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Notes

Cytotoxic Flavonol Glycosides from *Triplaris cumingiana*

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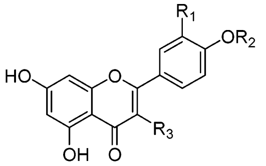
Three new compounds, 2-(3,4-dihydroxyphenyl)-5,7-dihydroxy-4-oxo-4*H*-chromen-3-yl-4,6-bis-*O*-*β*-D-(3,4,5-trihydroxybenzoyl)glucopyranoside (**1**), 5,7-dihydroxy-2-(4-hydroxyphenyl)-4-oxo-4*H*-chromen-3-yl-5-*O*-α-L-(3,4,5-trihydroxybenzoyl)arabinofuranoside (**2**), and 2-hydroxy-4-*O*-α-L-(3,5,7-trihydroxy-4-oxo-4*H*-chromen-2-yl)phenylarabinofuranoside (**3**), were isolated from the young leaves of *Triplaris cumingiana,* together with two known compounds, quercetin 3-*O*-α-L-(5″-*O*-galloyl)arabinofuranoside (**4**) and quercetin 3-*O*-*β*-D-(6″-*O*-galloyl)glucopyranoside (**5**). The structures of **1**−**3** were established by spectroscopic methods. Compounds **1**−**5** were evaluated for their cytotoxic activities against the MCF-7, H-460, and SF-268 human cancer cell lines.

As part of the Panama ICBG (International Cooperative Biodiversity Group) program aimed at discovering *inter alia* novel potential antitumor agents, an ethyl acetate-soluble extract of the young leaves of *Triplaris cumingiana* showed cytotoxic activity against the MCF-7, H-460, and SF-268 human cancer cell lines. The genus *Triplaris* (Polygonaceae) comprises approximately 20 species in South and Central America. *Triplaris cumingiana* Fisch. & C.A. Mey. ex Mey. is widely distributed in Panama[1] with no reports on this species having been found in the literature. Bioassay-guided fractionation of the EtOAc extract of *T. cumingiana* young leaves, using the MCF-7 (breast), H-460 (lung), and SF-268 (CNS) human cancer cell lines for monitoring fractionation, afforded three new compounds, 2-(3,4-dihydroxyphenyl)-5,7-dihydroxy-4-oxo-4*H*-chromen-3-yl-4,6-bis-*O*-*β*-D-(3,4,5-trihydroxybenzoyl)-glucopyranoside (**1**), 5,7-dihydroxy-2-(4-hydroxyphenyl)-4-oxo-4*H*-chromen-3-yl-5-*O*-α-L-(3,4,5-trihydroxybenzoyl)-arabinofuranoside (**2**), and 2-hydroxy-4-*O*-α-L-(3,5,7-trihydroxy-4-oxo-4*H*-chromen-2-yl)phenylarabinofuranoside (**3**). Also isolated were two known compounds, quercetin 3-*O*-α-L-(5″-*O*-galloyl)arabinofuranoside (**4**)[2] and quercetin 3-*O*-*β*-D-(6″-*O*-galloyl)glucopyranoside (**5**) (tellimoside).[3]



1 R_1 = OH R_2 = H R_3 = *O*-β-glc-4′′,6′′-digallate
2 R_1 = H R_2 = H R_3 = *O*-α-ara-5′′-gallate
3 R_1 = OH R_2 = O-α-ara R_3 = OH
4 R_1 = OH R_2 = H R_3 = *O*-α-ara-5′′-gallate
5 R_1 = OH R_2 = H R_3 = *O*-β-glc-6′′-gallate

Compound **1** was obtained as a yellow amorphous powder. The HRFABMS of **1** showed a $[M + 1]^+$ peak at *m/z* 769.12477, corresponding to the molecular formula $C_{35}H_{28}O_{20}$. Absorption maxima at 267 and 359 nm in the UV spectrum were characteristic of a flavonol skeleton.[4] The ^{1}H and ^{13}C NMR spectra (see Table 1) showed signals attributable to quercetin, two gallate groups [two singlets, each integrating for two protons, at δ 7.07 (H-6‴ and H-2⁗), 6.92 (H-2‴ and H-6⁗), and two carbonyl signals at δ_C 168.7 and 168.3], and signals of a glucose unit. The occurrence of a glucose unit was confirmed by acid hydrolysis and co-TLC with a reference sample. The above data indicated the presence of a quercetin glucoside esterified with two gallic acid units. The coupling constant of the anomeric proton ($J = 7.8$ Hz) and the ^{13}C NMR data indicated a *β*-glucopyranoside substituent. Substitution of the glucose unit at C-3 was indicated by the HMBC correlations between H-1″/C-3. The two gallate groups were positioned at C-4″ and C-6″, as evidenced from HMBC correlations of H-4″ and H-6″ with the gallate carbonyls (δ_C 168.7, 168.3) and the low-field shifted signals of H-4″ and H-6″ at 5.17 and 4.20 ppm, respectively. Furthermore, the ^{1}H−^{1}H COSY spectrum demonstrated correlations of H-4″/H-5″, H-3″ and H-6″/H-5″. On the basis of the above data, the structure of the new compound **1** was assigned as 2-(3,4-dihydroxyphenyl)-5,7-dihydroxy-4-oxo-4*H*-chromen-3-yl-4,6-bis-*O*-*β*-D-(3,4,5-trihydroxybenzoyl)glucopyranoside.

Compound **2** was isolated as an amorphous yellow powder. The molecular formula of **2** was established as $C_{27}H_{22}O_{14}$ by HRFABMS. The UV spectrum in different shift reagents again indicated the presence of a flavonol skeleton.[4] The ^{1}H and ^{13}C NMR data of **2** (see Table 1) showed signals of kaempferol aglycone, a gallate group, and arabinose, which was supported by acid hydrolysis and co-TLC with all three reference compounds. The coupling constant of the anomeric proton ($J = 0.9$ Hz) and a careful

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Table 1. ^{13}C and ^{1}H NMR Data (δ values) of Compounds **1−3**[a]

	1		**2**		**3**	
position	δ_C	δ_H	δ_C	δ_H	δ_C	δ_H
2	160.1 s		157.6 s		159.3 s	
3	136.0 s		134.2 s		135.7 s	
4	179.9 s		179.8 s		180.8 s	
5	163.5 s		160.6 s		163.8 s	
6	100.7 d	6.15 d (2.3)	99.3 d	6.27 d (2.0)	100.7 d	6.22 d (2.0)
7	166.5 s		164.9 s		166.8 s	
8	95.7 d	6.30 d (2.3)	94.3 d	6.50 d (2.0)	95.6 d	6.41 d (2.0)
9	159.0 s		158.1 s		160.1 s	
10	106.3 s		105.1 s		106.4 s	
1′	124.4 s		122.1 s		123.9 s	
2′	118.0 d	7.58 d (1.5)	116.1 d	8.04 d (8.7)	117.7 d	7.52 d (2.2)
3′	147.2 s		131.3 d	7.03 d (8.7)[c]	147.1 s	
4′	150.4 s		132.6 s		150.6 s	
5′	116.7 d	6.73 d (9.3)	131.3 d	7.03 d (8.7)[c]	117.2 d	6.92 d (8.5)
6′	123.7 d	7.60 dd (9.0, 1.5)	116.1 d	8.04 d (8.7)	123.8 d	7.52 dd (8.5, 2.2)
1″	104.9 d	5.35 d (7.8)	108.9 d	5.62 d (0.9)	110.3 d	5.48 s
2″	76.7 d	3.86[b]	84.7 d	4.05 m	84.1 d	4.35 dd (3.0, 1.1)
3″	74.5 d	3.86[b]	78.8 d	4.03 m	79.5 d	3.92 dd (6.0, 3.0)
4″	73.0 d	5.17 t (9.8)	84.9 d	4.42 m	88.7 d	3.89 m
5″	74.5 d	3.70 t (9.8)	64.2 t	4.26 dd (11.8, 4.1)	63.4 t	3.51 m
6″	64.3 t	4.20 m				
1‴	121.9 s		121.1 s			
2‴, 6‴	111.2 d	7.07 s	109.9 d	7.06 s		
3‴, 5‴	146.7 s		145.6 s			
4‴	140.8 s		138.5 s			
CO	168.7 s		166.8 s			
1⁗	121.8 s					
2⁗, 6⁗	111.1 d	6.92 s				
3⁗, 5⁗	146.5 s					
4⁗	140.5 s					
CO	168.3 s					

[a] Compounds **1** and **3** measured in MeOD and compound **2** in acetone-d_6. Coupling constants are (J in Hz) in parentheses. Assignments were made on the basis of ^{1}H−^{1}H-COSY, HMQC, and HMBC. Multiplicities were determined by DEPT 135 experiment. [b,c] Overlapping signals.

analysis of the ^{13}C NMR data[5−8] in addition to the NOESY correlations between H-1″/H-3″, H-4″ indicated the presence of an α-arabinofuranoside unit. Attachment of the arabinose at C-3 was deduced from the HMBC correlations between H-1″/C-3. The gallate group was positioned at C-5″, as evidenced from the low-field shifted H-5″ signal at 4.26 ppm and the HMBC correlation of H-5″ with the carbonyl carbon at δ_C 166.8 of the gallate unit. Thus, **2** was assigned as the new compound 5,7-dihydroxy-2-(4-hydroxyphenyl)-4-oxo-4*H*-chromen-3-yl-5-*O*-α-L-(3,4,5-trihydroxybenzoyl)arabinofuranoside.

The molecular formula of **3** was established by HRFABMS as $C_{20}H_{18}O_{11}$. The UV spectrum and the results of acid hydrolysis, in addition to the ^{1}H and ^{13}C NMR data (see Table 1), indicated the presence of quercetin as the aglycone attached to an arabinose unit. The position of the arabinose substituent at C-4′ was evidenced from UV spectra run in different shift reagents and the HMBC correlation between H-1″/C-4′. Thus, **3** was assigned as 2-hydroxy-4-*O*-α-L-(3,5,7-trihydroxy-4-oxo-4*H*-chromen-2-yl)phenylarabinofuranoside.

Compound **4** was identified as quercetin 3-*O*-α-L-(5″-*O*-galloyl)arabinofuranoside[2] and compound **5** as quercetin 3-*O*-β-D-(6″-*O*-galloyl)glucopyranoside (tellimoside),[3] by comparison of their spectral data reported in the literature.

Table 2 shows the GI_{50} values of compounds **1−5** when tested against a panel of three cell lines. Compound **1** showed cytotoxic activity against the H-460 (lung) cell line (GI_{50} = 3 μg/mL), while compound **4** was active against all three cell lines [GI_{50} = 1.4, 1.2, and 2.3 μg/mL in MCF-7, H-460, and SF-268, respectively]. Table 2 also shows the more potent activity of quercetin 3-*O*-α-L-arabinofuranoside-5″-gallate (**4**) in comparison with quercetin 3-*O*-β-D-glucopyranoside-6″-gallate (**5**), which may indicate the effect of the presence of an arabinose substituent relative to glucose.

Table 2. Cytotoxic Activities of Compounds **1−5**[a]

	GI_{50} (μg/mL)		
compound	MCF-7	H-460	SF-268
1	>10	3.0	>10
2	9.0	>10	>10
3	9.1	7.3	>10
4	1.4	1.2	2.3
5	>10	>10	>10
adriamycin	6.2×10^{-7}	3.6×10^{-7}	5.3×10^{-7}

[a] For the cell lines used, see the Experimental Section.

Experimental Section

General Experimental Procedures. Melting points are uncorrected. Optical rotations were measured with a Perkin-Elmer 141 polarimeter. UV spectra were measured with a Perkin-Elmer Model Lambda 2 UV/vis spectrometer. IR spectra were recorded on a Perkin-Elmer 1310 spectrophotometer. NMR spectra were recorded using a Brüker Avance 300 spectrometer in acetone-d_6 or MeOD at 300 MHz for ^{1}H and 75.0 MHz for ^{13}C NMR. Mass spectra were obtained on a Kratos MS50TC mass spectrometer. Silica gel [Merck, Kieselgel 60 (0.063−0.200 mm) and (0.015−0.040 mm)], LiChroprep RP-18 [prepacked column size B (31 × 2.5 cm), 40−63 μm, Merck, 9303], and Sephadex LH-20 (Sigma, 904-37-6) were used for column chromatography. Silica gel plates (Merck, Kieselgel 60 F_{254s}) were used for TLC. β-D-Glucose (Sigma) and α-L-arabinose (Sigma) were used as reference compounds.

Plant Material. Young leaves of *T. cumingiana* were collected from Soberania National Park (N 9°14′26″, W 79°39′30″), in Panama, November 2002. Voucher specimens

(52304) are deposited in the Herbarium of the University of Panama (PMA).

Cytotoxicity Bioassay. The cytotoxic activity was determined against breast (MCF-7), lung (H-460), and central nervous system (SF-268) human cancer cell lines according to the method given by Monks et al.[9] During the isolation process, the activity of all fractions was monitored using the three cell lines. Adriamycin was used as reference compound.

Extraction and Isolation. Fresh young leaves of *T. cumingiana* (280 g) were extracted and subjected to solvent partitioning in a manner described before.[10] Briefly, fresh young leaves of *T. cumingiana* were homogenized in MeOH for 30 s in a Waring blender followed by treatment with a Polytron homogenizer (Brinkmann Instruments). After filtration, the mark was washed with EtOAc. The crude MeOH/EtOAc extract [(25.99 g; GI$_{50}$ >10 μg/mL (MCF-7), 10 μg/mL (H-460), and 1.8 μg/mL (SF-268)] was partitioned between CH_2Cl_2 and H_2O, and the aqueous layer was further partitioned with EtOAc. The activity was retained in the EtOAc phase [5.8 g; percentage of growth (%G) 44.0, 42.1, and 39.7 of MCF-7, H-460, and SF-268, respectively]. Chromatography on a C$_{18}$-RP Lobar column using MeOH/H_2O as solvent (1:1, 2000 mL) yielded two fractions (1; 450 mL, 2; 1550 mL). Tannins and sugars were eluted in fraction 1, which was not cytotoxic. Fraction 2 (1.5 g; %G, 40.0, 36.7, 46.2) containing flavonoids was chromatographed on a C$_{18}$-RP Lobar column using as solvent system MeOH/H_2O (6:4, 1500 mL), which afforded fractions A (50−300 mL, 200 mg), B (350−600 mL, 162 mg), C (700−800 mL, 132 mg), and D (900−1500 mL, 250 mg), respectively. Fraction A was chromatographed separately on a Sephadex LH-20 column (60 × 2.5 cm) using 10% aqueous EtOH (500 mL), collecting 30 mL of each fraction, and combined fractions 4−7 yielded **4** (15 mg, 0.0053%). Fraction B was chromatographed under the same conditions as above, with combined fractions 2−4 affording **1** (20 mg, 0.0071%). Fraction D was chromatographed as above, and combined fractions 6−8 yielded **3** (50 mg, 0.01785%). Fraction C was also chromatographed as above, and fractions 2−4 yielded **2** (10 mg, 0.00357%), while fractions 5−7 yielded **5** (8 mg, 0.00285%).

2-(3,4-Dihydroxyphenyl)-5,7-dihydroxy-4-oxo-4*H*-chromen-3-yl-4,6-bis-*O*-*β*-D-(3,4,5-trihydroxybenzoyl)glucopyranoside (1): yellow amorphous powder, $[\alpha]^{28}_{D}$ +3.6° (*c* 0.14, MeOH); UV (MeOH) λ_{max} (log ϵ) 267 (4.71), 359 (4.39) nm; (MeOH + NaOMe) 273, 325, 410 nm; (MeOH + $AlCl_3$) 267, 300, 381 nm; (MeOH + $AlCl_3$ + HCl) 270, 290 (sh), 361, 405 nm; (MeOH + NaOAc) 267, 285 (sh), 359 nm; IR 3600−3000 (br), 1620, 1560, 1350, 1180 cm^{-1}; ^{1}H NMR (300 MHz, MeOD) and ^{13}C NMR (75 MHz, MeOD), see Table 1; FABMS *m/z* 769 $[M + 1]^+$ (3), 613 (3), 460 (3), 391 (3), 307 (25), 235 (3), 219 (3), 154 (100), 136 (66); HRFABMS *m/z* 769.12477 $[M + H]^+$ (calcd for $C_{35}H_{29}O_{20}$, 769.12522).

5,7-Dihydroxy-2-(4-hydroxyphenyl)-4-oxo-4*H*-chromen-3-yl-5-*O*-α-L-(3,4,5- trihydroxybenzoyl)arabinofuranoside (2): yellow amorphous powder; $[\alpha]^{28}_{D}$ −98.3° (*c* 0.06, MeOH); UV (MeOH) λ_{max} (log ϵ) 267 (4.00), 345 (3.76) nm; (MeOH + NaOMe) 274, 321, 390 nm; (MeOH + $AlCl_3$) 274, 300, 345, 395 nm; (MeOH + $AlCl_3$ + HCl) 274, 345, 390 nm; (MeOH + NaOAc) 267, 345 nm; ^{1}H NMR (300 MHz, acetone-*d*$_6$) and ^{13}C NMR (75 MHz, $CDCl_3$), see Table 1; FABMS *m/z* 769 $[M + 1]^+$ (3), 613 (3), 460 (3), 391 (3), 307 (25), 235 (3), 219 (3), 154 (100), 136 (66); HRFABMS *m/z* 571.11206 $[M + H]^+$ (calcd for $C_{27}H_{23}O_{14}$, 571.10878).

2-Hydroxy-4-*O*-L-(3,5,7-trihydroxy-4-oxo-4*H*-chromen-2-yl)phenylarabinofuranoside (3): yellow amorphous powder; $[\alpha]^{28}_{D}$ −106.3° (*c* 0.08, MeOH); UV (MeOH) λ_{max} (log ϵ) 256 (4.43), 360 (4.34) nm; (MeOH + NaOMe) 272, 325 (sh), 400 nm; (MeOH + $AlCl_3$) 274, 300 (sh), 425 nm; (MeOH + $AlCl_3$ + HCl) 268, 300 (sh), 362, 424 nm; (MeOH + NaOAc) 264, 397 nm; ^{1}H NMR (300 MHz, MeOD) and ^{13}C NMR (75 MHz, MeOD), see Table 1; FABMS *m/z* 435 $[M + 1]^+$ (4), 391 (10), 303 (10), 185 (61), 149 (10), 115 (10), 93 (100); HRFABMS *m/z* 435.09184 $[M + H]^+$ (calcd for $C_{20}H_{19}O_{11}$, 435.09274).

Acid Hydrolysis of 1−3. Five to 10 milligrams of each compound was added to 10% H_2SO_4 (5 mL) and left overnight at room temperature with stirring. The resulting reaction mixtures were neutralized and partitioned with EtOAc. The aqueous layers were freeze-dried using (Labconco), and the residues were dissolved in MeOH and co-TLC with authentic sugars *β*-D-glucose (Sigma) and α-L-arabinose (using silica gel, EtOAc/H_2O/formic acid/acetic acid (100:27:11:11), detection 10% H_2SO_4 in EtOH). Acid hydrolysis of **1**−**3** gave quercetin gallic acid and *β*-D-glucose (R_f 0.18), kaempferol, gallic acid and arabinose (R_f 0.25), and quercetin and α-L-arabinose, respectively.

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References and Notes

(1) Woodson, R. F.; Shery, R. W. *Ann. Missouri Bot. Gard.* **1960**, *47*, 338−339.
(2) Kitanaka, S.; Matsuzaki, K. Jpn. Kokai Tokkyo Koho: JP 2001316398 A2 20011113, 2001.
(3) Collins, F. W.; Bohm, B. A.; Wilkins, C. K. *Phytochemistry* **1975**, *14*, 1099−1102.
(4) Mabry, T. J.; Markham, K. R.; Thomas, B. M. *The Systematic Identification of Flavonoids*; Springer: New York, 1970; Chapter 5, pp 39−61.
(5) Yasukawa, K.; Ogawa, H.; Takido, M. *Phytochemistry* **1990**, *5*, 1707−1708.
(6) Iwagawa, T.; Kawasaki, J.-I.; Hase, T.; Sako, S.; Okubo, T.; Ishida, M.; Kim, M. *Phytochemistry* **1990**, *29*, 1013−1014.
(7) Markham, K. R.; Andersen, Ø. M. *Phytochemistry* **1990**, *29*, 3919−3920.
(8) Markham, K. R.; Whitehouse, L. A.; Webby, R. F. *J. Nat. Prod.* **1987**, *50*, 660−663.
(9) Monks, A.; Scudiero, D. A.; Johnson, G. S.; Pauli, K. D.; Sausville, E. A. *Anticancer Drug Des.* **1997**, *12*, 533−541.
(10) Hussein, A. A.; Bozzi, B.; Correa, M.; Capson, T. L.; Kursar, T. A.; Coley P. D.; Solis, P. N.; Gupta, M. P. *J. Nat. Prod.* **2003**, *66*, 858−860.

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