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Research Article

Secondary metabolites from *Campylospermum oliverianum* (Farron), *Campylospermum glaucum* (Tiegh) and *Campylospermum dybowskii* (Van Tiegh)

[*]Dominique Serge Ngono Bikobo[1], Auguste Abouem A Zintchem[1,2], Norbert Mbabi Nyemeck II[1], Alex de Théodore Atchade[1], Gaetan Bayiha Ba Njock[1], Paul Mosset[3] and Dieudonné Emmanuel Pegnyemb[1]

[1]*Department of Organic Chemistry, Faculty of Science, University of Yaoundé 1, P.O Box 812, Yaoundé, Cameroon.*
[2]*Department of Chemistry, Higher Training College, University of Yaoundé 1, P.O Box 47, Yaoundé, Cameroon.*
[3]*Université de Rennes 1, Institut des Sciences Chimiques de Rennes, CNRS UMR 6226, Avenue du Général Leclerc, 35042 Rennes Cedex, France*

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ABSTRACT
According to some specialists, the *Ouratea* genus has traditionally included the genera *Campylospermum*, *Rhabdophyllum* and *Idertia* of the Ochnoideae clade. Moreover, some other authors consider this relationship as controversial, partially due to the lack of a robust phylogenetic framework. Secondary metabolites can appear as chemotaxonomic markers; thus, a bridge can be applied in phylogeny between genetics and morphology. In order to evaluate chemotaxonomic position of *Campylospermum* and *Ouratea* genera**,** detailed chemical investigations on *C. oliverianum*, *C. glaucum* and *C. dybowskii* afforded several classes of known compounds (**3**, **4**, **5**, **7**, **13**, **14**, **15**, **16**, **18, 21**, **29**, **30** and **31**), most of them were previously isolated from the *Ouratea* genus, hence strengthening the generic concept which suggests that the *Campylospermum* genus seems to be closed to *Ouratea*'s one.

Keywords: *Campylospermum*; *Ouratea*; Ochnaceae; chemotaxonomic significance; flavonoids; indole alkaloids.

INTRODUCTION

The Ochnaceae family is a woody pantropical one, comprising 500 species and 27 genera. The highest diversity is found in the Neotropics with 15 genera and about 300 – 350 species. Africa has nine genera and about 150 species[1]. In this family, the tribe Ochneae displays particular traits; this latter one is characterized by three most genera: *Ouratea*, *Ochna* and *Campylospermum*[2].

The *Ouratea* genus is represented by trees or shrubs, usually completely glabrous with sepals caduceus. The *Campylospermum* genus (Ochnaceae) includes 50 species, trees or shrubs with sepals persistent, mainly occurring in tropical zone of Africa, Madagascar and extending to South West of Asia[3,4]. It is a member of the subfamily Ochnoideae, tribe Ochneae, subtribe Ouratinae[5]. The whole subtribe contains three other genera: *Rabdophyllum*, *Ouratea* and *Idertia*[5], confirmed by a recent study on the newest phylogeny classification of the pantropical Ochnaceae[2]. According to some botanical reports, the use of the broader genus concept in which *Ouratea* gathers the genera *Campylospermum*, *Idertia*[6,7,8] and sometimes *Rhabdophyllum*[3] has a preference. Other specialists adopt a conservative approach simply based on morphology which enables a demarcation among these four members of the subtribe Ouratinae[4]. Nowadays, *Ouratea* Aubl. seems to be confined to South America, when all Old World species are said to be considered as either *Campylospermum* Tiegh., *Rhabdophyllum* Tiegh. or *Idertia* Farron ones[9]. Despite this insufficient resolution in the phylogenetic tree and poor taxonomic treatment of this subfamily[2], many species belonging to the *Campylospermum* genus are used in folk medicine in the treatment of gastric pains, gonorrhea, icterus, whitlow and as aphrodisiac[10].

The objective of this study was to isolate compounds that might be used as taxonomic markers to enable an elucidation of the relationships in systematic position between *Campylospermum* and *Ouratea* genera.

EXPERIMENTAL

Optical rotations were measured on a Perkin–Elmer 341 polarimeter. NMR spectra were run on a Brüker instrument equipped with a 5 mm ^{1}H and ^{13}C probe operating at 400 and 100 MHz, respectively, with TMS as internal standard. ^{1}H assignments were made using 2D-COSY and NOESY (mixing time 500 ms) while ^{13}C assignments were made using 2D-HSQC and HMBC experiments. For this latter, the delay was 70 ms. Melting points were measured on a Büchi apparatus and are uncorrected. IR data were measured on a JASCO FTIR-300E spectrometer with KBr

*Author for Correspondence

pellets. The HR-ESI mass spectra were run on an Applied Biosystems API Q-STAR PULSAR. The EIMS was recorded on a JEOL JMSD-300 instrument. Column chromatography was carried out using silica gel of 70–230 mesh (Merck) and Sephadex LH-20. Aluminum sheets precoated with silica gel 60 F_{254} (20 x 20 cm, 0.2 mm thick; E-Merck were used for TLC to check the purity of the compounds and were visualized under UV light (254 and 366 nm), followed by iodine vapor revelation. The respective solvents hexane, CH_2Cl_2 and EtOAc were used in the partitioning of the extracts. The solvent systems for elution were: CH_2Cl_2/MeOH at increasing polarity (from pure CH_2Cl_2 to 100% MeOH) and pure MeOH.

The leaves of *C. oliverianum* (Farron) were collected at Countryside in south Region of Cameroon in December 2005, leaves of *C. glaucum* (Tiegh) were harvested at Sok Elle in Centre Region of Cameroon in December 2004 and leaves of *C. dybowskii* (Van Tiegh) were collected at Akonolinga in December 2012 in Centre Region of Cameroon, all species were identified by the senior botanist Victor Nana, being a voucher for each species (No 27057/HNC), (No. 28192/SRF/CAM) and (No. 30053/HNC), respectively, were deposited at the National Herbarium of Cameroon, Yaoundé**.**

In this paper, we report the isolation of compounds belonging to various classes from the leaves of *C. oliverianum*, *C. glaucum* and *C. dybowskii*. All these compounds (Fig. **1**) were identified by IR, ^{1}H and ^{13}C NMR, associated to 2D-dimensional techniques and their structural elucidation was confirmed by literature data.

Dried and powdered leaves of *C. olivieranum* (450 g) were extracted with MeOH at room temperature. The extract was filtered and concentrated *in vacuo* to obtain a residue (46 g) which was partitioned with hexane, CH_2Cl_2 and EtOAc. The EtOAc part (21 g) was successively fractionated on a silica gel column, eluting with a gradient solvent system (CH_2Cl_2/MeOH) giving four main fractions O_1 (4.5 g), O_2 (2.1 g) O_3 (5.4 g) and O_4 (9.0 g). Fraction I, O_1 (4.5 g) was chromatographed in a silica gel (500 g) CC using the solvent system CH_2Cl_2/MeOH (from 30/1 to 15/1) to give three sub-fractions O_{1a} (0.88 g), O_{1b} (1.66 g) and O_{1c} (1.96). Sub-fraction O_{1b} (1.66 g) was further purified by Sephadex LH-20 (MeOH) and preparative TLC (CH_2Cl_2/MeOH: 15/1) to yield menisdaurin (**4**, 14 mg)[11].

Sub-fraction O_{1c} (1.96 g) was purified on a silica gel (180 g) CC using CH_2Cl_2/ MeOH (from 30/1 to 10/1) to furnish dhurrin (**5**, 6 mg)[12]. Fraction II, O_2 (2.1 g) was chromatographed in a silica gel (200 g) CC using the solvent system CH_2Cl_2/ MeOH (from 25/1 to 8/1) to give three sub-fractions O_{2a} (0.81 g), O_{2b} (0.62 g) and O_{2c} (0.68 g). Sub-fraction O_{2b} (0.62 g) was subjected to Sephadex LH-20 (MeOH) CC rendering serotobenine (**7**, 18 mg)[13]. Using the same process as above, fraction IV, O_4 (9.0 g) gave four sub-fractions O_{4a} (0.94 g), O_{4b} (1.28 g) O_{4c} (3.98 g) and O_{4d} (2.8 g). Sub-fraction O_{4d} (2.8 g) was repeatedly subjected on Sephadex LH-20 (MeOH) to yield a mixture of steroids identified as campesterol, sistosterol, and stigmasterol-3-*O*-β-D-glucopyranoside (**29, 30** and **31**, 11 mg) [14].

Dried and powdered leaves of *C. glaucum* (600 g) were extracted as previously described. The EtOAc part (18 g) was fractionated by column chromatography on silica gel using the same solvent system as mentioned above to give four Fraction G_1 (2.79 g), G_2 (4.29. g) G_3 (1.24 g) and G_4 (9.68 g). Fraction I, G_1 (2.79 g) gave three sub-fractions G_{1a} (0.72 g), G_{1b} (1.02 g) and G_{1c} (1.05 g). Sub-fraction G_{1b} (1.02 g) was in turn subjected to a silica gel CC using the solvent system CH_2Cl_2/ MeOH (from 25/1 to 8/1) to afford 6''-*O*-acetylvitexin (**13**, 8 mg)[15] and isoliquiritigenin (**16**, 5 mg)[16]. Fraction II, G_2 (4.29 g) was subjected to CC on silica gel (400 g) and eluted with the solvent system CH_2Cl_2/ MeOH (from 30:1 to pure MeOH) to give four sub-fractions G_{2a} (1.46 g), G_{2b} (0.92 g), G_{2c} (0.98 g) and G_{2d} (0.93 g). Sub-fraction G_{2c} (0.98 g) was submitted to a silica gel (120 g) CC eluted with CH_2Cl_2/ MeOH (20/1 to 8/1); further chromatographic analyses using repeated preparative TLC (CH_2Cl_2/ MeOH: 15/1 to 8/1) afforded lanceolin C (**3**, 12 mg)[17] and serotobenine (**7**, 12 mg)[13]. Sub-fraction G_{2d} (0.93 g) was subjected to repeatedly CC to Sephadex LH-20 using MeOH to provide amentoflavone (**18**, 7 mg)[18].

Dried and powdered leaves of *C. dybowskii* (400 g) were also extracted as above. The resulting EtOAc part (18 g) was silica gel chromatographed using a binary gradient solvent system (CH_2Cl_2/MeOH: 30/1 to 8/1) producing four fractions D_1 (3.2 g), D_2 (2.8 g) D_3 (7.1 g) and D_4 (4.9 g). Fraction II, D_2 (2.8 g) was in turn subjected to a silica gel CC and to Sephadex LH-20 CC one (200 g) to provide three sub-fractions D_{2a} (0.71 g), D_{2b} (1.0 g) and D_{2c} (1.09 g). Sub-fraction D_{2b} (1.0 g) was submitted to a silica gel (100 g) CC eluted with CH_2Cl_2/ MeOH (20/1 to 15/1) to afford 2''-*O*-acetyl-7-*O*-methylvitexin (**14**, 12 mg)[19] and 4-*O*-methylvitexin (**15**, 9 mg)[20]. Fraction III, D_3 (7.1 g) was subjected to CC on silica gel (700 g) and eluted with the solvent system CH_2Cl_2/ MeOH (from 20/1 to pure MeOH) to give four sub-fractions D_{3a} (0.94 g), D_{3b} (1.87 g), D_{3c} (2.59 g) and D_{3d} (1.7 g). Sub-fraction D_{3b} (1.87 g) was chromatographed using a silica gel (200 g) CC with the solvent system CH_2Cl_2/ MeOH (from 20/1 to 8/1) giving sitosterol-3-*O*-β-D-glucopyranoside (**30**, 12 mg)[21]. Sub-fraction D_{3c} (2.59 g) was subjected to successively Sephadex LH-20 (MeOH) CC rendering lanceolin C (**3,** 3 mg)[17] and serotobenine (**7,** 25 mg)[13]. Sub-fraction D_{3d} (1.7 g) was chromatographed using a silica gel (200 g) CC with the solvent system CH_2Cl_2/ MeOH (from 15/1 to 8/1) affording amentoflavone (**18**, 4 mg)[18] and robustaflavone (**21**, 9 mg)[22].

Compound **3** was identified as Lanceolin C[17], an enantiomer of Campyloside A[23], through comparison of its spectral data (Table 1 and experimental section) with the previously published ones: White solid, $[\alpha]^{25}_{D}$ +28° (c 0.1, MeOH); ,m.p 297-299°C, IR υ^{KBr}_{max} cm^{-1} 3320, 2215, 1718, 1623, 1602, 1501; TLC Rf: 0.35 (CH_2Cl_2/MeOH: 95/5); HR-ESI MS m/z: 452.162 $[M+H]^+$ ^{1}H-NMR (DMSO-d_6, 400 MHz) δ_H : 5.77 (1H, s, H-2), 4.74 (1H, dd,, J = 8.5, 1.5 Hz, H-4), 4.79 (1H, dd, J = 8.5 Hz, H-5), 3.08 (1H, m, H-6), 2.23, 1.96 (2H, m, H-7), 4.87 (1H, dd, J = 10.2, H-8); Sugar: δ_H : 4.38 (1H, d, J = 7.5 Hz, H-1''); Aromatic ring: δ_H range 8.04-7.66. ^{13}C-NMR (DMSO-d_6, 100 MHz): δ_C:

117.7 (C-1), 96.3 (C-2), 164.9 (C-3), 69.2 (C-4), 79.2 (C-5), 66.9 (C-6), 35.3 (C-7), 75.3 (C-8); Sugar moiety: δ_C: 102.2 (C-1''), 78.3 (C-5''), 77.9,(C-3''), 74.6 (C-2''), 71.2 (C-4'') and 62.3 (C-6''): Aromatic ring: δ_C: 166.5 (C-7'), 134.6 (C-4'), 131.3 (C-1'), 130.8 (C-2', C-6'), 129.9 (C-3', C-5').

Compound **4** was identified as menisdaurin, through comparison of its spectral data (Table 1 and experimental section) with those from literature[11]. White solid, m.p 297-299°C, IR υ_{max}^{KBr} cm^{-1} 3340, 2220, 1750, 1040; TLC Rf: 0.30 (CH_2Cl_2/MeOH: 95/5); HR-ESI-TOF MS m/z: 336.1059 $[M+Na]^+$ ^{1}H-NMR (DMSO-d_6, 400 MHz). δ_H : 6.28 (1H,d, J = 9.1 Hz, H-4), 6.22 (1H, ddd, J = 9.1, 3.3, 1.3 Hz, H-5); Sugar: δ_H : 4.58 (1H, d, J = 7.5 Hz, H-1''); ^{13}C-NMR (DMSO-d_6, 100 MHz): δ_C: 118.0 (C-1), 125.9 (C-4), 141.2 (C-5); Sugar moiety: δ_C: 99.8 to 62.6 (C-1'-C6').

Compound **5** was identified as dhurrin through comparison of its spectral data (Table 1 and experimental section) with the previously published ones[12]. White amorphous solid, IR υ_{max}^{KBr} cm^{-1} 3320, 2106, 1510, 1095; TLC Rf: 0.38 (CH_2Cl_2/MeOH: 95/5); EI-MS m/z: 311 $[M]^+$. ^{1}H-NMR (DMSO-d_6, 400 MHz). δ_H: 5.90 (1H, s, H-2); Aromatic ring: δ_H 7.39 (2H, d, J = 8.6 Hz, H-4,8), 6.79 (2H, d, J = 8.6 Hz, H-5,7); Sugar moiety: δ_H : 4.62 (1H, d, J = 7.6 Hz, H-1'). ^{13}C-NMR (DMSO-d_6, 100 MHz): δ_C: 118.8 (C-1), 68.3 (C-2), 126.0 (C-3), 130.7 (C-4,8), 116.4 (C-5,7), 104.0 (C-6).); Sugar moiety: δ_C 100.8 (C-1'), 73.2 (C-2'), 76.9 (C-3'), 71.2 (C-4'), 77.3 (C-5'), 61.9 (C-6').

Compound **7** was identified as Serotobenine through comparison of its spectral data (Table 1 and experimental section) with the previously mentionned ones[13]. White solid m.p 284-286°C, IR υ_{max}^{KBr} cm^{-1} 3398, 2950, 1670, 1520; TLC Rf: 0.52 (CH_2Cl_2/MeOH: 95/5); HR-ESI-TOF MS m/z: 349.1079 $[M-H]^-$ ^{1}H-NMR (DMSO-d_6, 400 MHz). δ_H : 7.16 (1H, s, H-2), 7.20 (1H, d, J = 8.6 Hz, H-6), 6.67 (1H, d, J = 8.6 Hz, H-7); 3.05 and 2.91 (2H, t, J = 9.6, 3.5 Hz, H-10), 4.10, 4.35 (2H, t, J = 9.6, 7.9, 3.5 Hz, H-11), 6.14 (1H, d, J = 9.8 Hz, H-15), 4.77 (1H, d, J = 9.8 Hz, H-14); Aromatic ring: δ_H range 6.78-6.99 (H-1'-H-6'), 3.75 (3H, s, CH_3O-). ^{13}C-NMR (DMSO-d_6, 100 MHz): δ_C: 125.1 (C-2), 111.3 (C-3), 113.9 (C-4), 151.8 (C-5), 110.5 (C-6), 104.0 (C-7), 132.5 (C-8), 122.5 (C-9), 29.4 (C-10), 40.1 (C-11), 170.5 (C-13), 53.2 (C-14), 84.2 (C-15) and 55.6 (CH_3O-); Aromatic ring: δ_C range 147.6 - 110.6 (C-1'-C6').

Compound **13** was identified as 6''-O-acetylvitexin through comparison of its spectral data (Table 1 and experimental section) with the previously reported ones[15]. Yellow amorphous powder, IR υ_{max}^{KBr} cm^{-1} 3280, 2950, 1745, 1520, 1105; TLC Rf: 0.40 (CH_2Cl_2/MeOH: 95/5); EI-MS m/z: 474 $[M]^+$. ^{1}H-NMR (DMSO-d_6, 400 MHz). Flavonoid moiety: δ_H: 6.72 (1H, s, H-3), 6.02 (1H, d, s, H-6), 7.02 (2H, d, J = 8.5 Hz, H-2',6'), 6. 72 (2H, d, J = 8.5 Hz, H-3',5'); Sugar moiety: δ_H:4.91 (1H,d, J = 7.5 Hz, H-1"), 3.76 (1H, m, H-2") 3.42 (1H, m, H-3"), 3.38 (1H, m, H-4"), 3.86 (1H, m, H-5"), 4.32, 4.05 (2H, dd, J = 10.5, 2.0 Hz**,** H-6''); Acetyl moiety: : δ_H: 2.03 (CH_3-). ^{13}C-NMR (DMSO-d_6, 100 MHz): δ_C: 161.9 (C-2), 104.1 (C-3), 182.3 (C-4), 161.5 (C-5), 98.1 (C-6), 162.9 (C-7), 108.1 (C-8) 105.1 (C-9), 158.9 (C-10), 122.9 (C-1'), 126.8 (C-2',6'), 116.9 (C-3',5'), 160.3 (C-4'); Sugar moiety: δ_C: 78.2 (C-1''), 72.9 (C-2''), 76.2 (C-3''), 73.4 (C-4''), 77.0 (C-5''), 64.9 (C-6''); Acetyl moiety : δ_C: 170.1 (C-1'''), 20.3 (CH_3-)

Compound **14** was identified as 2''-*O*-acetyl-7-*O*-methylvitexin through comparison of its spectral data (Table 1 and experimental section) with the literature ones[19]. Yellow amorphous powder, IR υ_{max}^{KBr} cm^{-1} 3305, 1745; TLC Rf: 0.43 (CH_2Cl_2/MeOH: 95/5); EI-MS m/z: 488 $[M]^+$. ^{1}H-NMR (DMSO-d_6, 400 MHz). Sugar moiety; δ_H: 4.85 (1H, s, H-1''), 4.39 (1H, m, H-2'') 3.81 (1H, m, H-3''), 3.32 (1H, m, H-4''), 3.80 (1H, m, H-5''), 3.82, 3.54 (2H, dd, J= 10.4, 2.0, H-6''), 3.73 (CH_3O-). Acetyl: 2.02 (CH_3-). ^{13}C-NMR (DMSO-d_6, 100 MHz): δ_C: 96.8 (C-6), 165.2 (C-7), 106.8 (C-8), 122.9 (C-1'), 56.4 (CH_3O-); Sugar moiety: δ_C: 76.1 (C-1''), 74.1 (C-2''), 75.2 (C-3''), 69.9 (C-4''), 77.2 (C-5''), 62.9 (C-6''); Acetyl moiety : δ_C: 170.1 (C-1'''), 20.9 (CH_3-).

Compound **15** was identified as 4-*O*-methylvitexin through comparison of its spectral data (Table 1 and experimental section) with those from literature[20]. Yellow amorphous powder, IR υ_{max}^{KBr} cm^{-1} 3305; 2930 TLC Rf: 0.42 (CH_2Cl_2/MeOH: 95/5); EI-MS m/z: 448 $[M]^+$. ^{1}H-NMR (DMSO-d_6, 400 MHz). Flavonoid moiety: δ_H: 6.82 (1H, s, H-3), 6.08 (1H, d, s, H-6), 7.52 (2H, d, J = 8.5 Hz, H-2',6'), 6. 76 (2H, d, J = 8.5 Hz, H-3',5'), 3.79 (CH_3O-); Sugar moiety: δ_H:4.71 (1H,d, J = 7.5 Hz, H-1"), 3.96 (1H, m, H-2") 3.42 (1H, m, H-3"), 3.40 (1H, m, H-4"), 3.36 (1H, m, H-5"), 4.12, 3.95 (2H, dd, J = 10.5, 2.0 Hz**,** H-6''). ^{13}C-NMR (DMSO-d_6, 100 MHz): Flavonoid moiety: δ_C: 162.0 (C-2), 104.1 (C-3), 182.3 (C-4), 160.9 (C-5), 98.1 (C-6), 162.8 (C-7), 106.2 (C-8), 158.9 (C-9), 105.1 (C-10), 122.9 (C-1'), 127.2 (C-2',6'), 116.8 (C-3',5'), 160.3 (C-4'), 56.9 (CH_3O-);Sugar moiety : δ_C range 76.0 -.61.9 (C-1'-C6').

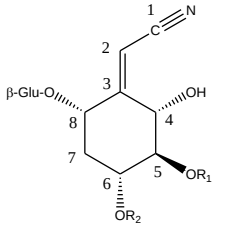
Compound **16** was identified as Isoliquiritigenin through its spectral data (Table 1 and experimental section)[16]. Yellow solid powder, m.p 209-212°C. IR υ_{max}^{KBr} cm^{-1} 3250, 3008, 1508, 1109; TLC Rf: 0.38 (CH_2Cl_2/MeOH: 95/5); EI-MS m/z: 256 $[M]^+$. ^{1}H-NMR (DMSO-d_6, 400 MHz). δ_H:7.08 (H, d, H-2,6), 6.69 (2H, d, H-3,5), 7.46 (1H, d, J = 15.4 Hz, H-α), 7.92 (1H, d, J = 15.4 Hz, H-β), 6.19 (1H, d, J = 2.4 Hz, H-3'), 6.32 (1H, dd, J = 8.6, 2.4 Hz, H-5'), 7.28 (1H, d, J = 8.6 Hz, H-6'). ^{13}C-NMR (DMSO-d_6, 100 MHz): δ_C: 186.2 (CO), 124.4 (C-1), 127.8 (C-2,6), 115.9 (C-3,5), 159.9 (C-4), 122.6 (C-α), 144.8 (C-β), 119.9 (C-1'), 160.9 (C-2'), 101.9 (C-3'), 162.0 (C-4'), 102.9 (C-5'), 129.9 (C-6').

Compound **18** was identified as amentoflavone[18]. Yellow amorphous powder, TLC Rf: 0.29 (CH_2Cl_2/MeOH: 95/5); HR-ESI-TOF MS m/z: $[M+H]^+$ à *m/z* 539,0929. ^{1}H-NMR (Acetone 400 MHz). δ_H : 6.72 (1H, s, H-3), 6.23 (1H, d, J = 2.1 Hz, H-6), 6.51 (1H, d, J = 2.1 Hz, H-8); 8.16 (1H, d, J = 2.4 Hz, H-2'), 7.20 (H, d, J = 8.7 Hz, H-5'), 8.01 (1H, dd, J = 8.7, 2.4 Hz, H-6'), 6.64 (1H, s, H-3''), 6.44 (1H, s, H-6''), 7.64 (2H, d, J = 8.8 Hz, H-2''',H-6'''), 6.82 (2H, d, J = 8.8 Hz, H-3''',H-5''') ; ^{13}C-NMR (Acetone, 100 MHz): δ_C: 163.5 (C-2), 104.4 (C-3), 184.6 (C-4), 163.5 (C-5), 99.7 (C-6), 164.4. (C-7), 94.6 (C-8), 161.8 (C-9), 104.2

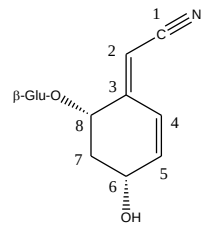
(C-10), 122.3 (C-1'), 132.6 (C-2'), 116.7 (C-3'), 159.2 (C-4'), 117.8 (C-5'), 128.8 (C-6'), 164.2. (C-2''), 103.8 (C-3''), 182.7 (C-4''), 162.5 (C-5''), 99.8 (C-6''), 163.3 (C-7''), 103.6 (C-8''), 155.5 (C-9''), 104.5 (C-10''), 122.5 (C-1'''), 129.3 (C-2''', 6'''), 116.8 (C-3''', 5''').

Compound **21** was identified as robustaflavone (Table 1 and experimental section)[22]. Yellow amorphous powder, TLC Rf: 0.29 (CH_2Cl_2/MeOH: 95/5); HR-ESI-TOF MS m/z: $[M+H]^+$ à *m/z* 539,0899. δ_H : 6.68 (1H, s, H-3), 6.19 (1H, d, J = 2.1 Hz, H-6), 6.52 (1H, d, J = 2.2 Hz, H-8); 8.11 (1H, d, J = 2.2 Hz, H-2'), 7.09 (H, d, J = 8.3 Hz, H-5'), 7.92 (1H, dd, J = 8.4, 2.2 Hz, H-6'), 6.69 (1H, s, H-3''), 6.04 (1H, s, H-6''), 7.44 (2H, d, J = 8.6 Hz, H-2''', H-6'''), 6.68 (2H, d, J = 8.6 Hz, H-3''', H-5'''); ^{13}C-NMR (Acetone, 100 MHz): δ_C: 163.7 (C-2), 104.5 (C-3), 182.4 (C-4), 163.7 (C-5), 98.7 (C-6), 164.9. (C-7), 94.2 (C-8), 160.1 (C-9), 103.5 (C-10), 121.3 (C-1'), 128.6 (C-2'), 116.2 (C-3'), 155.9 (C-4'), 126.9 (C-5'), 128.2 (C-6'), 163.7 (C-2''), 104.3 (C-3''), 182.1 (C-4''), 162.9 (C-5''), 105.9 (C-6''), 164.0 (C-7''), 96.9 (C-8''), 159.5 (C-9''), 104.8 (C-10''), 121.9 (C-1'''), 128.3 (C-2''', 6'''), 116.1 (C-3''', 5''').

Compounds **29, 30** and **31** were obtained throughout a mixture which was identified as campesterol, sistosterol, and stigmasterol-3-*O*-β-D-glucopyranoside (Table 1 and experimental section)[14]. White amorphous solid, TLC Rf: 0.41 (CH_2Cl_2/MeOH: 95/5); HR-ESI-TOF MS m/z: 599.4282, 597.4130 and 585.4133 $[M+Na]^+$. ^{1}H-NMR (DMSO-d_6, 400 MHz). δ_H: 5.32 (1H, *brs*, H-6), 3.52 (1H, m, H-3), for campesterol and sistosterol (H-22 is not observed), 1.24 (1H, m, H-23) for stigmasterol 5.15 (1H, m, H-22), 5.03 (1H, m, H-23); Sugar moiety: δ_H: 4.25 (1H, d, J = 7.5 Hz, H-1') 4.22 (1H, d, J = 7.5 Hz, H-1') and 4.18 (1H, d, H-1'). ^{13}C-NMR (DMSO-d_6, 100 MHz): δ_C: 76.2 (C-3), 140.5 (C-5), 121.0 (C-6), 12.0 (C-29) for campesterol δ_C 14.1 (C-28). For stigmasterol δ_C 137.9 (C-22), 128.7 (C-23). Sugar moiety: δ_C: 100.7 (C-1' sitosterol), 100.5 (C-1' campesterol) and 100.4 (C-1' stigmasterol), 72.8 (C-2'), 74.2 (C-3'), 71.5 (C-4'), 76.6 (C-5'), 64.2 (C-6').



1 R_1= Bz, R_2= H (4S, 5S)
2. R_1= pyrrol-2-ylcarbonyl, R_2= Bz
3 R_1= Bz, R_2= H [enantiomer of **1**, (4R, 5R)]



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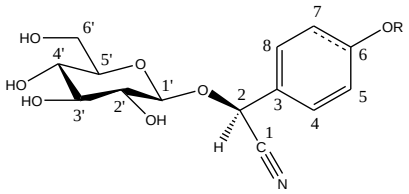
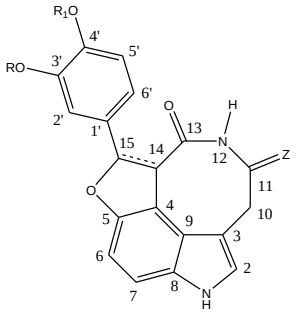
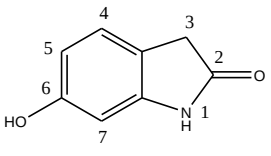


5 R= H, $\Delta^{6,7}$
6 R= H, 6,7-dihydro



7 R= H, R_1= CH_3-, Z= H, H; 14, 15-dihydro
8 R= R_1= -CH_2-, Z= H, H; 14, 15-dihydro
9 R= H, R_1= CH_3-, Z=O; 14, 15-dihydro
10 R= H, R_1= CH_3-, Z=O; $\Delta^{14,15}$



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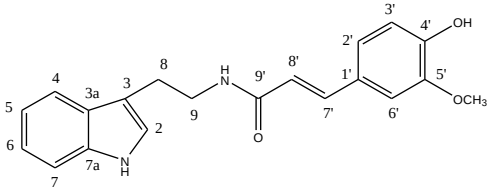

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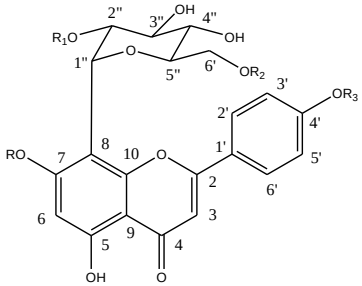
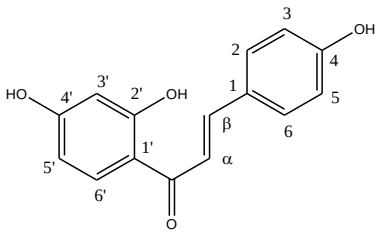


13 R= H, R_1= H, R_2= CH_3CO-, R_3= H
14 R= CH_3-, R_1= CH_3CO-, R_2= H, R_3= H
15 R= H, R_1= CH_3CO-, R_2= H, R_3= CH_3-,



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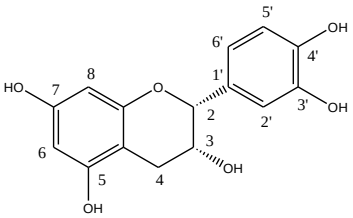

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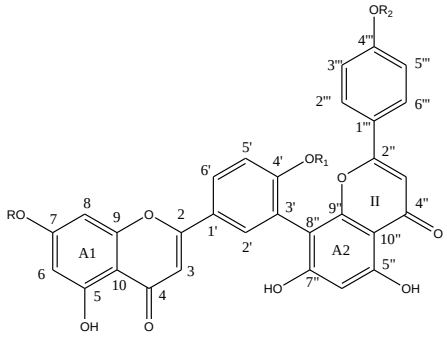
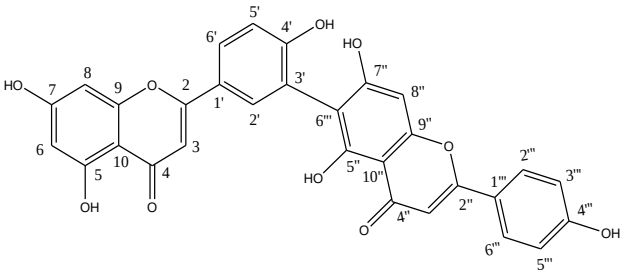


18 R= H, R_1= H, R_2= H
19 R= CH_3-, R_1= H, R_2= H
20 R= CH_3-, R_1= H, R_2= CH_3-



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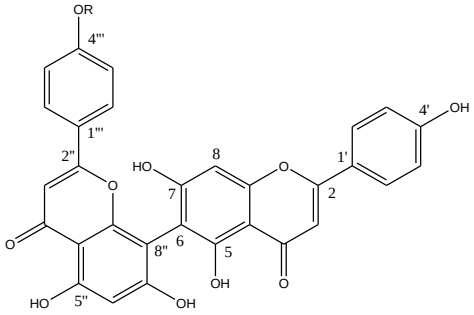
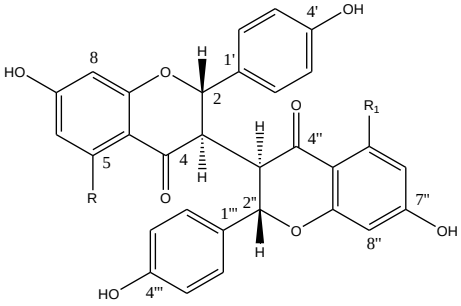
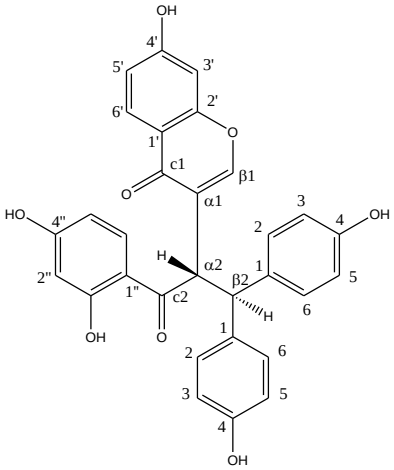


22 R= H
23 R= CH_3-



24 R= R_1= H
25 R= OH, R_1= H
26 R= R_1= OH



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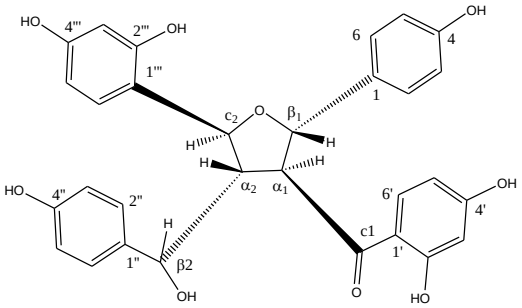

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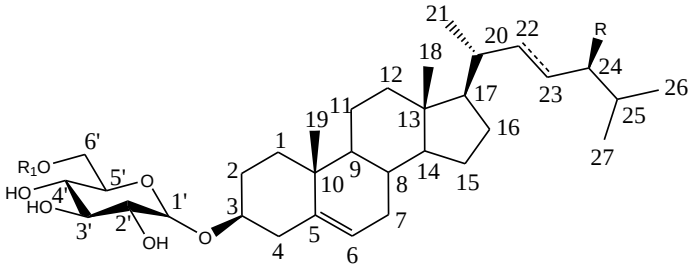
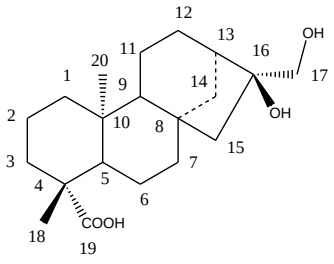


29 R= CH_3-, R_1= H, 22, 23-dihydro
30 R= CH_3CH_2-, R_1= H, 22, 23-dihydro
31 R= CH_3CH_2-, R_1= H, $\Delta^{22,23}$
32 R= CH_3CH_2-, R_1= 2-(palmitelaidoyloxy)-β-Glu-, 22, 23-dihydro
33 R= CH_3CH_2-, R_1= 2-(palmitelaidoyloxy)-β-Glu-, $\Delta^{22,23}$



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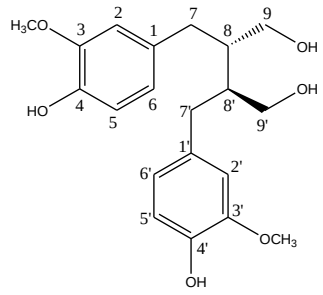

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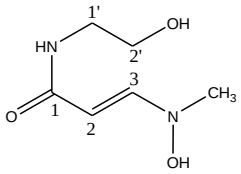

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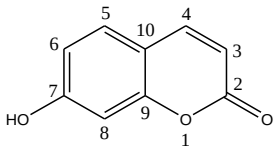

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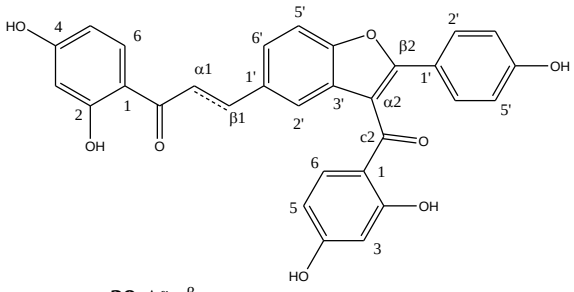
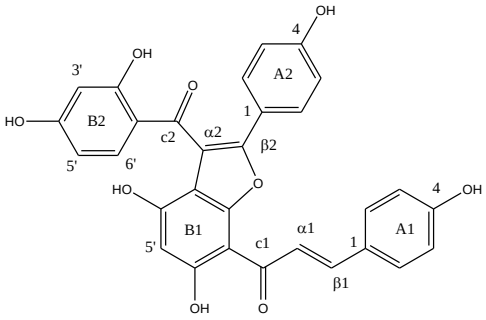


38 $\Delta^{\alpha_1,\beta_1}$
39 α_1, β_1-dihyd



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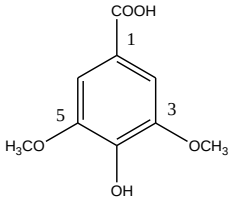

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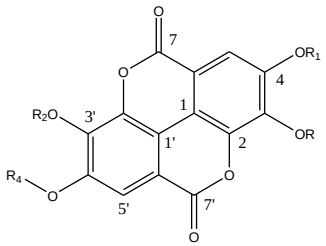

42 R= CH_3-, R_1= H, R_2= H, R_4= α-Rha
43 R= CH_3-, R_1= H, R_2= CH_3-, R_4= β-Xyl

Fig. 1. Compounds isolated from *Campylospermum* and *Ouratea* genus which rendered serotobenine by classes.

Table 1: Chemical data on the genus *Campylospermum* published gradually.

Species	Part studied	Compounds isolated	References
C.glaucum	Stem roots	1 Campyloside A 2 Campyloside B 18 Amentoflavone 27 Lophirone A	Abouem et al., 2008
	Leaves	3 Lanceolin C, 7 Serotobenine 13 6”-*O*-acetylvitexin 16 Isoliquiritigenin 18 Amentoflavone	This study
C.mannii	Leaves	18 Amentoflavone 21 Robustaflavone 24 Campylospermone A 25 Campylospermone B 26 Chamaejasmin	Elo Manga et al., 2009
C.flavum	Leaves	5 Dhurrin 11 6-hydroxyindolin-2-one 14 2’’-*O*-acetyl-7-*O*-methylvitexin 15 4-*O*-methylvitexin 18 Amentoflavone 22 Agathisflavone 23 4’’’-*O*-methylagathisflavone 28 Flavumchalcone	Ndongo et al., 2010

		30 Sitosterol-3-*O*-β-D-glucopyranoside	
	Stem bark	6 2-*O*-β-D-glucopyranosyloxy-*p*-hydroxy-6,7-dihydromandelonitrile 7 Serotobenine 9 Flavumindole 14 2''-*O*-acetyl-7-*O*-methylvitexin 17 Epicatechin,	
C.densiflorum	Leaves	4 Menisdaurin 7 Serotobenine 8 Decursivine 12 *N*-feruloyltriptamine 29 Campesterol-3-*O*-β-D-glucopyranoside 30 Sitosterol-3-*O*-β-D-glucopyranoside 31 Stigmasterol-3-*O*-β-D-glucopyranoside 32 Densiflosides A 33 Densiflosides B 34 ent-16α, 17-dihydroxykauran-19-oic acid 35 Secoisolariciresinol 36 Campylospermine 37 Ombelliferone	Ngono et al., 2011; 2014
C.calanthum	Leaves	10 Calanthumindole 18 Amentoflavone 19 Sequoiaflavone 20 Podocarpusflavone B	Bayiha Ba Njock et al., 2013
C. oliverianum	Leaves	4 Menisdaurin 5 Dhurrin, 7 Serotobenine 29 Campesterol-3-*O*-β-D-glucopyranoside 30 Sitosterol-3-*O*-β-D-glucopyranoside 31 Stigmasterol-3-*O*-β-D-glucopyranoside	This study
C. dybowskii	Leaves	3 Lanceolin C 7 Serotobenine 14 2''-*O*-acetyl-7-*O*-methylvitexin 15 4-*O*-methylvitexin 18 Amentoflavone 21 Robustaflavone 30 Sitosterol-3-*O*-β-D-glucopyranoside	This study

Table 2: Chemical data of *Ouratea* species which rendered serotobenine published gradually.

Species	Part studied	Compounds isolated	references
O. turnarea	Leaves	7 Serotobenine 18 Amentoflavone 27 Lophirone A 38 Lophirone C, 39 Isolophirone C 40 Caledonin B	Abouem et al., 2008
	Leaves	7 Serotobenine 13 6''-*O*-acetylvitexin 18 Amentoflavone 22 Agathisflavone	Bayiha Ba Njock et al., 2011

O. gilgiana		30 Sistosterol-3-*O*-β-D-glucopyranoside 41 Syringic acid
	Stem bark	42-Ellagic-3-*O*-methyl-4'-*O*-α-L-rhamnoside acid 43 Ellagic -4'-*O*-β-D-xylopyranoside-3,3'-dimethylether acid

RESULTS AND DISCUSSION

Compounds like flavonoids, terpenoids, steroids, cyanoglycosides and biflavonoids have been widely reported from the Ochnaceae family[17, 18, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31]. In the particular case of the *Campylospermum* genus and apart from species of this study, derivatives of vitexin or sitosterol are noticeable in *C. flavum*[27]. (Table 1). Meanwhile, cyanoglycosides like menisdaurin (**4**), dhurrin (**5**), or the stereoisomers campyloside A (**1**) and lanceolin C (**3**) have already been isolated from *C. densiflorum*, *C. flavum* and *C. glaucum*[23, 27, 28], their occurrence in *C. oliverianum*, *C. dybowskii* and once more in *C. glaucum* suggest their taxonomical meaning.

Indole alkaloids as seen in Table 1 appeared as well as other already mentioned classes as major components of the genus *Campylospemum* genus[23, 27, 28, 31]. Serotobenine (**7**) for example is a secondary metabolite appearing in five *Campylospermum* species (Table 1); it is gradually appearing as a chemotaxonomic marker of the genus as well as flavonoids and biflavonoids (**18**, **21** and **22**) and their methyl ether derivatives (**19**, **20** and **23**) are taxonomic markers of the *Ouratea* genus[26, 30, 32, 33]. Hitherto, serotobenine (**7**) was isolated from two *Ouratea* species (*O. turnarea* and *O. gilgiana*)[15, 23] (Table 2). The occurrence of indole alkaloids in many *Campylospermum* species but in few *Ouratea* ones (Table 2) cannot really suggest at this stage whether these two genera might be judged con-specific as described by Verdcourt (2005) or different as described by Bissiengou (2013), due to insufficient reports. Moreover, this study reveals some supplementary data regarding the taxonomical value of the isolated compounds; this is possible through the founding of classes not yet described before.

In the present study, secondary metabolites are used to perform chemotaxonomy study of *Campylospermum* and *Ouratea* genera and to compare with previous studies on molecular data. Analyzing Tables 1, 2 and Figure 1, it appears that the chemistry of both *Ouratea* and *Campylospermum* genera seems the same. This assertion is expected regarding the isolation of few markers of the *Ouratea* genus and their methyl derivatives from the *Campylospermum* one (**18**, **19**, **20**, **21**, **22**, **23**, **24**, **25** and **26**), and other compounds (**4**, **7** and **30**) described above too.

The present results are in accordance with recent phylogenetic studies, which suggest the two genera (*Ouratea* and *Campylospermum*) to be closed based on mainly molecular and morphological data[2].

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