Phenolic Glycosides from the Stem Bark of Caryocar villosum and C. glabrum

Abdulmagid Alabdul Magid, Laurence Voutquenne-Nazabadioko, Dominique Harakat, Christian Moretti, Catherine Lavaud

Phenolic Glycosides from the Stem Bark of *Caryocar villosum and C. glabrum*

Abdulmagid Alabdul Magid,[†,*] Laurence Voutquenne-Nazabadioko,[†] Dominique Harakat,[‡] Christian Moretti,[§] and Catherine Lavaud[†].

Laboratoire de Pharmacognosie, Faculté de Pharmacie, FRE CNRS 2715, IFR 53 Biomolécules, Bâtiment 18, BP 1039, 51687 Reims Cedex 2, France, UMR CNRS 6519, Réactions Sélectives et Applications, Bât. 18, Moulin de la Housse, BP 1039, 51687 Reims cedex 2, France, and IRD, Unité S84 Biodival, Technoparc, 5 rue du Carbone, 45072 Orléans Cedex 2, France

To whom correspondence should be addressed Tel.: + 33 3 26 91 82 08. Fax: +33 3 26 91 35 96. E-mail: abdulmagid.alabdulmagid@univ-reims.fr

[†] FRE CNRS 2715.

[‡] UMR CNRS 6519.

[§] IRD.

Seven new phenolic glycosides (**1**-**7**), along with seventeen known compounds (**8-24**) were isolated from the polar extracts of the stem bark of *Caryocar villosum* and *C. glabrum*. The structures of these compounds were established on the basis of spectroscopic methods including 1D and 2D NMR analysis, HRESIMS and also by comparison with literature experimental data for known compounds. Mushroom tyrosinase inhibitory activity of the methanol extracts and some fractions has been assessed.

The genus *Caryocar* is one of the two genera belonging to the small, tropical and New World family Caryocaraceae. The magnificent trees of *Caryocar* genus are exploited for the fruit oil, which is used for cooking and in the cosmetic industry. Traditionally, the fruit and the stem bark are used by the Indian tribes of French Guyana, Colombia, Venezuela, and Brazil as a remedy for skin problems and as fishing poison.[1] In the course of a phytochemical investigation on *Caryocar* species, we recently have reported the structural elucidation of seven triterpenoid saponins[2] and of two dihydroisocoumarin glucosides[3] purified from the stem bark of *C. villosum* (Aubl.) Pers and *C. glabrum* (Aubl.) Pers, respectively. The present study led to the isolation and identification of seven new phenolic glycosides (**1**-**7**) together with seventeen known compounds (**8**-**24**) from the MeOH extract of the stem bark of these two species. Compounds **1**-**2** (ellagic acid derivatives) were solely present in *C. villosum*, whereas compounds **3**-**5** **(**3,4,5-trimethoxyphenyl glucosides derivatives), **6** (3,4-dimethoxyphenyl glucoside), and **7** (a galloylhamamelose) were only isolated from the *C. glabrum*. Tyrosinase is a copper containing enzyme involved in melanogenesis, and its inhibitors do interest cosmetic, pharmaceutical and food industry, and also can be used in controlling insect pests.[4] In a search for tyrosinase inhibitors from natural sources, the *in vitro* mushroom tyrosinase inhibitory activities of the crude fractions of stem bark of *C. villosum* and *C. glabrum* were determined.

Compound **1** was obtained as a yellowish amorphous powder. A molecular formula of $C_{23}H_{20}O_{13}Na$ was assigned on the basis of the HRESIMS giving an $[M + Na]^+$ ion peak at *m/z* 527.0810 measured in positive ion mode. The ESIMSMS experiment of the negative $[M - H]^-$ ion peak of **1**, observed at *m/z* 503, gave fragments at *m/z* 443 and 428 due to the successive losses of an acetic acid and of a methyl group. Another fragment observed at *m/z* 315 was attributed to the loss of a monoacetylated desoxyhexose unit suggesting that the acetyl group

was located on the sugar part of **1**. The ^{1}H NMR spectrum of **1** showed two aromatic protons at δ_H 7.50 (s) and 7.83 (s), two three proton singlets due to one methoxy (δ_H 4.12) and one acetyl groups (δ_H 2.14), together with six signals corresponding to the eight protons arising from a 6-desoxyhexose (Table 1). The ^{13}C NMR spectrum of **1** exhibited signals for twelve aromatic carbons (Table 1, $C_{1\text{-}6}$ and $C_{1'\text{–}6'}$), three carbonyl carbons at δ_C 159.1, 159.2 and 172.6 due to α,β-unsaturated lactones and acetoxy group, one methoxy carbon (δ_C 61.1), one acetyl carbon (δ_C 20.4), and six carbons for the 6-desoxyhexose (Table 1). Analysis of the HSQC, HMBC and NOESY spectra and comparison of the ^{1}H and ^{13}C NMR chemical shifts with those of analogous compounds in the literature established a methylellagic acid structure for the aglycone of **1**.[5] The methoxy group was assigned at position C-3 to account for its high field chemical shift (δ_C 61.1)[6] in ^{13}C NMR and the lack of a NOE correlation between the methoxy protons and the aromatic proton signal at δ_H 7.50 (H-5). The sugar moiety with its anomeric proton resonating at δ_H 5.62 (d, J = 1.6 Hz) was determined as an α-L-rhamnopyranose ($\delta_{H6''}$ 1.25, d, J = 6.3 Hz) by analysis of the coupling patterns of its proton signals and COSY spectrum (Table 1), and sugar analysis of the optical rotations and thin layer chromatographies. The observed small coupling constant of the anomeric proton and the chemical shift of C-5″ (δ_C 69.0) indicated the usual α configuration for this sugar.[7] The location of the acetyl group at position 3 was suggested by the pronounced downfield shift of H-3″ (δ_H 5.13) (Table 1) and this was confirmed by the HMBC experiment which exhibited cross-peaks between H-3″ of rhamnose and the carbonyl carbon (δ 172.6) of the acetoxy group. In the HMBC spectrum of **1**, a long-range correlation was observed between H-1″ of rhamnose and one of the oxygenated aromatic carbons (δ_C 146.3) of the 3-*O*-methylellagic acid moiety. This carbon could be either C-3' or C-4' of the 3-*O*-methylellagic acid, since in the HMBC experiment, correlations were observed between the proton H-5' and C-7', and the two oxygenated aromatic carbons (C-3' and C-4'). Comparison of the ^{13}C NMR spectra of **1**

with those of 3-*O*-methyl-3'-*O*-rhamnopyranosylellagic acid ($\delta_{C-3'}$138.2 and $\delta_{C-4'}$ 154.0)[8] and of 3-*O*-methyl-4'-*O*-rhamnopyranosylellagic acid ($\delta_{C-3'}$ 141.8 and $\delta_{C-4'}$ 146.4)[9] indicated that the rhamnose unit was linked to 4'-position of ellagic acid moiety in **1** ($\delta_{C-3'}$ 141.3 and $\delta_{C-4'}$ 146.3). The site of glycosylation was confirmed by the NOESY experiment which exhibited NOE effect between H-1′′ of rhamnose and H-5' of methylellagic acid. The structure of compound **1** was elucidated as 3-*O*-methyl-4'-(3"-*O*-acetyl)-*α*-L-rhamnopyranosylellagic acid.

Compound **2** displayed a pseudomolecular ion peak $[M + Na]^+$ at *m/z* 527.0803 in the positive HRESIMS, thus appearing to be an isomer of **1** with a $C_{23}H_{20}O_{13}Na$ molecular formula. The ESIMSMS experiment of the negative $[M - H]^-$ ion peak of **2** observed at *m/z* 503, gave the same fragments as those obtained for **1** indicating the presence of a methoxy group, and of a monoacetylated desoxyhexose unit. The ^1H and ^{13}C NMR data and HMBC experiments for **2** were similar to those of **1** except for the position of the acetyl group on the rhamnose moiety, suggesting that **2** was a regioisomer of **1** (Table 1). The positions of the 3-*O*-methyl and the 4'-*O*-rhamnosyl groups on ellagic acid were substantiated by HMBC and NOESY correlations. In the HMBC spectrum, the correlation of the down-field methine proton at $\delta_{H-4''}$ 5.09 of rhamnose with the carbonyl carbon of acetoxy group at δ_C 171.1, showed that the rhamnose was acetylated in position 4. The other correlations led to the assignment of **2** as 3-*O*-methyl-4'-(4"-*O*-acetyl)-*O*-*α*-L-rhamnopyranosylellagic acid.

Similar rhamnosides of ellagic acid were previously reported in the literature but none with this particular substitution pattern for the rhamnose unit, the methoxy and the acetyl groups.[5,8,9]

Compound **3** displayed a pseudomolecular ion peak $[M + Na]^+$ at *m/z* 673.1379 in the positive HRESIMS, in accordance with an empirical molecular formula of $C_{29}H_{30}O_{17}Na$. Its ^1H NMR spectrum showed an anomeric proton signal at δ_H 5.27 (d, J = 7.9 Hz) correlated in the HSQC spectrum with an anomeric carbon at δ_C 102.1. The sugar unit was identified by

analysis of COSY, NOESY and HSQC experiments as a *β*-D-glucopyranose esterified at positions 2 and 3 (Table 2). In the ^{1}H NMR spectrum, three additional aromatic proton singlets were observed at δ_H 6.36, 7.02 and 7.07, each integrating for 2H, and correlating in the HSQC spectrum with the methine carbons at δ_C 96.6 and 110.3. Supplementary carbon atoms were revealed as ten quaternary oxygenated aromatics, two carbonyl esters near δ_C 168, two quaternary aromatic carbons at δ_C 121.7, and three methoxy groups. Further analysis of the ^{1}H and ^{13}C NMR, HSQC and HMBC spectra of **3** showed the presence of a 3',4',5'-trimethoxyphenyl moiety[10] and of two galloyl moieties[11] (Table 2). The 3',4',5'-trimethoxyphenyl group was attached to the anomeric position of the *β*-D-glucopyranose as deduced from the NOE effect between Glc-H-1 and both H-2' and H-6' (δ_H 6.36), and the HMBC correlation between Glc-H-1 and C-1' (δ_C 156.0) of the aromatic ring. The location of the two galloyl groups at positions 2 and 3 of the glucose was suggested by the pronounced downfield shifts of Glc-H-2 (δ_H 5.36) and Glc-H-3 (δ_H 5.51) (Table 2) and was confirmed by the HMBC correlation between Glc-H-2 and the carbonyl ester group (δ 167.3) of one galloyl moiety, and Glc-H-3 and the carbonyl ester group (δ 168.8) of the other galloyl moiety. Therefore, the structure of **3** was elucidated as 3',4',5'-trimethoxyphenyl-(2,3-di-*O*-galloyl)-*β*-D-glucopyranoside.

The positive HRESIMS of compound **4** showed an $[M + Na]^+$ ion peak at *m/z* 521.1261, in accordance with an empirical molecular formula of $C_{22}H_{26}O_{13}Na$ and suggesting the loss of one galloyl group compared to **3**. Analysis of the ^{1}H and ^{13}C NMR, HSQC, and HMBC spectra of **4** confirmed the presence of a 3',4',5'-trimethoxyphenyl moiety, and a *β*-D-glucopyranose unit as in **3,** and with one galloyl group (Table 2). In the HMBC experiment, cross-peaks were observed between H-1 of glucose (δ_H 5.06) and C-1' of 3',4',5'-trimethoxyphenyl moiety and between H-2 of glucose (δ_H 5.13) and the carbonyl carbon of

the galloyl moiety (δ 167.7). Therefore the structure of **4** was elucidated as 3',4',5'-trimethoxyphenyl-(2-*O*-galloyl)-β-D-glucopyranoside.

The positive HRESIMS of compound **5** showed a $[M + Na]^+$ ion peak at *m/z* 623.0667, in accordance with an empirical molecular formula of $C_{22}H_{25}O_{16}SNa_2$, suggesting that **5** possessed an additional sodium sulphate group compared to **4**. The spectral features indicated that **5** was closely related to **4** except for the signals belonging to the sugar moieties (Table 2). In the HMBC spectrum of **5** the long-range correlations observed between Glc-H-1 (δ_H 4.94) and C-1' of the 3',4',5'-trimethoxyphenyl group, and between the downfield shifted Glc-H-3 (δ_H 5.26) and the carbonyl carbon C-7" (δ_C168.1), indicated that the galloyl moiety was linked at position 3 of glucose in **5** (Table 2). An important downfield shift ($\Delta\ \delta_C$ + 5.7 ppm) was observed on C-6 of the β-D-glucose (Table 2) but no inter-residue HMBC or NOE correlations involving H-6 of this sugar unit were observed. These data indicated that the sodium sulphate group, previously detected in HRESIMS, was attached at this position.. Based on the above evidences, the structure of **5** was established as 3',4',5'-trimethoxyphenyl-(3-*O*-galloyl-6-*O*-sodium sulphate)-β-D-glucopyranoside.

Compound **6** with its pseudomolecular ion peak $[M + Na]^+$ at *m/z* 593.0638 in the positive HRESIMS had a molecular formula of $C_{21}H_{23}O_{15}SNa_2$ indicating the loss of a methoxy group compared to **5**. A quick survey of the ^1H and ^{13}C NMR, HSQC and HMBC spectra showed that **6** contained a β-D-glucose disubstituted by a galloyl moiety in position 2 (δ_H 5.15) as in **4** and by a sodium sulphate in position 6 (δ_C 68.4) as in **5** (Table 2). In the ^1H NMR spectrum of **6**, three proton signals characteristic of a 1,2,4-trisubstituted aromatic ring were observed at δ_H 6.55 (dd, J = 8.5, 2.6 Hz), 6.71 (d, J = 2.6 Hz), and 6.82 (d, J = 8.5 Hz), and two methoxy protons signals at δ_H 3.74 and 3.77. These spectral data were in accordance with a 3',4'-dimethoxyphenyl radical (Table 2).[12] This group was attached to the anomeric position of the glucose as observed by the long-range correlation between Glc-H-1 (δ_H 5.01)

and C-1' of the 3',4'-dimethoxyphenyl group in the HMBC spectrum. Thus, compound **6** is 3',4'-dimethoxyphenyl-(2-*O*-galloyl-6-*O*-sodium sulphate)-*β*-D-glucopyranoside.

Compound **7** displayed a pseudomolecular ion peak $[M + Na]^+$ at *m/z* 507.0758 in accordance with the molecular formula $C_{20}H_{20}O_{14}Na$ in the positive HRESIMS. Analysis of the ^1H and ^{13}C NMR spectra indicated the presence of two galloyl groups as in **3**-**6** and a sugar unit different from the *β*-D-glucose of most common gallotannins.[11] After observation of connectivities in COSY, HSQC, HMBC, and NOESY experiments, the sugar residue of **7** was identified as hamamelofuranose.[13] The 1D NMR spectra of **7** displayed one anomeric proton (δ_H 6.19, s) attached to the anomeric carbon (δ_C 101.4) in the HSQC spectrum. The ^{13}C NMR spectrum also showed two hydroxymethines at δ_C 74.2 (C-3) and 83.0 (C-4), two hydroxymethylenes at δ_C 64.2 (C-2') and 66.9 (C-5), and one quaternary carbon bearing hydroxyl group at δ_C 82.1 (C-2). The HMBC spectrum, showing long-range correlations between H-1/C-2, C-2', and C-4 confirmed the structure of this monosaccharide. The NOE effects between H-2'/H-3, and H-3/H-5, and the lack of correlation between H-1/H-3 indicated a *β* configuration. The presence of D-hamamelofuranose in hamamelotannins **7, 11** and **12**, was confirmed by the isolation of this sugar after alkaline hydrolysis of the fraction from which they were purified; followed by measurement of its ^1H NMR spectrum and optical rotation. The sites of the esterification by the two galloyl groups in **7** were deduced from the long-range correlations observed in the HMBC spectrum between the anomeric proton of hamamelose (δ_H 6.19) and the carbonyl carbon of the first galloy group ($\delta_{C7''}$ 166.6), and between both H-5a and H-5b of hamamelose (δ_H 4.22, 4.58) and the carbonyl carbon of the second galloyl group ($\delta_{C7'''}$ 168.2). Therefore the structure of **7** was elucidated as 1,5-di-*O*-galloyl-*β*-D-hamamelofuranose.

The known compounds **8**-**20** were isolated from *C. villosum* and identified by measurements of 1D and 2D NMR, ESIMS and comparison of their spectral data with the

literature values as 3-*O*-methylellagic acid-4′-(2"-*O*-acetyl)-*O*-α-L-rhamnopyranoside (**8**),[5] 3-*O*-methylellagic acid-4′-*O*-α-L-rhamnopyranoside (**9**), ellagic acid (**10**),[5,9] 5-*O*-galloyl-D-hamamelofuranose (**11**),[13] 2'-*O*-galloyl-D-hamamelofuranose (**12**),[14] 2',5-di-*O*-galloyl-D-hamamelofuranose (**13**),[13] 2',3,5-tri-*O*-galloyl-D-hamamelofuranose (**14**),[15] 1,2',3,5-tetra-*O*-galloyl-β-D-hamamelofuranose (**15**),[15] 1,2,3,4,6-penta-*O*-galloyl-β-D-glucose (**16**),[11] corilagin (**17**),[16] tercatain (**18**),[17] chebulagic acid (**19**),[16,18] and putranjivain A (**20**).[19] Whereas the known compounds isolated from *C. glabrum* were identified in the same manner as nikoenoside (**21**),[20] 3,4,5-trimethoxyphenyl-β-D-glucopyranoside (**22**),[10] caryocaroside II-22 (**23**) and caryocaroside II-23 (**24**).[2]

The mushroom tyrosinase inhibitory activity of the methanol extracts, the H_2O-fraction of *C. glabrum*, and the two phenol-rich fractions A and B of *C. villosum* has been assessed. The methanol extract and the H_2O-fraction of *C. glabrum* exhibited weak activity against the enzyme with IC_{50} values of 1.60 and 1.86 mg/mL respectively, when compared to the standard tyrosinase inhibitor kojic acid (IC_{50} 0.1 mg/mL, 0.68 mM). Similarly, the methanol extract and fractions A and B of *C. villosum* exhibited low tyrosinase inhibitions with IC_{50} values of 1.42, 1.28, and 0.98 mg/mL respectively. Although we were rather looking for applications with crude extracts and standardisation with pure compounds, the relatively high values obtained for this activity and the low chemical diversity of the isolated compounds did not encourage us to pursue the measurements on the pure compounds.

Experimental Section

General Experimental Procedures. Optical rotations were measured on a Perkin-Elmer 241 polarimeter. UV spectra were obtained using a Philips PU 8720 spectrophotometer. NMR spectra were recorded on a Bruker Avance DRX-500 spectrometer

at 500 MHz for ^{1}H NMR and 125 MHz for ^{13}C NMR. ESIMS and HRESIMS were recorded on a ESI-Q-TOF Micromass spectrometer. Semipreparative HPLC was performed on a Dionex apparatus equipped with a Chromeleon software, an ASI-100 automated sample injector, a STH 585 column oven, a P580 pump, a UVD 340S diode array detector at 275 nm using a prepacked C_{18} column (Thermo Electron corporation hyperprep HS, 10µ, 21.2 x 250 mm) with a binary gradient elution: solvent A (H_2O-TFA 0.0025%), and solvent B (CH_3CN-H_2O 1:1) with a flow rate of 5 $mL.min^{-1}$. Column chromatography was performed on Sephadex LH-20 (Pharmacia Biotech AB), Kieselgel 60 (63 - 200 µm, Merck), LiChroprep C_{18} (40 - 63 μm, Merck) or Amberlite IRN-77 ion exchange resin. TLC analysis was run on 60 F_{254} precoated silica gel plates (Merck) and spots were visualized by heating after spraying with 50% H_2SO_4. Mushroom tyrosinase (EC 1-14-18-1), kojic acid, L-Dopa, L-tyrosine, and all chemicals and reagents used for the tyrosinase inhibitory assays were purchased from Sigma Chemical Company, Ltd.

Plant Material. The stem bark of *C. villosum* was collected in May 1998 near Regina at Saint Georges de l'Oyapock station, Cayenne Island in French Guyana. The species was identified by P. Grenand of the botany laboratory of the IRD centre of Cayenne, and Herbarium specimen (Grenand P. 3076) was deposited in the Herbarium of Guyana. The stem bark of *C. glabrum* were collected in French Guyana near Matoury in the Amirande forest, and in the Ecerex forest station near Sinnamary, in October 2001. The species was identified by M.F. Prevost of the botany laboratory of the IRD Centre of Cayenne, and a herbarium specimen (Prevost MFP 4864) was deposited in the Herbarium of Guyana.

Extraction and Isolation. The dried and powdered stem bark (400 g) of *C. villosum* was refluxed for 3 h with 3.5 L of MeOH, then concentrated to dryness to give a gummy residue

(37.5 g) of which 32 g was subjected to VLC on RP-18 eluted with MeOH-H_2O (4:6, 6:4, 8:2, and 10:0) yielding fractions A (18 g), B (2.8 g), C (0.87 g) and D (2 g), respectively.

5 g of fraction A was submitted to Sephadex LH-20 CC (MeOH) affording 22 fractions (100 mL each) of which fractions 4, 5, 8, 10-12, and 17-19 were purified by semi-prep. HPLC to give compounds **8**-**20**. Compounds **10** (11 mg) and **13** (54 mg) were purified from fraction 4 eluted with solvents B-A (15:85). Compound **11** (14 mg) was purified from fraction 5 eluted with solvents B-A (2:8). Compounds **8** (3 mg) and **9** (3.5 mg) were obtained from fraction 8 eluted with solvents B-A (22:78). Compounds **14** (37 mg), **18** (4 mg), **20** (4 mg), **12** (3 mg), **15** (20 mg), **19** (14 mg), and **17** (7 mg) were purified from fractions 10-12 eluted with solvents B-A (3:7). Compound **16** (15 mg) was obtained from fractions 17-19 eluted with solvents B-A (35:65).

800 mg of fraction B were submitted to two successive CC on silica gel eluted with $CHCl_3$ containing increasing amounts of MeOH. Fractions eluted with $CHCl_3$-MeOH (8:2 and 7:3) were purified by semi-prep. HPLC eluted with a gradient of solvents B-A (12:88 to 15:85) for 20 min.to afford **9** (8 mg), **2** (8 mg), and **1** (6 mg).

The dried and powdered stem bark (396 g) of *C. glabrum* was refluxed for 3 h with 1 L MeOH, then filtered and concentrated to provide a brown pasty residue (29 g). This residue was suspended in H_2O, and successively partitioned with *n*-hexane and EtOAc to give a H_2O soluble fraction (15 g),. 14 g of the H_2O layer was subjected to Sephadex LH-20 CC eluted successively with MeOH-H_2O (0:10, 1:1, 8:2, and 10:0) affording 22 fractions (100 mL each). Fractions 2-4 were first subjected to a silica gel CC eluted with a gradient of $CHCl_3$-MeOH-H_2O (8:2:0 to 14:6:1) and fractions xx were further purified by semi-prep. HPLC eluted with solvents A- B (2:8) to give **22** (15 mg) and **21** (21 mg), whereas fractions xx afforded **3** (9 mg) by semi-prep. HPLC eluted with the gradient of solvents A-B (3-7 to 1:1) for 25 min. Fractions 6-7 were subjected to a silica gel CC eluted with a gradient of $CHCl_3$-MeOH-H_2O

(7:3:0 to 14:6:1) followed by a semi-prep. HPLC eluted with solvents A-B (28:72). to afford **5** (27 mg), **4** (12 mg) and **6** (3.5 mg). Compounds **11** (34 mg) and **12** (60 mg) were obtained from fractions 8-9 by semi-prep. HPLC eluted with solvents A-B (5:95). Fractions 10-13 were purified by a RP-18 CC, using a gradient of MeOH-H_2O (3:7 to 8:2), followed by a semi-prep. HPLC eluted with solvents A-B (1:9) to afford **11** (8 mg), **12** (60 mg), and **7** (8 mg). Fractions 14-17 were subjected to a RP-18 CC eluted with MeOH-H_2O (3:7 to 8:2) followed by a silica gel CC, using a gradient of $CHCl_3$-MeOH-H_2O (8:2:0 to 14:6:1), to obtain 5 mg of **23** and 6 mg of **24**.

Compound 1: Yellow amorphous powder; $[\alpha]^{20}_D$ -30 (*c* 0.02, MeOH); ^1H and ^{13}C NMR (CD_3OD), see Table 1; ESIMS (positive ion mode) *m/z* 527 $[M+Na]^+$ (100); ESIMS (negative ion mode) *m/z* 503 $[M-H]^-$ (100); ESIMS-MS *m/z* 443 $[M-H-CH_3COOH]^-$ (10), 428 $[M-H-CH_3COOH-CH_3]^-$ (7), 315 $[M-H-(Rha-CH_3COOH)]^-$ (5); HRESIMS (positive ion mode) *m/z* 527.0810 $[M+Na]^+$ (calcd. for $C_{23}H_{20}O_{13}Na$, 527.0802).

Compound 2: Yellow amorphous powder; $[\alpha]^{20}_D$ -20 (*c* 0.16, MeOH); ^1H and ^{13}C NMR (CD_3OD), see Table 1; ESIMS (positive ion mode) *m/z* 527 $[M+Na]^+$ (100); ESIMS (negative ion mode) *m/z* 503 $[M-H]^-$ (100); ESIMS-MS *m/z* 443 $[M-H-CH_3COOH]^-$ (10), 428 $[M-H-CH_3COOH-CH_3]^-$ (7), 315 $[M-H-Rha-CH_3COOH]^-$ (5); HRESIMS (positive ion mode) *m/z* 527.0803 $[M+Na]^+$ (calcd. for $C_{23}H_{20}O_{13}Na$, 527.0802).

Compound 3: Pale yellow powder; $[\alpha]^{20}_D$ -23 (*c* 0.25, MeOH); ^1H and ^{13}C NMR (CD_3OD), see Table 2; HRESIMS (positive ion mode) *m/z* 673.1379 $[M+Na]^+$ (calcd. for $C_{29}H_{30}O_{17}Na$, 673.1381).

Compound 4: Amorphous brown powder; $[\alpha]^{20}_{D}$ -30 (*c* 0.57, MeOH); ^{1}H and ^{13}C NMR (CD_3OD), see Table 2; HRESIMS (positive ion mode) *m/z* 521.1261 $[M+Na]^+$ (calcd. for $C_{22}H_{26}O_{13}Na$, 521.1271).

Compound 5: Amorphous brown powder; $[\alpha]^{20}_{D}$ -20 (*c* 0.25, MeOH); ^{1}H and ^{13}C NMR (CD_3OD), see Table 2; HRESIMS (positive ion mode) *m/z* 623.0667 $[M+Na]^+$ (calcd. for $C_{22}H_{25}O_{16}SNa_2$, 623.0659).

Compound 6: Amorphous brown powder; $[\alpha]^{20}_{D}$ -15 (*c* 0.57, MeOH); ^{1}H and ^{13}C NMR (CD_3OD), see Table 2; HRESIMS (positive ion mode) *m/z* 593.0638 $[M+Na]^+$ (calcd. for $C_{21}H_{23}O_{15}SNa_2$, 593.0631).

Compound 7: Amorphous brown powder; $[\alpha]^{20}_{D}$ +8 (*c* 0 .5, MeOH); ^{1}H NMR (CD_3OD 500 MHz) δ 3.78 (1H, d, *J* = 11.5 Hz, H-2'a), 3.90 (1H, d, *J* = 11.5 Hz, H-2'b), 4.11 (1H, d, *J* = 7.8 Hz, H-3), 4.22 (1H, dd, *J* = 11.7, 7.2 Hz, H-5a), 4.33 (1H, ddd, *J* = 7.8, 7.2, 3.3 Hz, H-4), 4.58 (1H, dd, *J* = 11.7, 3.3 Hz, H-5b), 6.19 (1H, s, H-1), 7.04 (4H, s, H-2", H-2"', H-6", H-6"'); ^{13}C NMR (CD_3OD, 125 MHz) δ 64.2 (C, C-2'), 66.9 (CH_2, C-5), 74.2 (CH, C-3), 82.1 (C, C-2), 83.0 (CH, C-4), 101.4 (CH, C-1), 110.2 (CH, C-2", CH, C-2"', CH, C-6", CH, C-6"'), 121.0 (C, C-1"'), 121.1 (C, C-1"), 139.9 (C, C-4"), 140.2 (C, C-4"'), 146.4 (C, C-3", C, C-5"), 146.5 (C, C-3"', C, C-5"'), 166.6 (C, C-7"), 168.2 (C, C-7"'); HRESIMS (positive ion mode) *m/z* 507.0758 $[M+Na]^+$ (calcd. for $C_{20}H_{20}O_{14}Na$, 507.0751).

Sugar analysis. 100 mg of fractions A and B of *C. villosum*, and of the MeOH extract of *C. glabrum*, were separately refluxed, with 2N HCl for 4 h. After extraction with EtOAc, the aqueous layer was neutralized with 0.5 M NaOH and freeze-dried. Rhamnose and glucose

were identified by comparison with authentic samples by TLC in MeCOEt-*iso*-PrOH-Me_2CO-H_2O (20:10:7:6). 50 mg of fraction 8 obtained from the first column chromatography of H_2O soluble extract of *C. glabrum*, was dissolved in 3 mL of 3% methanolic KOH; after 24 hours at room temperature, the solution is neutralized through a column of Amberlite IRN-77 ion exchange resin, and then evaporated. After preparative TLCs of the sugar mixtures in MeCOEt-*iso*-PrOH-Me_2CO-H_2O (20:10:7:6), the optical rotation of each purified sugar was recorded to afford L-rhamnose: $[\alpha]_D^{20}$ +10 (*c* 0.1, H_2O), D-glucose: $[\alpha]_D^{20}$ + 11 (*c* 0.12, H_2O), and D-hamamelose: $[\alpha]_D^{20}$ - 1 (*c* 0.8, H_2O).

Tyrosinase Inhibition Assay. The assay for activity against mushroom tyrosinase was performed according to a slight modification of a previous described method using L-tyrosine and L-DOPA as substrate.[21] Samples were dissolved in DMSO and five sample dilutions with respective concentrations of 0.1, 0.5, 1, 2.5 and 5 mg/mL were prepared in Na-phosphate buffer. The L-tyrosine and L-DOPA were dissolved in Na-phosphate buffer (pH 6.8) to obtain a concentration of 0.18 mg/mL and 0.2 mg/mL respectively. Solutions of L-tyrosine (333 µL; 0.18 mg/mL) and L-DOPA (333 µL; 0.20 mg/mL) were first pre-incubated with the tested compounds (333 µL) for 10 min at 25 °C. Then, the tyrosinase (EC 1-14-18-1) (33 µl; 2400 U/mL) was added to the reaction mixture and the enzyme reaction was monitored by measuring the change in absorbance at 480 nm due to the formation of DOPA chrome, during 5 min at 25 °C. The 100% activity was obtained with 333 µL of L-tyrosine, L-DOPA, Na-phosphate buffer, and then 33 µL of tyrosinase. The percent inhibition of the enzyme was calculated by using MS Excel® based program as follows: percent inhibition = [(B-S)/B] × 100 where B and S are the absorbances measured for the blank and the samples, respectively. After screening of the fractions, the IC_{50} was calculated. All the studies have been carried out in triplicate with kojic acid as the positive control.[4]

Acknowledgments. The authors are very grateful to P. Grenand and M.F. Prevost of the Botany Department, Research Institute for the Development (IRD), Cayenne (French Guyana), for help with the identification of the plant and to Dr. G. Massiot from the ISTMT-Laboratoires Pierre Fabre, for critical reading of the manuscript.

References and Notes

(1) (a) Passos, X. S.; Castro, A. C. M.; Pires, J. S.; Garcia, A. C.-F.; Campos, F.-C.; Fernandes, O. F. L.; Paula, J. R.; Ferreira, H. D.; Santos, S. C.; Ferri, P. H.; Silva, M. D. R. R. *Pharm. Biol.* **2003**, *41*, 319-324. (b) Grenand, P.; Moretti, C.; Jacquemin, H.; Prévost, M. F. In *Pharmacopées Traditionnelles en Guyane, Créoles, Wayasi, Palikur*; IRD Editions: Paris, 2004; pp 293-298.

(2) Alabdul Magid, A.; Voutquenne-Nazabadioko, L.; Renimel, L.; Harakat, D.; Moretti, C.; Lavaud, C. *Phytochemistry* **2006**, *67*, 2096-2102.

(3) Alabdul Magid, A.; Voutquenne, L.; Moroy, G.; Moretti, C.; Lavaud, C. *Phytochemistry* **2007**, *68*, 2439-2443.

(4) Seo, S.-Y., Sharma, V. K., Sharma, N. *J. Agric. Food Chem.* **2003**, *51*, 2837-2853.

(5) Guo, Q.-M.; Yang, X.-W. *Pharmazie* **2005**, *60*, 708-710.

(6) Massiot, G.; Chen, X.-F.; Lavaud, C.; Le Men-Olivier, L.; Delaude, C.; Viari, A.; Vigny, P.; Duval, J. *Phytochemistry*, **1992**, *31*, 3571-3576.

(7) Kasai, R.; Okihara, M.; Asakawa, J.; Mizutani, K.; Tanaka, O. *Tetrahedron* **1979**, *35*, 1427-1432.

(8) Elkhateeb, A.; Subeki, K.; Takahashi, K.; Matsuura, H.; Yamasaki, M.; Yamato, O.; Maede, Y.; Katakura, K.; Yoshihara, T.; Nabeta, K. *Phytochemistry* **2005**, *66*, 2577-2580.

(9) Xue, P.-F.; Zhao, Y.-Y; Wang, B.; Liang, H. *Biochem. Sys. Eco*. **2006**, *34*, 825-828.

(10) Steinbeck, C.; Schneider, C.; Rotscheidt, K.; Breitmaier, E. *Phytochemistry* **1995**, *40*, 1313-1315.

(11) Wang, K.-J.; Yang, C.-R.; Zhang, Y.-J. *J. Food Chemistry* **2007**, *101*, 365-371.

(12) Pan, H.; Lundgren, L.N. *Phytochemistry* **1995**, *39*, 1423-1428.

(13) Nonaka, G.-I.; Ageta, M.; Nishioka, I. *Chem. Pharm. Bull*. **1985**, *33*, 96-101.

(14) Schilling, G.; Keller, A. *Z. Naturforsch.* **1986**, *41*, 253-257.

(15) Nonaka, G.-I.; Ishimaru, K.; Tanaka, T. *Chem. Pharm. Bull*. **1984**, *32*, 483-489.

(16) Hatano, T.; Yoshida, T.; Shingu, T.; Okuda, T. *Chem. Pharm. Bull*. **1988**, *36*, 3849-3856.

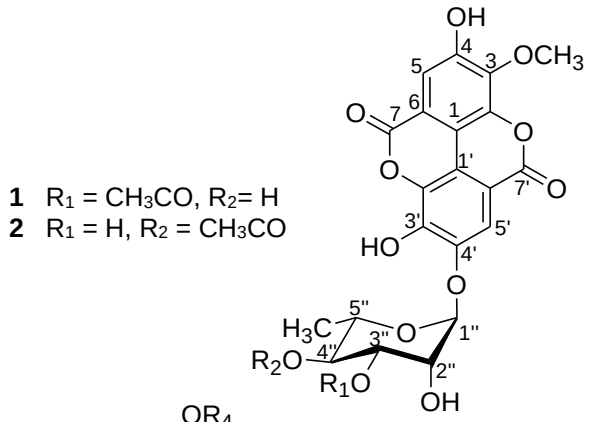
(17) Lee, S.-H.; Tanaka, T.; Nonaka, G.-I.; Nishioka, I.; Zhang, B. *Phytochemistry* **1991**, *30*, 1251-1253.

(18) Tanaka, T.; Kouno, I.; Nonaka, G.-I. *Chem. Pharm. Bull*. **1996**, *44*, 34-40.

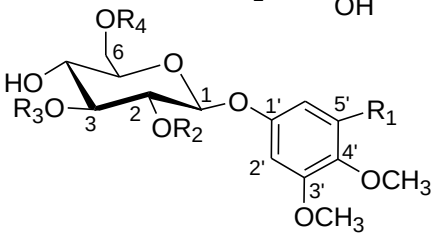
(19) Yoshida, T.; Itoh, H.; Matsunaga, S.; Tanaka, R.; Okuda, T. *Chem. Pharm. Bull*. **1992**, *40*, 53-60.

(20) Morikawa, T.; Tao, J.; Ueda, K.; Matsuda, H.; Yoshikawa, M. *Chem. Pharm. Bull*. **2003**, *51*, 62-67.

(21) Renimel, I.; Olivier, M.; André, P. FR. Patent 2,757,394, 1998.



1 $R_1 = CH_3CO$, $R_2 = H$
2 $R_1 = H$, $R_2 = CH_3CO$



3 $R_1 = -OCH_3$, $R_2 = R_3 =$ Galloyl, $R_4 = H$
4 $R_1 = -OCH_3$, $R_2 =$ Galloyl, $R_3 = R_4 = H$
5 $R_1 = -OCH_3$, $R_2 = H$, $R_3 =$ Galloyl, $R_4 = SO_3Na$
6 $R_1 = H$, $R_2 =$ Galloyl, $R_3 = H$, $R_4 = SO_3Na$

7

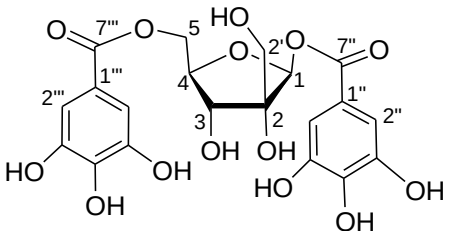


Table 1. NMR Spectral Data of Compounds **1** (in acetone-d_6), and **2** (in CD_3OD).

	1			**2**		
position	δ_C, mult.	δ_H (J in Hz)	HMBC[a]	δ_C, mult	δ_H (J in Hz)	HMBC[a]
Ellagic acid						
1	111.5, qC	-		111.5	-	
2	141.5, qC	-		141.1	-	
3	140.4, qC	-		140.1	-	
4	152.5, qC	-		152.6	-	
5	111.6, CH	7.50, s	1, 2, 3, 4, 6, 7	111.5	7.54, s	1, 2, 3, 4, 6, 7
6	113.0, qC	-		113.0	-	
7	159.2, qC	-		159.1	-	
1′	114.6, qC	-		114.6	-	
2′	136.0, qC	-		136.1	-	
3′	141.3, qC	-		141.2	-	
4′	146.3, qC	-		146.2	-	
5′	111.6, CH	7.83, s	1′, 3′, 4′, 6′, 7′	112.1	7.85, s	1′, 3′, 4′, 6′, 7′
6′	107.3, qC	-		107.4	-	
7′	159.1, qC	-		159.1	-	
3-OCH_3	61.1, CH_3	4.12, s	3	60.6	4.20, s	3
α-L-rhamnopyranose						
1″	99.7, CH	5.62, d (1.6)	4′	100.0	5.63, s	4′
2″	67.7, CH	4.38, dd (3.6, 1.6)		70.3	4.26, m	
3″	74.2, CH	5.13, dd (9, 3.6)		68.6	4.21, dd (9.1, 3.4)	
4″	69.9, CH	3.78, t (9.5)		73.7	5.09, t (9.7)	
5″	69.0, CH	3.78, m		67.6	3.93, dq (9.5, 6.3)	
6″	17.1, CH_3	1.25, d (6.3)		16.4	1.19, d (6.2)	
Acetyl group						
1‴ (CO)	172.6, qC	-		171.1	-	
2‴ (CH_3)	20.4, CH_3	2.14, s	1‴, 3″	19.6	2.14, s	1‴, 4″

[a] Key HMBC correlations are from proton(s) stated to the indicated carbon.

Table 2. NMR Spectral Data of Compounds **3 - 6** (in CD_3OD).

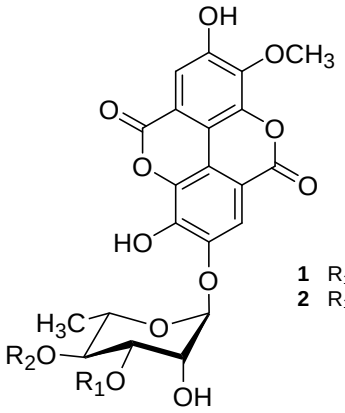
	3			**4**			**5**			**6**		
Position	δ_C, mult	δ_H (*J* in Hz)	HMBC [a]	δ_C, mult	δ_H (*J* in Hz)	HMBC [a]	δ_C, mult	δ_H (*J* in Hz)	HMBC [a]	δ_C, mult	δ_H (*J* in Hz)	HMBC [a]
β-D-glucopyranose												
1	102.1, CH	5.27, d (7.9)	1′	102.6, CH	5.06, d (8.1)		103.2, CH	4.94, d (7.8)		102.6, CH	5.01, d (8.1)	
2	73.5, CH	5.36, dd (9.4, 7.9)	7″	75.5, CH	5.13, dd (9.4, 8.1)	7″	73.4, CH	3.71, dd (9.4, 7.8)		75.5, CH	5.15, dd (9.3, 8.1)	7″
3	76.8, CH	5.51, t (9.4)	7‴	76.2, CH	3.76, t (9.2)		78.7, CH	5.26, t (9.4)	7″	76.2, CH	3.75, t (9.3)	
4	69.6, CH	3.83, t (9.5)		71.8, CH	3.48, dd (9.7, 9)		70.1, CH	3.65, t (9.5)		71.7, CH	3.50, t (9.0)	
5	78.6, CH	3.82, m	1	78.7, CH	3.56, ddd (9.7, 6.6, 2.1)	1	76.1, CH	3.87, m	1	78.7, CH	3.60, m	1
6a	62.4, CH_2	3.81, brd (12)		62.7, CH_2	3.75, dd (12.1, 6.6)		68.4, CH_2	4.15, dd (10.7, 7.2)		68.4, CH_2	4.17, dd (10.5, 6.8)	
6b		4.40, d (12.2, 1.8)			3.99, dd (12.1, 2.1)			4.44, brd (10.2)			4.56, brd (11.0)	
3′,4′,5′-trimethoxyphenyl											**3',4'-dimethoxyphenyl**	
1′	156.0, qC	-		156.0, qC	-		156.0, qC	-		153.9, qC	-	
2′	96.6, CH	6.36, s	1′, 3′, 4′, 6′	96.6, CH	6.33, d (2.1)	1′, 3′, 4′, 6′	96.1, CH	6.54, s	1′, 3′, 4′, 6′	103.8, CH	6.71, d (2.6)	1′, 3′, 4′, 6′
3′	154.8, qC	-		154.8, qC	-		154.8, qC	-		151.1, qC	-	
4′	139.4, qC	-		134.8, qC	-		134.4, qC	-		145.9, qC	-	
5′	154.8, qC	-		154.8, qC	-		154.8, qC	-		113.9, CH	6.82, d (8.5)	1′, 3′, 4′, 6′
6′	96.6, CH	6.36, s	1′, 2′, 4′, 5′	96.6, CH	6.33, d (2.1)	1′, 2′, 4′, 5′	96.1, qC	6.54, s	1′, 2′, 4′, 5′	109.0, CH	6.55, dd (8.5, 2.6)	1′, 2′, 4′, 5′
3′-OCH₃	56.5, CH_3	3.74, s	3′	56.4, CH_3	3.71, s	3′	56.8, CH_3	3.86, s	3′	56.5, CH_3	3.77, s	3′
4′-OCH₃	61.2, CH_3	3.70, s	4′	61.2, CH_3	3.68, s	4′	61.2, CH_3	3.73, s	4′	57.2, CH_3	3.74, s	4′
5′-OCH₃	56.5, CH_3	3.74, s	5′	56.4, CH_3	3.71, s	5′	56.8, CH_3	3.86, s	5′	-	-	5′
Galloyl-I												
1″	121.7, qC	-		121.5, qC	-		121.7, qC	-		121.5, q C	-	
2″	110.3, CH	7.02, s	1″, 3″, 4″, 6″, 7″	110.3, CH	7.13, s	1″, 3″, 4″, 6″, 7″	110.3, CH	7.17, s	1″, 3″, 4″, 6″, 7″	110.3, CH	7.14, s	1″, 3″, 4″, 6″, 7″
3″	146.3, qC	-		146.6, qC	-		146.4, qC	-		146.6, CH	-	
4″	139.9, qC	-		140.0, qC	-		139.7, qC	-		139.8, qC	-	
5″	146.3, qC	-		146.6, qC	-		146.4, qC	-		146.6, qC	-	
6″	110.3, CH	7.02, s	1″, 2″, 4″, 5″, 7″	110.3, CH	7.13, s	1″, 2″, 4″, 5″, 7″	110.3, CH	7.17, s	1″, 2″, 4″, 5″, 7″	110.3, CH	7.14, s	1″, 2″, 4″, 5″, 7″
7″	167.3, qC	-		167.7, qC	-		168.1, qC	-		167.7, qC	-	
Galloyl-II												
1‴	121.7, qC	-										
2‴	110.3, CH	7.07, s	1‴, 3‴, 4‴, 6‴, 7‴									
3‴	146.5, qC	-										
4‴	139.9, qC	-										
5‴	146.5, qC	-										
6‴	110.3, CH	7.07, s	1‴, 2‴, 4‴, 5‴, 7‴									
7‴	168.8, qC	-										

[a] Key HMBC correlations are from proton(s) stated to the indicated carbon.

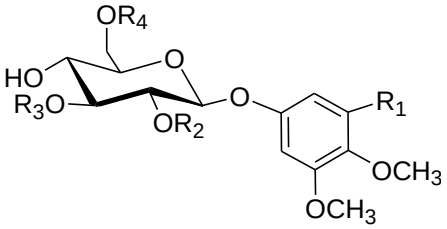
Table of Contents Graphic

Phenolic Glycosides from the Stem Bark of *Caryocar villosum* and *C. glabrum*

Abdulmagid Alabdul Magid, Laurence Voutquenne-Nazabadioko, Dominique Harakat, Christian Moretti, and Catherine Lavaud



1 $R_1 = CH_3CO$, $R_2 = H$
2 $R_1 = H$, $R_2 = CH_3CO$



3 $R_1 = -OCH_3$, $R_2 = R_3 =$ Galloyl, $R_4 = H$
4 $R_1 = -OCH_3$, $R_2 =$ Galloyl, $R_3 = R_4 = H$
5 $R_1 = -OCH_3$, $R_2 = H$, $R_3 =$ Galloyl, $R_4 = SO_3Na$
6 $R_1 = H$, $R_2 =$ Galloyl, $R_3 = H$, $R_4 = SO_3Na$