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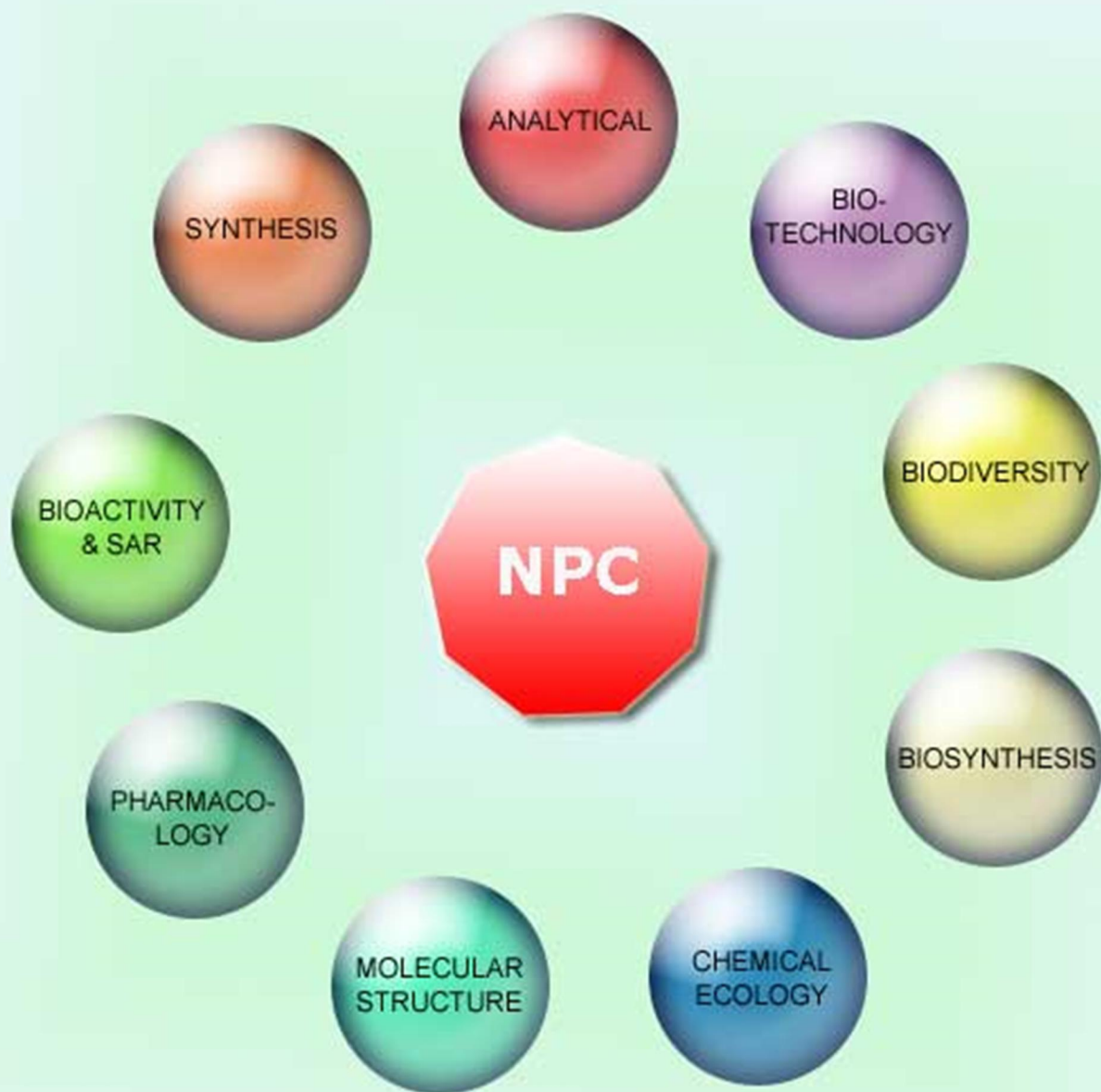
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New Flavonol Glycoside from the Leaves of *Ventilago africana*Diane Patricia Apie Gossan^{a,b}, Abdulmagid Alabdul Magid^{*a}, Philomène Akoua Yao-Kouassi^b, Damien Le Faucheur^a, Antoine Ahibo Coffy^b, Dominique Harakat^c and Laurence Voutquenne-Nazabadioko^a^aICMR-UMR CNRS 7312, Groupe Isolement et Structure, Campus Sciences, Bat. 18, BP 1039, 51687 Reims Cedex 2, France^bLaboratoire de Chimie Organique Biologique, 01 BPV 34 Abidjan 01, Université Félix-Houphouët Boigny d'Abidjan-Cocody, Côte d'Ivoire^cICMR-UMR CNRS 7312, Service Commun d'Analyses, Campus Sciences, Bat. 18, BP 1039, 51687 Reims Cedex 2, France

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A new flavonol diglycoside, rhamnocitrin-3-*O*- α -L-rhamnopyranosyl-(1 \rightarrow 6)- β -D-galactopyranoside, was isolated from the leaves of *Ventilago Africana*, in addition to 11 known flavonoids. Their structures were determined by spectroscopic methods including 1D- and 2D-NMR, and HR-ESI-MS analysis. The isolated compounds were evaluated for their antioxidant activity by using DPPH radical-scavenging assay. Compounds 4, 7-9 have discrete to good antioxidant potential with EC₅₀ values ranging from 20.9 to 40.4 μ M, compared with ascorbic acid (EC₅₀ 60 μ M) used as positive control.

Keywords: *Ventilago africana*, Rhamnaceae, Flavonoids; DPPH free radical scavenging activity.

Ventilago africana Exell is a liane with a thick, soft, woody stem belonging to the family Rhamnaceae [1]. It is distributed in evergreen forests, especially marshy sites and mangroves, from Guinea-Bissau to Nigeria, and across the Congo basin to Uganda and Angola [2]. *V. africana* has been used in traditional medicine for the treatment of *dysmenorrhoea* and as a febrifuge [2]. Previous chemical studies of the genus *Ventilago* have shown the presence of a variety of anthraquinones, naphthoquinones, quinones, and benzisochromanquinones [3]. In the course of our search for interesting bioactive substances in nature, the 80% aqueous methanol extract of the leaves of *V. africana* was investigated. As a result, a new flavonol diglycoside was identified, together with eleven known flavonoids. Free radical scavenging activities of the isolated compounds are also described in this paper.

The *n*-BuOH-soluble fraction of the 80% MeOH extract of the leaves of *V. africana* was separated by a combination of chromatographic methods to obtain a new flavonol diglycoside **1**, in addition to eleven known compounds (**2-12**) (Figure 1). Compounds **2-12** were elucidated as kaempferol-3-*O*- α -L-rhamnopyranosyl-(1 \rightarrow 6)- β -D-glucopyranoside (**2**) [4], kaempferol-3-*O*-(2,6-di-*O*- α -L-rhamnopyranosyl- β -D-glucopyranoside (**3**) [5], kaempferol-3-*O*- α -L-rhamnopyranosyl-(1 \rightarrow 6)- β -D-galactopyranoside (**4**) [6], rhamnocitrin-3-*O*- α -L-rhamnopyranosyl-(1 \rightarrow 6)- β -D-glucopyranoside (**5**) [6], rhamnocitrin-3-*O*-(2,6-di-*O*- α -L-rhamnopyranosyl- β -D-glucopyranoside (**6**) [7], quercetin (**7**) [8], quercetin-3-*O*- α -L-rhamnopyranosyl-(1 \rightarrow 6)- β -D-glucopyranoside (**8**) [9], quercetin-3-*O*-(2,6-di-*O*- α -L-rhamnopyranosyl- β -D-glucopyranoside (**9**) [9], rhamnetin-3-*O*- α -L-rhamnopyranosyl-(1 \rightarrow 6)- β -D-glucopyranoside (**10**) [10], isorhamnetin 3-*O*- α -L-rhamnopyranosyl-(1 \rightarrow 6)- β -D-glucopyranoside (**11**) [10], and rhamnazin-3-*O*- α -L-rhamnopyranosyl-(1 \rightarrow 6)- β -D-glucopyranoside (**12**) [11]. To the best of our knowledge, this is the first report of flavonoids in the genus *Ventilago*.

Compound **1** was isolated as an amorphous, yellow powder, and its molecular formula was determined to be C₂₈H₃₂O₁₅ by HR-ESI-MS

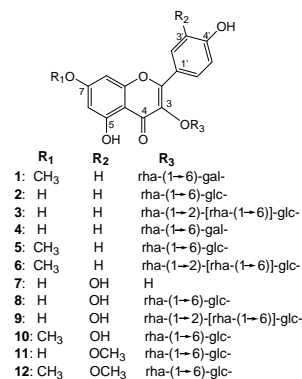


Figure 1: Chemical structure of compounds **1-12**, isolated from *V. africana*.

from the ion at m/z 631.1636 [M + Na]⁺ (C₂₈H₃₂O₁₅Na, calcd 631.1639). The UV spectrum revealed absorption bands at 266 and 346 nm, suggesting a flavonol skeleton. The ¹H and ¹³C NMR spectra of **1** comprised resonances corresponding to aromatic and glycosidic protons and carbons, and an OCH₃ (δ_C 56.5; δ_H 3.92). Four aromatic doublets corresponded to H-6 [δ_H 6.38 (1H, J = 1.1 Hz)], *meta*-coupled with H-8 [δ_H 6.65 (1H, J = 1.1 Hz)] on the A ring, and to H-2', H-6' [δ_H 8.14 (2H, J = 8.0 Hz)], *ortho*-coupled with H-3', H-5' [δ_H 6.92 (2H, J = 8.0 Hz)] on the B ring. Full identification of the aglycone was finally achieved by 2D-NMR spectroscopy. The HMBC correlation between δ_H 3.92 (OCH₃) and δ_C 167.5 (C-7) placed the OCH₃ at C-7 of the aglycone, which led to the rhamnocitrin structure (7-methoxy 3,5,4'-trihydroxy-flavone) [7]. Furthermore, two anomeric proton resonances corresponding to *O*-linked sugars were displayed in the ¹H NMR spectrum as two doublets at $\delta_{H-1''}$ 5.10 (d, J = 7.8 Hz) and $\delta_{H-1''}$ 4.54, J = 1.2 Hz. Based on the results of acid hydrolysis of the flavonoids mixture, the magnitudes of their $J_{1,2}$ coupling constants and the analysis of 1D and 2D-NMR data, the sugar units were elucidated as β -D-galactopyranose ($\delta_{H-1''}$ 5.10; $\delta_{C-1''}$ 105.3) and α -L-rhamnopyranose ($\delta_{H-1''}$ 4.54; $\delta_{C-1''}$ 101.9) [12]. The β -D-gal was characterized by the

large coupling constants $J_{H-1,H-2}$ and $J_{H-2,H-3}$ (> 7.8 Hz) and the small coupling constant $J_{H-3,H-4}$ (3.3 Hz), whereas the α -L-rha was characterized by the small coupling constants $J_{H-1,H-2}$ (1.2 Hz) and its methyl group at δ_{H-6} 1.20 (d, $J = 6.0$ Hz) and δ_{C-6} 18.0. The glycosidic linkage was established by the HMBC correlations between $\delta_{H-1''}$ 5.10 and δ_C 135.8 (C-3) and between $\delta_{H-1''}$ 4.54 and $\delta_{C-6''}$ 67.5, indicating that the galactose unit was located at C-3 of the aglycone and the rhamnose unit was attached to C-6 of galactose. Thus, **1** was characterized as rhamnocitrin-3-*O*- α -L-rhamnopyranosyl-(1 \rightarrow 6)]- β -D-galactopyranoside.

In order to assess the antioxidative properties of **1-12**, the radical scavenging activity of these compounds on DPPH was measured. According to the results, summarized in Table 1, compounds **4**, **7**, **8**, and **9** exhibited a good antioxidant potential (EC_{50} values ranging from 20.9 to 40.4 μ M) compared with ascorbic acid, which was used as a positive control (EC_{50} 60 μ M) (Table 1).

Compounds **7-10** were found to be active, which indicated that the di-OH substitution at 3' and 4' of the B-ring is particularly important to the antiradical activity of a flavonoid. Compounds **2** and **4** shared a common aglycone mono-OH substituted in the B-ring. The only difference was the nature of the disaccharide chain linked at C-3, rha-(1 \rightarrow 6)-glc- (**2**) and rha-(1 \rightarrow 6)-gal- (**4**), suggesting that the galactose might contribute to the antioxidant activity.

Table 1: DPPH radical scavenging activity of compounds **1-12**.

Compds	$EC_{50} \pm S.D.$ (μ M)	Compds	$EC_{50} \pm S.D.$ (μ M)
1	- ^a	8	40.2 \pm 0.7
2	- ^a	9	36.5 \pm 0.6
3	- ^a	10	65.5 \pm 1.9
4	40.4 \pm 0.9	11	- ^a
5	- ^a	12	- ^a
6	- ^a	Ascorbic acid ^b	60.2 \pm 1.2
7	20.9 \pm 0.5		

^a 50% inhibition not achieved at the concentration of 200 μ g/mL. ^b Used as a positive control.

Comparison of the antioxidant activity of compounds **1** with **4** and **8** with **10** indicated that the methoxy group at C-7 in **1** or **10** decreased activity. The free 3'-OH was also found to be important for antioxidant activity by comparison of the antioxidant activity of **8** (3'-OH) with **11** (3'-OCH₃). The same result was found by comparison of activity of **10** and **12**. Thus, the hindrance effect (methoxy groups at C-7 and/or C-3'), the nature of the saccharide chain linked at C-3, and the B ring OH substitution (4'-OH or 3',4'-di-OH) in the flavonoid structures are determinant in the scavenging of free radicals.

Experimental

General: NMR, Bruker Avance DRX III 500; HR-ESI-MS, Micromass Q-TOF micro instrument; Optical rotations, Perkin-Elmer 341 polarimeter; UV, Shimadzu UV-2450 spectrophotometer in MeOH; TLC, pre-coated silica-gel 60 F₂₅₄; CC, Kieselgel 60 (63-200 mesh) or LiChroprep RP-18 (40-63 μ m); Flash chromatography, Grace Reveleris system with dual UV and ELSD detection equipped with a 12g RP-C₁₈ column. The mobile phase was water and methanol with a flow rate of 30 mL/min and the effluents were monitored at 205, 225, 250, and 360 nm. HPLC was performed on a Dionex apparatus equipped with an ASI-100 autosampler, an Ultimate 3000 pump, a diode array detector UVD 340S and a Chromeleon software. RP-C₁₈ column (Phenomenex 250x15 mm, Luna 5 μ) was used for semi preparative HPLC with a binary gradient eluent (H₂O (pH 2.4 with TFA); MeCN) and a flow rate of 6 mL/min; the chromatogram was monitored at 205, 225, 250, and 350 nm. Absorbance values in the DPPH free radical

scavenging assay were read on a Fluostar omega microplate reader (BMG labtech).

Plant material: The leaves of *V. africana* were collected in Adiopodoumé, Ivory Coast, in January 2011. A voucher specimen (Aké-Assi-21254) has been deposited in the herbarium of the National Center of Floristics of FHB University of Cocody (Ivory Coast).

Extraction and isolation: The powdered dry leaves of *V. africana* (800 g) were percolated with 14 L of 80% MeOH. After methanol evaporation and concentration to 2 L, the aqueous residue was successively partitioned with light petroleum, CH₂Cl₂, and *n*-BuOH. The *n*-BuOH extract (43.8 g) was subjected to Diaion HP20 macroporous adsorbent resin CC using a MeOH-H₂O gradient (25, 50, 75, and 100% MeOH) to yield fractions I-IV, successively. Fraction II (9.7 g) was subjected to RP-C₁₈ CC with a gradient system of MeOH-H₂O (25-45% MeOH) to afford 12 fractions (B₁-B₁₂). Further purification of B₄ (0.42 g) (eluted with MeOH-H₂O, 3:7) by RP-C₁₈ chromatography using a MeOH-H₂O gradient (6:4-7:3) and then by semi-prep HPLC (14% MeCN) afforded compounds **9** (*rt* 10.9 min, 9 mg), **8** (*rt* 11.9 min, 10 mg), and **3** (*rt* 14.0 min, 19 mg). Further purification of B₇ (0.61 g) (eluted with MeOH-H₂O, 35:65) by RP-C₁₈ chromatography using a MeOH-H₂O gradient (5:5-6:4) and then by semi-prep HPLC using a binary gradient (16-20% MeCN in 30 min) yielded compounds **4** (*rt* 16.5 min, 5 mg), **2** (*rt* 17.5 min, 26 mg), and **11** (*rt* 20.4 min, 4 mg). Fraction III (12 g) was subjected to silica gel VLC eluted successively with CHCl₃-MeOH-H₂O (95:5:0, 9:1:0, 85:15:0, 8:2:0, 75:25:0, 7:3:0.5) to yield fractions C₁-C₆. Fraction C₂ (0.28 g) was further separated by RP-C₁₈ CC eluted with a gradient of MeOH:H₂O (4:6-8:2) to afford 16 mg of **13** and 33 mg of **7**. Fraction C₄ (0.84 g) was further purified by RP-C₁₈ flash chromatography using a binary gradient (30-80% MeOH in 60 min) and then by semi-prep HPLC (27-32% MeCN in 50 min) to afford compound **5** (*rt* 12.5 min, 11 mg). Fraction C₆ (1.1 g) was further purified by RP-C₁₈ VLC and then by semi-prep HPLC (24-26% MeCN in 50 min) to yield compounds **6** (*rt* 11.1 min, 12 mg) and **12** (*rt* 13.3 min, 8 mg) or isocratic elution with 14% MeCN to give compounds **10** (*rt* 13.8 min, 10 mg) and **1** (*rt* 21.3 min, 11 mg).

Rhamnocitrin-3-*O*- α -L-rhamnopyranosyl-(1 \rightarrow 6)]- β -D-galactopyranoside (**1**)

$[\alpha]_D$: -27.2 (*c* 0.13, MeOH).

UV/Vis λ_{max} (MeOH) nm (Abs.): 266 (0.58), 348 (0.50).

¹H NMR (500 MHz, MeOH-*d*₄): 1.20 (1H, d, $J = 6.0$ Hz, H-6''), 3.30 (1H, t, $J = 9.4$ Hz, H-4'''), 3.42 (1H, dd, $J = 10.3$ -6.1 Hz, H-6''), 3.51 (1H, dd, $J = 9.4$ -3.2 Hz, H-3'''), 3.54 (1H, m, H-5'''), 3.56 (1H, dd, $J = 9.0$ -3.3 Hz, H-3''), 3.60 (1H, dd, $J = 3.2$ -1.2 Hz, H-2'''), 3.64 (1H, brdt, $J = 6.1$ Hz, H-5''), 3.74 (1H, dd, $J = 10.3$ -5.5 Hz, H-6''), 3.79 (1H, d, $J = 3.2$ Hz, H-4''), 3.82 (1H, dd, $J = 9.0$ -7.8 Hz, H-2''), 3.92 (3H, s, 7-OMe), 4.54 (1H, d, $J = 1.2$ Hz, H-1'''), 5.10 (1H, d, $J = 7.8$ Hz, H-1''), 6.38 (1H, s, H-6), 6.65 (1H, s, H-8), 6.92 (2H, d, $J = 8.0$ Hz, H-3',5'), 8.14 (2H, d, $J = 8.0$ Hz, H-2',6').

¹³C NMR (125 MHz MeOH-*d*₄): 18.0 (CH₃, C-6'''), 56.5 (7-OCH₃), 67.5 (CH₂, C-6''), 69.7 (CH, C-5''), 70.2 (CH, C-4''), 72.1 (CH, C-2''), 72.3 (CH, C-3'''), 73.0 (CH, C-2''), 73.9 (CH, C-4''), 75.0 (CH, C-3''), 75.4 (CH, C-5''), 93.3 (CH, C-8), 99.2 (CH, C-6), 101.9 (CH, C-1'''), 105.3 (CH, C-1''), 106.5 (C, C-10), 116.2 (CH, C-3',5'), 122.6 (C, C-1'), 132.6 (CH, C-2',6'), 135.8 (C, C-3), 158.5 (C, C-9), 159.7 (C, C-2), 162.0 (C, C-4'), 163.8 (C, C-5), 167.5 (C, C-7), 179.8 (C, C-4).

HR-ESI-MS: *m/z* [M + Na⁺] calcd for C₂₈H₃₂O₁₅Na: 631.1639; found: 631.1636.

Acid hydrolysis: Acid hydrolysis of the *n*-BuOH soluble fraction was realized as previously described [13]. Briefly, 1 g was refluxed with 50 mL of 2M HCl for 4 h. After extraction with ethyl acetate (3 x 30 mL), the aqueous layer was evaporated to furnish the monosaccharide residue (300 mg). Three sugars were identified as D-glucose, D-galactose and L-rhamnose by comparison with authentic samples on TLC and by measurement of the optical rotation of each purified sugar.

DPPH free radical scavenging assay: The scavenging activity of isolated compounds against DPPH was investigated by spectrophotometric methodology, as previously described [13]. Briefly, 5 μ L of either the standard or sample solutions (dissolved in DMSO) was mixed with 95 μ L of DPPH solution (158 μ M, dissolved in absolute EtOH). After mixing gently and incubating for

30 min at 37°C, the optical density was measured at λ 515 nm. The percentage of absorbance inhibition at λ 515 nm was calculated using the following equation: % inhibition $[(Ab_{\text{control}} - Ab_{\text{sample}})/Ab_{\text{control}}] \times 100$. DPPH solution in EtOH was used as a control. The curve of the % scavenging activity against the concentration of sample was prepared by the MSeExcel based program to obtain the EC₅₀. All the tests were conducted in triplicate. The experimental data were expressed as mean \pm standard deviation.

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