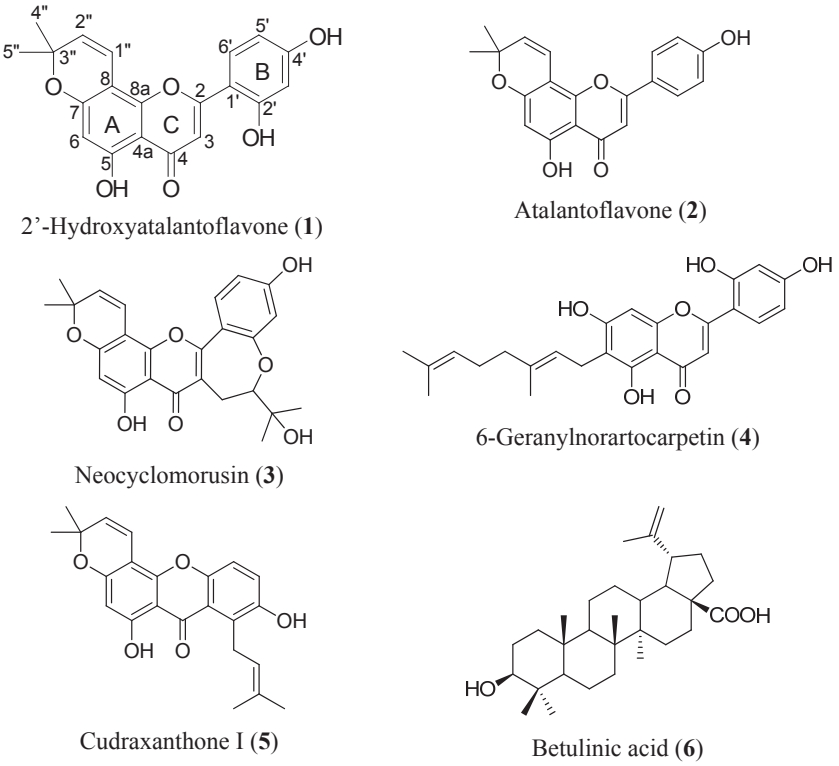

Fig. 2. Chemical structures of the compounds isolated from the roots of *M. excelsa*.

the root bark of *Cudrania tricuspidata*, another species of Moraceae (Hano *et al.*, 1990). Betulinic acid (**6**) was isolated from *Alangium lamarckii* and firsly reported in 1968 by Pakrashi *et al.* It may be speculated that the newly identified metabolite **1** represents the biogenetic precursor of neocyclomorusin (**3**), the formation of which could consist in a C-prenylation of position 3, followed by epoxidation and ring opening.

Conclusion

The majority of the isolated and identified secondary metabolites of *M. excelsa* are prenylated flavonoids, and the antimicrobial activity of this class of compounds is well documented (Edziri *et al.*, 2012; Chukwujekwu *et al.*, 2011; Yin *et al.*, 2004; Cushnie and Lamb, 2005). Therefore, the traditional use of this species could be justified by its content of these secondary metabolites. Moreover, some of the metabolites were reported to possess analgesic and anti-inflammatory activities which could explain why this plant is used to treat different painful ailments (Botta *et al.*, 2005).

Experimental

Instrumentation

Column chromatography (CC) and thin-layer chromatography (TLC) were performed over silica gel 0.035 – 0.070 mm (Merck, Darmstadt, Germany), 60A and $60F_{254}$, respectively. ^{13}C and 2D-NMR spectra were recorded on an AVANCE III-600 MHz spectrometer (Bruker, Karlsruhe, Germany) equipped with a 5-mm inverse TCI cryoprobe using standard pulse sequences. The IR spectrum was recorded on a Bruker Tensor 27 IR spectrometer equipped with a diamond ATR unit. The UV spectrum was recorded on an evolution 201 UV-visible spectrophotometer (Thermo Fischer Scientific, Waltham, MA, USA). HR-ESI-MS was carried out with a Q-ToF ULTIMA-III quadrupole TOF mass spectrometer (Waters, Eschborn, Germany).

Plant material

The roots of *M. excelsa* were collected on June 28, 2011 in Yaoundé, Cameroon, and identified by the staff of the national herbarium where a voucher specimen was deposited under the registration number HNC 57226.

Extraction and isolation

The plant material was cut into small pieces which were air-dried and crushed. The powder obtained (1.06 kg) was macerated in a mixture of dichloromethane (DCM)/methanol (1:1, v/v) for 72 h. The solution was evaporated *in vacuo* yielding 63 g of crude extract. The latter was poured onto water, and a liquid-liquid extraction was performed using successively DCM, ethyl acetate, and *n*-butanol to give three fractions, A (18 g), B (26 g), and C (12 g), respectively. Fraction A was chromatographed with a mixture of *n*-hexane and ethyl acetate in gradient conditions affording 100 sub-fractions. Cudraxanthone I (**5**) (3.0 mg) was obtained from the sub-fractions eluted with a mixture of *n*-hexane/ethyl acetate (85:15). Compound **1** (2.5 mg) was isolated from the sub-fractions eluted with a mixture of *n*-hexane/ethyl acetate (65:35). The sub-fractions obtained from *n*-hexane/ethyl acetate (95:5) were further purified in isocratic conditions with a mixture of DCM/MeOH (99:1) as eluent, affording atalantoflavone (**2**) (5.0 mg) and betulinic acid (**6**) (4.0 mg). Moreover, a second chromatographic purification of the sub-fractions obtained from *n*-hexane/ethyl acetate (9:1) was performed using isocratic conditions with a mixture of DCM/MeOH (98:2) as eluent, yielding neocyclomorusin (**3**) (2.0 mg). Fraction B was chromatographed in gradient conditions with a mixture of DCM/MeOH giving 180 sub-fractions. 6-Geranylnorartocarpetin (**4**) (6.0 mg) was obtained from the sub-fractions eluted with DCM/MeOH (35:1).

2'-Hydroxyatalantoflavone (**1**): Reddish gum. – UV (MeOH): λ_{max} (log ε) = 203 (4.31), 220 (4.27), 234 (4.24), 254 (4.18), 273 (4.20), 345 (4.03) nm. – IR: ν = 2923, 1655, 1595, 1570, 1347, 1240 cm^{-1}. – HR-ESI-MS (positive mode): *m/z* = 353.1024 (calcd. for $[C_{20}H_{16}O_6 + H]^+$, 353.1025).

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