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

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Mushroom tyrosinase inhibitory activity of methanol extracts and polar fractions of the stem bark of *Caryocar villosum* and *C. glabrum* has been assessed. Seven new phenolic glycosides (**1–7**) were isolated from the most active fractions, along with 15 known compounds (**8–22**). The structures of these compounds were established on the basis of spectroscopic methods including 1D and 2D NMR analysis, HRESIMS, and comparison with literature experimental data for known compounds.

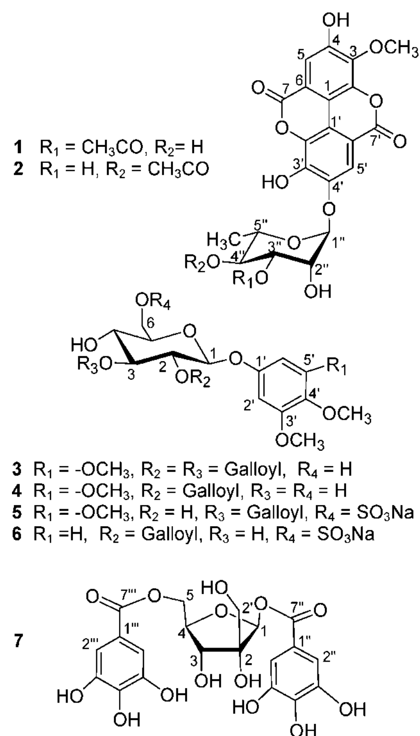
The genus *Caryocar* is one of the two genera belonging to the small, tropical, and New World family Caryocaraceae. The magnificent trees of the *Caryocar* genus are exploited for the fruit oil, which is used for cooking and in the cosmetic industry. Traditionally, the fruit and the stem bark are used by the Indian tribes of French Guyana, Colombia, Venezuela, and Brazil as a remedy for skin problems such as mycosis, microfilaria, and external tumors.[1]

In a search for tyrosinase inhibitors from natural sources, the *in vitro* mushroom tyrosinase inhibitory activities of the extracts of stem bark of *C. villosum* (Aubl.) Pers and *C. glabrum* (Aubl.) Pers were determined. Tyrosinases are copper-containing enzymes involved in melanogenesis, and their inhibitors interest cosmetic and pharmaceutical industries due to their preventive effect on pigmentation disorders. The literature presents contradictory results on their implication in cancer, suggesting a tumor-suppressing effect or a possible role in mutagenicity due to production of phenolic and quinoid compounds.[2] The H_2O-soluble extract of *C. glabrum* and the polar aqueous methanolic fraction of *C. villosum* exhibited low but definitive activity against the enzyme with IC_{50} values of 1.86 and 0.98 mg/mL, respectively.

Other than previous work from our laboratory on *Caryocar* species,[3,4] little is known about the phenolic compounds of this genus. Further fractionation of extracts has resulted in the isolation of seven new phenolic glycosides (**1–7**). Investigation of less active fractions of the MeOH extracts of stem bark of these two species led to the isolation of 15 known compounds (**8–22**).

A MeOH extract of stem bark of *C. villosum* was submitted to chromatographic separation and finally to semipreparative HPLC to afford compounds **1** and **2**, whereas a series of chromatographic steps of the aqueous extract of *C. glabrum* gave compounds **3–7**.

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 $[M + Na]^+$ peak at *m/z* 527.0810 measured in the positive ion mode. The negative ESIMSMS experiment on the negative $[M - H]^-$ ion peak of **1**, observed at *m/z* 503, gave fragments at *m/z* 443 and 428 due to successive losses of acetic acid and 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 ^1H NMR spectrum of **1** showed two aromatic protons at δ_H 7.50 (s) and 7.83 (s), two three-proton singlets due to methoxy (δ_H



4.12) and acetyl groups (δ_H 2.14), and six signals corresponding to eight protons arising from a 6-desoxyhexose moiety. The ^{13}C NMR spectrum of **1** exhibited signals for 12 aromatic carbons (C1–6 and C1′–6′), three carbonyl carbons (δ_C 159.1, 159.2, and 172.6) due to α,β-unsaturated lactones and an acetoxy group, one methoxy carbon (δ_C 61.1), one acetyl carbon (δ_C 20.4), and six carbons for the 6-desoxyhexose. Analysis of the HSQC, HMBC, and NOESY spectra and comparison of the ^1H 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 located at position C-3 to account for its high-field ^{13}C NMR chemical shift (δ_C 61.1)[6] 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 to be an α-L-rhamnopyranose (δ_H 6″ 1.25, d, J = 6.3 Hz) by analysis of the coupling patterns of its proton signals, the correlations in the COSY spectrum, and acid hydrolysis of the extracts. The 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] Position 3 for the acetyl group was suggested by the pronounced downfield

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Table 1. NMR Spectral Data of Compounds **3–6** (in CD_3OD)

	3		**4**		**5**		**6**	
position	δ_C, mult	δ_H (J in Hz)	δ_C, mult	δ_H (J in Hz)	δ_C, mult	δ_H (J in Hz)	δ_C, mult	δ_H (J in Hz)
β-D-glucopyranose								
1	102.1, CH	5.27, d (7.9)	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)	75.5, CH	5.13, dd (9.4, 8.1)	73.4, CH	3.71, dd (9.4, 7.8)	75.5, CH	5.15, dd (9.3, 8.1)
3	76.8, CH	5.51, t (9.4)	76.2, CH	3.76, t (9.2)	78.7, CH	5.26, t (9.4)	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	78.7, CH	3.56, ddd (9.7, 6.6, 2.1)	76.1, CH	3.87, m	78.7, CH	3.60, m
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	96.6, CH	6.33, d (2.1)	96.1, CH	6.54, s	103.8, CH	6.71, d (2.6)
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)
6′	96.6, CH	6.36, s	96.6, CH	6.33, d (2.1)	96.1, qC	6.54, s	109.0, CH	6.55, dd (8.5, 2.6)
3′-OCH_3	56.5, CH_3	3.74, s	56.4, CH_3	3.71, s	56.8, CH_3	3.86, s	56.5, CH_3	3.77, s
4′-OCH_3	61.2, CH_3	3.70, s	61.2, CH_3	3.68, s	61.2, CH_3	3.73, s	57.2, CH_3	3.74, s
5′-OCH_3	56.5, CH_3	3.74, s	56.4, CH_3	3.71, s	56.8, CH_3	3.86, s		
Galloyl-I								
1″	121.7, qC		121.5, qC		121.7, qC		121.5, qC	
2″	110.3, CH	7.02, s	110.3, CH	7.13, s	110.3, CH	7.17, s	110.3, CH	7.14, s
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	110.3, CH	7.13, s	110.3, CH	7.17, s	110.3, CH	7.14, s
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						
3‴	146.5, qC							
4‴	139.9, qC							
5‴	146.5, qC							
6‴	110.3, CH	7.07, s						
7‴	168.8, qC							

shift of H-3″ (δ_H 5.13), and this was confirmed by the HMBC experiment, which exhibited cross-peaks between H-3″ of rhamnose and the carbonyl carbon (δ_C 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 either be 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′ of the 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 yielded a NOE effect between H-1″ of rhamnose and H-5′ of methylellagic acid. The structure of compound **1** was thus elucidated as 3-*O*-methyl-4′-(3″-*O*-acetyl)-α-L-rhamnopyranosylellagic acid.

Compound **2** was an isomer of **1** ($C_{23}H_{20}O_{13}Na$), according to the pseudomolecular ion peak $[M + Na]^+$ at *m/z* 527.0803 in the positive HRESIMS. The ESIMSMS fragmentation of the $[M - H]^-$ 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 a monoacetylated desoxyhexose unit. The ^1H NMR, ^{13}C NMR, and HMBC experiments for **2** were similar to those of **1** except for the signals of the acetyl group on the rhamnose moiety, suggesting that **2** was a regioisomer of **1**. The positions of the 3-*O*-methyl and the 4′-*O*-rhamnosyl groups on ellagic acid were confirmed by HMBC and NOESY correlations. In the HMBC spectrum, the correlation of the downfield methine proton ($\delta_{H-4''}$ 5.09) of rhamnose with the carbonyl carbon of the acetoxy group (δ_C 171.1) indicated that the rhamnose was acetylated at 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 of methoxy and acetyl groups on a rhamnose unit.[5,8,9]

Compound **3** displayed a molecular ion peak $[M + Na]^+$ at *m/z* 673.1379 in the positive HRESIMS ($C_{29}H_{30}O_{17}Na$). Its ^1H NMR spectrum showed an anomeric proton signal (δ_H 5.27) 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 1). In the ^1H 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. Further carbon atoms appeared as 10 quaternary oxygenated aromatic carbons, two carbonyl esters near δ_C 168, two quaternary aromatic carbons at δ_C 121.7, and three methoxy groups. Complete analysis of the ^1H and ^{13}C NMR, HSQC, and HMBC spectra of **3** showed the presence of a 3′,4′,5′-trimethoxyphenyl moiety[10] and two galloyl moieties.[11] The 3′,4′,5′-trimethoxyphenyl group was linked 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) and was confirmed by the HMBC correlations between Glc-H-2 and the carbonyl ester group (δ_C 167.3) of one galloyl moiety and between Glc-H-3 and the carbonyl ester group (δ_C 168.8) of the other galloyl moiety. Therefore, the structure of **3** was elucidated as 1-*O*-3′,4′,5′-trimethoxyphenyl-(2,3-di-*O*-galloyl)-*β*-D-glucopyranoside.

The positive HRESIMS of compound **4** ($C_{22}H_{26}O_{13}Na$) suggested the lack of one galloyl group compared to **3**. The ^1H 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 one galloyl group. In the HMBC experiment, cross-peaks were

observed between H-1 of glucose (δ_H 5.06) and C-1′ of the 3′,4′,5′-trimethoxyphenyl moiety and between H-2 of glucose (δ_H 5.13) and the carbonyl carbon of the galloyl moiety (δ_C 167.7). Therefore, **4** was determined to be 1-*O*-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 $C_{22}H_{25}O_{16}SNa_2$, suggesting that **5** possessed an additional sodium sulfate group compared to **4**. Spectroscopic features indicated that **5** was closely related to **4** except for the signals belonging to the sugar moieties. 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_C 168.1) indicated that the galloyl moiety was linked at position 3 of glucose in **5**. The important downfield shift observed for the C-6 of glucose (δ_C 68.4 in **5** instead of 62.7 in **4**) and the absence of HMBC correlation with supplementary signals indicated that the sodium sulfate group was attached at C-6 of glucose. Thus, **5** was identified as the sodium salt of 1-*O*-3′,4′,5′-trimethoxyphenyl-(3-*O*-galloyl-6-*O*-sulfate)-β-D-glucopyranoside.

The molecular formula of compound **6** ($C_{21}H_{23}O_{15}SNa_2$) indicated the loss of a methoxy group compared to **5**. The ^{1}H and ^{13}C NMR, HSQC, and HMBC spectra showed that **6** contained a glucose substituted by a galloyl moiety at position 2 (δ_H 5.15), as in **4**, and by a sulfate at position 6 (δ_C 68.4) as in **5**. In the ^{1}H NMR spectrum of **6**, three proton signals characteristic of a 1,2,4-trisubstituted aromatic ring and two methoxy signals were observed. These data were in accordance with a 3′,4′-dimethoxyphenyl group[12] linked to the anomeric position of the glucose as proven 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 the sodium salt of 1-*O*-3′,4′-dimethoxyphenyl-(2-*O*-galloyl-6-*O*-sulfate)-β-D-glucopyranoside.

Compound **7** displayed a molecular ion peak $[M + Na]^+$ at *m/z* 507.0758 in accordance with the molecular formula $C_{20}H_{20}O_{14}Na$. Analysis of the ^{1}H and ^{13}C NMR spectra indicated the presence of two galloyl groups, as in **3**−**6**, and a sugar unit different from the glucose of most gallotannins.[11] The ^{13}C NMR spectrum of **7** showed the sugar moiety to have three hydroxymethines [δ_C 74.2 (C-3), 83.0 (C-4), and 101.4 (C-1)], two hydroxymethylenes [δ_C 64.2 (C-2′) and 66.9 (C-5)], and one quaternary OH-bearing carbon [δ_C 82.1 (C-2)]. Since the ^{1}H NMR spectrum showed that the anomeric proton correlating in the HSQC spectrum to the anomeric carbon at δ_C 101.4 resonated as a sharp singlet at δ_H 6.19, it was deduced that the carbon C-2 of the sugar unit was the quaternary carbon (δ_C 82.1). After analysis of correlations in COSY, HSQC, HMBC, and NOESY experiments, the sugar residue of **7** was identified as hamamelofuranose.[13] The HMBC spectrum showed long-range correlations between H-1 and C-2, C-2′, and C-4 and confirmed the structure of this monosaccharide. NOE effects between H-2′/H-3 and H-3/H-5 and the absence of correlation between H-1/H-3 indicated a β-configuration. Alkaline hydrolysis of the fraction from which **7** was obtained yielded D-hamamelofuranose, as determined by measurement of its ^{1}H 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. Therefore the structure of **7** was elucidated as 1,5-di-*O*-galloyl-β-D-hamamelofuranose.

The known compounds were identified by measurements of 1D and 2D NMR and ESIMS and comparison of their spectroscopic data with 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] putranjivain A (**20**),[19] nikoenoside (**21**),[20] and 1-*O*-3,4,5-trimethoxyphenyl-β-D-glucopyranoside (**22**).[10]

The mushroom tyrosinase inhibitory activity of the methanol extracts, of the H_2O fraction of *C. glabrum*, and of the two phenol-rich fractions of *C. villosum* was assessed. Although we were looking for development of crude extracts standardized with pure compounds, the relatively high values obtained for the IC_{50} (from 0.98 to 1.86 mg/mL) 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. IR spectra were recorded on a Nicolet Avatar 320 FT-IR spectrometer. 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 Chromeleon software, an ASI-100 automated sample injector, a STH 585 column oven, a P580 pump, and a UVD 340S diode array detector at 275 nm using a prepacked RP-18 column (Thermo Electron Corporation Hyperprep HS, 10 μm, 21.2 × 250 mm) with 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 (CC) was performed on Sephadex LH-20 (Pharmacia Biotech AB), Kieselgel 60 (63−200 μm, Merck), LiChroprep RP-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. 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. The stem bark of *C. glabrum* was collected in French Guyana near Matoury in the Amirande Forest and in the Ecerex Forest Station near Sinnamary, in October 2001, and identified by M. F. Prevost of the botany laboratory of the IRD Centre of Cayenne. Herbarium specimens (Grenand P. 3076 and Prevost MFP 4864) were 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 the solution was concentrated to dryness to give a gummy residue (37.5 g), 32 g of which was subjected to VLC on RP-18 eluted with MeOH−H_2O (4:6, 6:4, 8:2, and 10:0) to yield fractions A−D. Fraction B (800 mg) was submitted to successive CC on silica gel eluted with $CHCl_3$ containing increasing amounts of MeOH, and fractions eluted with $CHCl_3$−MeOH (8:2 and 7:3) were then purified by semipreparative HPLC to afford **1** (6 mg) and **2** (8 mg) (eluted with solvents B−A, 12:88 to 15:85 for 20 min). Fraction A [resulting from VLC eluted with MeOH−H_2O (4:6); 5 g] 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 semipreparative HPLC eluted with a mixture of solvents A (H_2O−TFA, 0.0025%) and B (CH_3CN−H_2O, 1:1) (5 mL/min) to give compounds **8**–**20**.

A portion of the dried and powdered stem bark (396 g) of *C. glabrum* was refluxed for 3 h with 1 L of MeOH. Then the solution was concentrated to provide a brown pasty residue (29 g), which was suspended in H_2O and then successively partitioned with *n*-hexane and EtOAc. The residue from the H_2O layer (14 g) 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 subjected to CC on silica gel eluted with $CHCl_3$−MeOH, and compound **3** (9 mg) was finally isolated by semipreparative HPLC (solvents A−B, 3–7 to 1:1, for 25 min) of column fractions eluted with $CHCl_3$−MeOH (7:3). Fractions 6 and 7 were subjected to CC on silica gel eluted with $CHCl_3$−MeOH−H_2O, and the resulting fractions eluted with $CHCl_3$−MeOH−H_2O (70:30:2) were purified by HPLC to afford **4** (12 mg), **5** (27 mg), and **6** (3.5 mg) (solvents A−B, 28:72).

Fractions 10–13 were submitted to RP-18 CC eluted with MeOH−H_2O, and the resulting fractions eluted with MeOH−H_2O (3:7) were finally purified by HPLC to afford **7** (8 mg) (solvents A−B, 1:9).

Compound 1: yellow, amorphous powder; $[\alpha]^{20}_D$ −30 (*c* 0.02, MeOH); UV (MeOH) λ_{max} (log ε) 256 (4.51), 354 (4.03) nm; IR (KBr) ν_{max} 3396, 2924, 1715, 1610, 1491, 1436, 1380, 1267, 1227 cm^{-1}; ^{1}H NMR (CD_3OD 500 MHz) δ 1.25 (1H, d, J = 6.3 Hz, H-6″), 2.14 (3H, s, H-2‴), 3.78 (1H, m, H-5″), 3.78 (1H, t, J = 9.5 Hz, H-4″), 4.12 (3H, s, 3-OCH_3), 4.38 (1H, dd, J = 3.6, 1.6 Hz, H-2″), 5.13 (1H, dd, J = 9.0, 3.6 Hz, H-3″), 5.62 (1H, d, J = 1.6 Hz, H-1″), 7.50 (1H, s, H-5), 7.83 (1H, s, H-5′); ^{13}C NMR (CD_3OD, 125 MHz) δ 17.1 (CH_3, C-6″), 20.4 (CH_3, C-2‴), 61.1 (CH3, 3-OCH_3), 67.6 (CH, C-2″), 69.0 (CH, C-5″), 69.9 (CH, C-4″), 74.2 (CH, C-3″), 99.1 (CH, C-1″), 107.3 (C, C-6′), 111.5 (C, C-1), 111.6 (2CH, C-5, C-5′), 113.0 (C, C-6), 114.6 (C, C-1′), 136.0 (C, C-2′), 140.4 (C, C-3), 141.3 (C, C-3′), 141.5 (C, C-2), 146.3 (C, C-4′), 152.5 (C, C-4), 159.1 (C, C-7′), 159.2 (C, C-7), 172.6 (C, C-1‴); ESIMS (positive ion mode) *m/z* 527 [M + Na]$^+$ (100); ESIMS (negative ion mode) *m/z* 503 [M − H]$^-$ (100); ESIMSMS *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); UV (MeOH) λ_{max} (log ε) 253 (4.52), 352 (4.02) nm; IR (KBr) ν_{max} 3405, 2920, 1731, 1605, 1488, 1437, 1361, 1268, 1227 cm^{-1}; ^{1}H NMR (CD_3OD 500 MHz) δ 1.19 (1H, d, J = 6.2 Hz, H-6″), 2.14 (3H, s, H-2‴), 3.93 (1H, dq, J = 9.5, 6.3 Hz, H-5″), 4.20 (3H, s, 3-OCH_3), 4.21 (1H, dd, J = 9.1, 3.4 Hz, H-3″), 4.26 (1H, m, H-2″), 5.09 (1H, t, J = 9.7 Hz, H-4″), 5.63 (1H, s, H-1″), 7.54 (1H, s, H-5), 7.85 (1H, s, H-5); ^{13}C NMR (CD_3OD, 125 MHz) δ 16.4 (CH_3, C-6″), 19.6 (CH_3, C-2‴), 60.6 (CH_3, 3-OCH_3), 67.6 (CH, C-5″), 68.6 (CH, C-3″), 70.3 (CH, C-2″), 73.7 (CH, C-4″), 100.0 (CH, C-1″), 107.4 (C, C-6′), 111.5 (C, C-1), 111.5 (CH, C-5), 112.1 (CH, C-5′), 113.0 (C, C-6), 114.6 (C, C-1′), 136.1 (C, C-2′), 140.1 (C, C-3), 141.1 (C, C-2), 141.2 (C, C-3′), 146.2 (C, C-4′), 152.6 (C, C-4), 159.1 (2C, C-7, C-7′), 171.1 (C, C-1‴); ESIMS (positive ion mode) *m/z* 527 [M + Na]$^+$ (100); ESIMS (negative ion mode) *m/z* 503 [M − H]$^-$ (100); ESIMSMS *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); UV (MeOH) λ_{max} (log ε) 237 (3.82), 271 (3.92) nm; IR (KBr) ν_{max} 3410, 2910, 1732, 1650, 1520, 1215, 1119 cm^{-1}; ^{1}H and ^{13}C NMR (CD_3OD), see Table 1; 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); UV (MeOH) λ_{max} (log ε) 239 (3.75), 272 (3.94) nm; IR (KBr) ν_{max} 3410, 2910, 1732, 1650, 1520, 1215, 1119 cm^{-1}; ^{1}H and ^{13}C NMR (CD_3OD), see Table 1; 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); UV (MeOH) λ_{max} (log ε) 274 (3.74), 237 (3.85) nm; IR (KBr) ν_{max} 3405, 2920, 1731, 1645, 1498, 1460, 1361, 1268, 1227 cm^{-1}; ^{1}H and ^{13}C NMR (CD_3OD), see Table 1; 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); UV (MeOH) λ_{max} (logε) 233 (3.69), 272 (3.77) nm; IR (KBr) ν_{max} 3533, 2921, 1731, 1522, 1488, 1460, 1359, 1270, 1230 cm^{-1}; ^{1}H and ^{13}C NMR (CD_3OD), see Table 1; 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); UV (MeOH) λ_{max} (log ε) 272 (3.65) nm; IR (KBr) ν_{max} 3364, 2921, 1732, 1612, 1488, 1361, 1268, 1227 cm^{-1}; ^{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″, C-2‴, C-6″, 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-5″), 146.5 (C, C-3‴, 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. Fractions A and B of *C. villosum* and the MeOH extract of *C. glabrum* (100 mg of each) were separately refluxed with 2 N 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−*i*-PrOH−Me_2CO−H_2O (20:10:7:6). A part of fraction 8 obtained from the first column chromatography of the H_2O-soluble extract of *C. glabrum* (50 mg) was dissolved in 3 mL of 3% methanolic KOH; after 24 h at room temperature, the solution was neutralized through a column of Amberlite IRN-77 ion-exchange resin and then evaporated. After preparative TLCs of the sugar mixtures in MeCOEt−*i*-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 previously described method using L-tyrosine and L-DOPA as substrate.[21]

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