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Biflavonoids constituents from the leaves of *Rhabdophyllum Calophyllum* (Hook. F.) Tiegh (Ochnaceae)

[1, 2,*]Baleba Mbanga Moise Roger, [1]Tih Ewola Anastasie and [1]Ghogomu Tih Raphael

[1]Department of Organic Chemistry, Faculty of Science, University of Yaounde I, P.O. Box 812, Yaounde, Cameroon
[2]Centre for Food and Nutrition Research, IMPM, P.O. Box 6163, Yaounde, Cameroon

ABSTRACT

Chemical investigation of the ethyl acetate extracts of the leaves of Rhabdophyllum calophyllum has resulted in the isolation of biflavonoids named Agathisflavone (1), Amentoflavone (2), 7-O-methylamentoflavone (3), and Campylospermone B (4), The structures of the these compounds were identified by comparaison of their MS, UV, IR, 1H and 13C NMR data with those reported in the literature as well as the acetylation for compound (4).

Keywords: Ochnaceae, *Rhabdophyllum calophyllum*, biflavonoïds.

Introduction

Rhabdophyllum Calophyllum (Hook.f.) Tiegh (Ochnaceae) is a shrub growing in wet areas. It is distributed in Cameroon, Nigeria, Gabon and the Democratic Republic of Congo, where it is harvested and used as herbal medicine [**1**]. In Congo Brazzaville, the leaves are used for the treatment of heart and belly aches. Rhabdophyllum is traditionally used in African folk medicine as an aphrodisiac and in the treatment of many illnesses such as hip disease, sore ribs and tiredness [**2]**. As a part of our ongoing effort to discover phenolic compounds in the family Ochnaceae **[3-5]** and to supply useful evidence for chemotaxonomy on related members of this family, we report herein the first phytochemical investigation on *R. calophyllum*. Biflavonoids from the family of Ochnaceae exhibit several biological and pharmacological effects such as antimicrobial**,** anti-inflammatory, anti-hepatotoxic and anti-ulcer properties **[6-8].** They also inhibit enzymes such as aldose reductase and xanthine oxidase **[9].** This article presents the isolation and characterisation of 4 biflavonoids, which were purified from the ethyl acetate extract of the dried leaves *of R. callophyllum*, as well as the acetylation of campylospermone B (4). In continuation of our study on Cameroonian medicinal species and in the aim to find secondary metabolites, we report here the isolation and the structures elucidation of biflavonoïds from the EtOAc fraction of the leaves of *Rhabdophyllum Calophyllum* from Cameroon. We describe here the identification on the basis of spectroscopic studies, acetylation and comparison with literature data.

MATERIALS AND METHODS

General Procedures

Melting points were uncorrected. The $[\alpha]_D$ values were obtained in MeOH at 20°C on a Perkin-Elmer 341 digital polarimeter. UV/Vis spectra were recorded on a Perkin-Elmer (Billerica, MA, USA) Lambda 15 UV/Vis spectrometer. IR spectra were recorded with an ATR platinium Diamond 1 Refl Spectrometer (Bruker Optics, Inc., Billerica, MA, USA), while the ^{1}H- and ^{13}C- NMR spectra where recorded in acetone d_6, at 400 and 100 MHz with a Bruker DPX-400, 400 spectrometers (Bruker, Rheinstetten, Germany). Chemical shifts (δ) were given in ppm using TMS as internal standard and coupling constant (J) are given in Hz. The High-resolution ESI-TOF in positive-ion

mode was acquired using a TSQ 700 (Finnigan MAT) instrument (Thermo Finningan MAT GmBH, Bremen, Germany). Silica gel (Kieselgel 60, 70-230 Mesh, Merck) was used for Column Chromatography. Size-exclusion chromatography was performed on Sephadex LH-20 (lipophilic Sephadex; Amersham Biosciences purchased from Sigma-Aldrich Chemie, Steinheim, Germany). TLC was conducted on precoated Merk Kieselgel 60 F254 plates (20*20 Cm, 0.25 mm). Spots were checked on TLC plates under UV light (254 mm), and developed with $KMnO_4$ reagents, followed by heating. Phenolic compounds were detected on TLC plates as dark blue spots after spraying with $FeCl_3$ reagents.

Plant material

The leaves of *Rhabdophyllum Calophyllum* were collected at Ndongo, a village in Nyong-and-Kelle division (Cameroon) in November 2014 and was identified by Mr Nana Victor, botanist at the National Herbarium, yaounde, Cameroon, where a voucher specimen (HNC 31405) documenting the collection was deposited.

Extraction and isolation

The air-dried and powdered leaves of *Rhabdophyllum Calophyllum* (1.5 kg) were percolated for 48 h with MeOH at room temperature, and afterwards the solution was filtered and concentrated under vacuum. The crude MeOH extract (158.7 g) was suspended in water and partitioned with *n*-hexane, ethyl acetate, and *n*-butanol successively in the same volume seven times to give 14.4, 28.6, and 14.3 g extract residues, respectively. The residual aqueous layer was finally evaporated and freeze-dried to obtain 58.4 g of residue. The EtOAc extract (28.6 g) was subjected to column chromatography (CC) on silica gel (CH_2Cl_2/MeOH gradient of increasing polarity) to give five fractions, R1–R5, on the basis of TLC composition. Fraction R2 (2.1 g) was subjected to repeated CC on silica gel eluted with CH_2Cl_2/MeOH (10:1) and on Sephadex LH-20 eluted with MeOH to yield 5 mg Amentoflavone (2). Fraction R3 (2.9 g) was firstly chromatographed on a silica gel column (CH_2Cl_2/MeOH, 10:1 and 5:1) to give subfractions R3A and R3B, which were further purified using the same procedure as before to yield 8 mg 7-O-methylamentoflavone (**3**). Fraction R5 (3.6 g) was purified by CC with CH_2Cl_2/MeOH (10:1, 8:1, and 5:1) to yield 4.5 mg agathisflavone (1). Further purification of fraction R1 was conducted by repeated silica gel column chromatography and preparative TLC to give 8 mg campylospermone B (4).

General procedure for the acetylation

A mixture of compound 4 (1mmole), acetic anhydride (1.5 mmole) and $Zr(HSO_4)_4$ (0.05 mmole) in pyridine (5mL) was stirred under fume hood. The progress of the reaction was monitored by TLC. After completion of the reaction, solvent was evaporated and water was added (2*10 mL). The organic layer was separated and washed with saturated $NaHCO_3$ (2*10mL) and water (5mL), dried over anhydrous $MgSO_4$. Evaporation of the solvent followed by column chromatography on silica gel afforded the pure acetate.

RESULTS AND DISCUSSION

From the EtOAc extract obtained from the leaves of *Rhabdophyllum Calophyllum*, four biflavonoids (1-4) (figure 1), were isolated by chromatographic methods identified on the basis of their IR,UV and NMR spectra data with the comparison of literature data for similar structures.

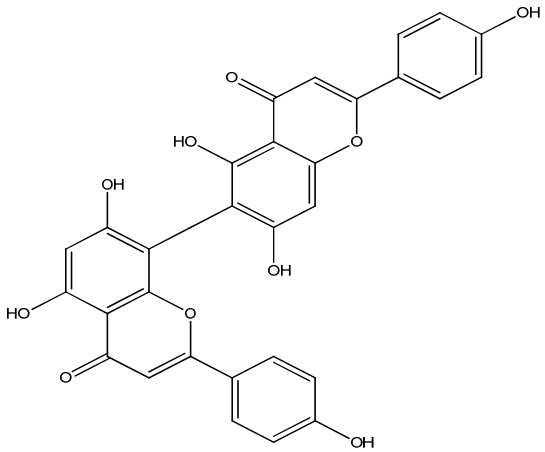
Compound 1: yellow powder soluble in MeOH with a melting point 167-169°C. the molecular formula of this compound was determined as [M+H]+ corresponding to $C_{30}H_{19}O_{10}$ on the basis of HR-MS+ spectrum at m/z 539.0989 (100%) which led to the formula $C_{30}H_{18}O_{10}$ indicating a compound containing 22 insaturations. UV λ_{max} (nm): MeOH, (c=0.1): 227, 275, 327. IR (KBr) Cm^{-1}: 3430, 1647, 1607, 1502, 1104. ^{1}H: NMR: (400 MHz Acetone-d6), δ ppm: 6.81 (1H, s, H-3); 13.02 (1H, s, OH-5); 6.68 (1H, s, H-8); 6.94(2H, d, J=8.8 Hz, H-3'/H-5'); 7.95 (2H, dd, J=8.8 Hz, H-2'/H-6'); 6.63 (1H, s, H-3''); 13.32 (1H, s, OH-5''); 6.35 (1H, s, H-6''); 7.87 (2H, d, d=8.7Hz, H-2'''/H-6'''); 6.92 (2H, d, J=8.7Hz, H-3'''/H-5'''). ^{13}C NMR (100 MHz, acetone-d6), δ ppm: 165.9 (s, C-2); 103.3 (d, C-3); 183,7 (s, C-4); 162.4 (s, C-5); 100,0 (d,C-6); 165.3 (s, C-7); 94.8 (s, C-8); 156,9 (s, C-9); 103.8 (s, C-10); 123.2 (s, C-1'); 129.1 (d, C-2'/C-6'); 116.8 (d, C-3'/C-5'); 164.4 (s, C-4'); 166.O (s, C-2''); 105.1 (d, C-3''); 184.1 (s, C-4''); 162.3 (s, C-5''); 100.5 (s, C-6''); 164.3 (s, C-7''); 104.8 (s, C-8''); 158.9 (s, C-9''); 103.6 (s, C-10''); 123.3 (s, C-1'''); 129.4 (d, C-2'''/C-6'''); 116.9 (d, C-3'''/C-5'''); 164.8 (s, C-4'''). This compound was identified as **agathisflavone** [**10**].

Compound 2: amorphous yellow solid soluble in MeOH. The molecular formula of this compound was determined as [M+H]+ corresponding to $C_{30}H_{19}O_{10}$ on the basis of HR-MS+ spectrum at m/z 539.0895 (100%) which led to the formula $C_{30}H_{18}O_{10}$ indicating a compound containing 22 insaturations. This compound was positive on phenol and flavonoid tests. IR (KBr) ν Cm^{-1}: 3318, 1682, 1628, 1508. ^{1}H: NMR: (400 MHz Acetone-d6), δ ppm: 6.80 (1H, s, H-3); 12.92 (1H, s, OH-5); 6.23 (1H, d, J=1.9Hz, H-6); 6.50 (1H, d, J=1.9Hz, H-8); 8.11 (1H, d, J=2.1 Hz, H-2'); 6.94 (1H, d, J=8.8Hz, H-5'); 7.95 (1H, dd, J=8.8 et 2.1 Hz, H-6'); 6.74 (1H,s, H-3'); 13.00 (1H, s, OH-5''); 6.64 (1H, s,

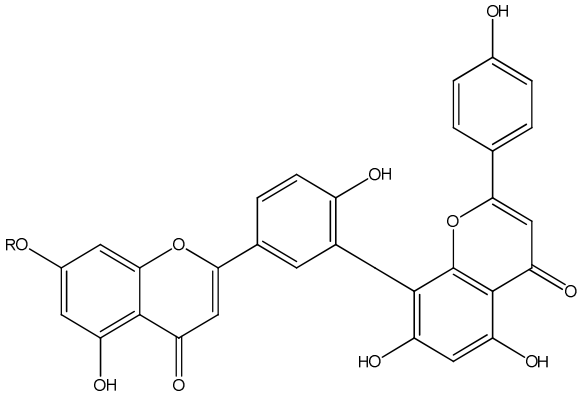
H-6''); 7.58 (2H, d, d=8.9Hz, H-2'''/H-6'''); 6.77 (2H, d, J=8.9Hz, H-3'''/H-5'''). ^{13}C NMR (100 MHz, acetone-d6), δ ppm: 163.9 (s, C-2); 103.1 (d, C-3); 182,1 (s, C-4); 162.4 (s, C-5); 100,0 (d,C-6); 162.3 (s, C-7); 95.4 (s, C-8); 158,9 (s, C-9); 103.8 (s, C-10); 123.3 (s, C-1'); 132.1 (d, C-2'); 116.9 (d, C-3'); 160.2 (s, C-4');122.9 (d, C-5'); 129.8 (d, C-6'); 166.1 (s, C-2''); 103.4 (d, C-3''); 184.3 (s, C-4''); 162.3 (s, C-5''); 100.1 (s, C-6''); 163.3 (s,C-7''); 105.6 (s, C-8''); 155.8 (s, C-9''); 105.3 (s, C-10''); 121.6 (s, C-1'''); 129.4 (d, C-2'''/C-6'''); 116.9 (d, C-3'''/C-5'''); 162.5 (s, C-4'''). This compound was identified as 3', 8''-Biapigenin named **amentoflavone** [**11**].

Compound 3: amourphous yellow powder soluble in MeOH. The molecular formula of this compound was determined as [M+H]+ corresponding to $C_{30}H_{21}O_{10}$ on the basis of HR-MS+ spectrum at m/z 539.0805 (100%) which led to the formula $C_{30}H_{20}O_{10}$ (553.1135Da) indicating a compound containing 22 insaturations. This compound was positive on phenol and flavonoid tests. IR (KBr) ν Cm^{-1}: 3321, 1654, 1632, 1504, 1103. ^{1}H: NMR: (400 MHz Acetone-d6), δ ppm: 6.92 (1H, s, H-3); 13.08 (1H, s, OH-5); 6.34 (1H, d, J=2.1Hz, H-6); 6.46 (1H, d, J=2.1Hz, H-8); 3.84 (3H, s, CH_3O-7); 7.95 (1H, d, J=2.3Hz, H-2'); 7.18 (1H, d, J=8.3Hz, H-5'); 8.04 (1H, dd, J=8.7 et 2.3 Hz, H-6'); 6.80 (1H,s, H-3''); 12.98 (1H, s, OH-5''); 6.44 (1H, s, H-6''); 7.56 (2H, d, d=8.9Hz, H-2'''/H-6'''); 6.72 (2H, d, J=8.9Hz, H-3'''/H-5'''). ^{13}C NMR (100 MHz, acetone-d6), δ ppm: 163.8 (s, C-2); 103.4 (d, C-3); 182,8 (s, C-4); 161.9 (s, C-5); 99.4 (d,C-6); 164.4 (s, C-7); 94.2 (d, C-8); 157,4 (s, C-9); 103.9 (s, C-10);55.9 (q, CH_3O-7); 121.4 (s, C-1'); 131.9 (d, C-2'); 116.6 (s, C-3'); 162.2 (s, C-4'); 122.6 (d, C-5'); 129.4 (d, C-6'); 164.4 (s, C-2''); 102.9 (d, C-3''); 184.1 (s, C-4''); 162.9 (s, C-5''); 100.1 (s, C-6''); 162.9 (s,C-7''); 105.4 (d, C-8''); 156.4 (s, C-9''); 104.1 (s, C-10''); 121.1 (d, C-1'''); 129.3 (d, C-2'''/C-6'''); 115.8 (d, C-3'''/C-5'''); 162.3 (s, C-4'''). This compound was caracterised as 7-O methylamentoflavone, named **sequoiaflavone** [**12**].

Compound 4: Amorphous white powder soluble in MeOH. the molecular formula of this compound was calculated as [M+H]+ corresponding to $C_{30}H_{23}O_9$ on the basis of HR-MS+ spectrum at m/z 527.1340 which led to the formula $C_{30}H_{22}O_9$ indicating a compound containing 14 insaturations. UV λ_{max} (nm): MeOH, (c=0.1): 237. IR (KBr) Cm^{-1}: 1795, 1518. $[\alpha]^{20}$ D= -64°, (c=0.3, MeOH). ^{1}H: NMR: (400 MHz Acetone-d6), δ ppm: 12.63 (1H, s, OH-5); 7.08 (2H, d, J=9.0Hz, H-2''/ H-6''); 6.54 (2H, d, J=9.2Hz, H-3''/H-5''); 5.98 (1H, d, J=1.8Hz, H-6); 5.96 (1H, d, J=1.8Hz, H-8); 7.77 (1H, d, J=8.6 Hz, H-5''); 7.08 (2H, J=9.1Hz, H-2'''/H-6'''); 6.49 (2H, J=9.1 Hz, H-3'''/H-5'''); 6.62 (1H, dd, J=8.6 and 2.2 Hz, H-6''); 6.45 (1H, d, J=2.2 Hz, H-8''); 5.73 (2*1H, d, J=12Hz, H-2/H-2''); 3.79 (2*1H, d, J=12.0 Hz, H-3/H-3'').). ^{13}C NMR (100 MHz, acetone-d6), δ ppm: 195.4 (s, C-4), 166.2 (s, C-7); 164.5 (s, C-5); 163.2 (s, C-9); 157.6 (s, C-4'); 129.4 (d, C-2'); 129.2 (d, C-6'); 127.8 (s, C-1'); 115.8 (d, C-3'); 115.9 (d, C-5'); 101.9 (s, C-10); 96.6 (d, C-6); 94.5 (d, C-8), 83.2 (d, C-2); 49.9 (d, C-3); 195.1 (s, C-4''); 165.4 (s, C-7); 164.3 (C-9''); 130.3 (d, C-5''); 110.9 (s, C-6''); 158.4 (s, C-4'''); 130.3 (s, C-1'''); 129.4 (C-2'''); 129.4 (C-6'''); 115.9 (d, C-3'''); 115.9 (d, C-5'''); 115.5 (s, C-10''); 103.8 (d, C-8''); 84.3 (d, C-2''), 52.1 (d, C-3''). This compound was identified as Campylospermone B [**13**]; his complete acetylation with acetic anhydride-pyridine mixture gave a penta-acetate derivative of formula $C_{40}H_{42}0_{13}$. CIMS (NH_3), $[M+H]^+$ at m/z 731. Its ^{1}H NMR (400 MHz) spectrum gave four sharp methyl signals for five acetyl groups at δ 2.19 (3H, CH_3), 2.22 (6H, $2CH_3$), 2.23 (3H, CH_3) and 2.24 (3H, CH_3) confirming its non-symetric structure. The large coupling constant (12Hz) measured between H-2 and H-3 on the first flavanone unit and H-2'' and H-3'' on the second flavanone unit establishes a trans-trans stereochemistry in **campylospermone B** [**13].**



Agathisflavone (1)



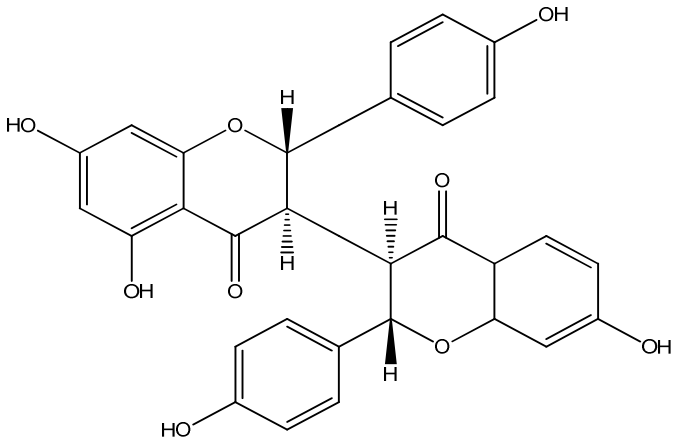
R=H Amentoflavone (2)

R=CH_3 7-*O*-methylamentoflavone (3)

Literature search revealed that all these compounds that have been isolated, have diverse biological activities such as Amentoflavone which is one of the bioactive biflavonoid obtained from polyphenol plants;it is one of the main active compound in aerial parts, leaves of variety of plants from the Ochnaceae family. It has been reported that it

has a variety of *in vitro* activities including antimalarial activity [**14]**, anticancer activity (which may, at least in part, be mediated by its inhibition of fatty acid synthase) [**15-17]**, and antagonist activity at the κ-opioid receptor [**18]**. All these biflavonoids isolated and there derivatives are common in edible plants used in traditional medicine to treat a wide variety of diseases. Agathisflavone demonstrated significant activity against influenza A and B viruses [**19]**.

The family Ochnaceae is known to be rich in flavonoids oxygenated at the 5 position giving regular biflavonoids with strongly chelated *peri* hydroxyl groups. This report of campylospermone B in *R. Calophyllum* adds to that on the Biflavonoids from *O. integerrima* **[20]** to show that the Ochnaceae family is among the sources of the rare class of biflavonoids lacking an oxygenated substituent in the 5 position.



Campylospermone B (4)

Figure 1: Structures of compounds (1–4) isolated from *R. Calophyllum.*

CONCLUSION

This study relates to the phytochemical investigation of the EtOAc extract obtained from the leaves of *R. Calophyllum.* This contribution led to the isolation of four known flavonoids named as Agathisflavone (1), Amentoflavone (2), 7-O-methylamentoflavone (3), and Campylospermone B (4).

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REFERENCES

[1]MSM Sosef, Révision du genre africain Rhabdophyllum Tiegh. (Ochnaceae), avec sa distribution au Cameroun et au Gabon., **2008**, Adansonia (30), 119–135.
[2] A Bouquet, Féticheurs et Médecines Traditionnelles du Congo (Brazzaville). Paris: ORSTOM, **1969**, 305.
[3] DE Pegnyemb, JN Mbing, AT Atchade, RG Tih,BL Sondengam,A Blond,B Bodo, *Phytochemistry*, **2005**, 66, 1922–1926.
[4]JN Mbing, MB Missi, JT Ndongo, G Bayiha ba Njock, AT Atchade, DE Pegnyemb, A Gueiffier, C Enguehard-Gueiffier, *Natural Product Research: Formerly Natural Product Letters*, 2014, 539 – 544.
[5]AA Zintchem, DN Bikobo, AT Atchade, JN Mbing, JG Pieboji, RG Tih, A Blond, DE Pegnyemb, B Bodo, *Phytochemistry*. **2008**, 69, 2209–2213.
[6]AE Tih, RT Ghogomu, BL Sondengam, C Caux, B Bodo, *J nat prod*, **2006**, *69*(8), 1206-1208.
[7]VJ Essa'a, RMM Baleba, GN Medoua, *J Food Proc*, **2015**, 1-9.
[8]G Bayiha Ba Njock, TA Bartholomeusz, M Foroozandeh, DE Pegnyemb, P Christens, JD. NASCAHMBC, *Phyto Anal*, 2011, 23, 126-130.
[9] JT Ndongo, M Shaaban, JN Mbing, DS Ngono Bikobo, AT Atchadé, DE Pegnyemb, H Laatsh, *Phytochemistry*, **2010,** 71, 1872-1878.
[10]C Souleles, N Argyriadou, S Philianos, *J Nat Prod*, **1987**, *50*(3), 510-511.
[11] SS Elo Manga, AE Tih, RT Ghogomu, A Blond, B Bodo, *Biochem system and ecol*, **2009**, 37(4), 402-404
[12] "Inhibitors of Plasmodium falciparum M1- Family Alanyl Aminopeptidase (M1AAP)".

[13]JS Lee, MS Lee, WK Oh,JY Sul, *Biol & Pharma Bull*, **2009**, 32(8), 1427–1432.
[14] S Wilsky, K Sobotta, N Wiesener, J Pilas, N Althof, T Munder, P Wutzler, A Henke, *Arch of Viro*, **2012**, 157(2), 259–269
[15]JS Lee, JY Sul, JB Park, MS Lee, EY Cha, IS Song, JR Kim, ES Chang, *Phyto Res*, **2013**, 27(5), 713–720.
[16] S Wilsky, K Sobotta, N Wiesener, J Pilas, N Althof, T Munder, P Wutzler, A Henke, *Arch of Viro*, **2012**, 157(2), 259–269
[17] JS Lee, JY Sul, JB Park, MS Lee, EY Cha, IS Song, JR Kim, ES Chang, *Phyto Res*, **2013**, 27(5), 713–720.
[18] PL Katavic, K Lamb, H Navarro, TE Prisinzano, *J Nat Prod.*, **2007**, 70(8), 1278–1282.
[19] YM Lin, MT Flavin, R Schure, FC Chen, R Sidwell, DL Barnard, JH Huffman, ER Kern, *Planta Med*, **1999**, 65, 120–125
[20] C Ichino, H Kiyohara, N Soonthornchareonnon, W Chuakul, A Ishiyama, H Sekiguchi, H Yamada, *Planta med*, **2006**, *72*(7), 611-614.