Two Novel Flavanones from *Greigia sphacelata*

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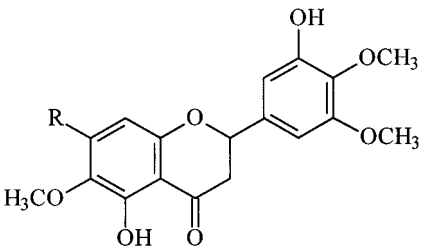
As part of our continuing phytochemical investigations of plants from arid environments in Chile, the aerial parts of *Greigia sphacelata* were examined. Two novel flavanones, 5,7,3′-trihydroxy-6,4′,5′-trimethoxyflavanone (**1**) and 5,3′-dihydroxy-6,7,4′,5′-tetramethoxyflavanone (**2**), as well as eight known compounds—1,3-*O*-di-*trans*-*p*-coumaroylglycerol (**3**), 1-*O*-*trans*-*p*-coumaroylglycerol (**4**), a mixture of 1-(ω-feruloyldocosanoyl)glycerol (**5**) and 1-(ω-feruloyltetracosanoyl)glycerol (**6**), *trans*-ferulic acid 22-hydroxydocosanoic acid ester (**7**), arborinone (**8**), arborinol (**9**), and isoarborinol (**10**)—were isolated.

Greigia sphacelata (R. *et* P.) Regel, locally known in Chile as "chupón" or "quiscal", is a member of the Bromeliaceae.[1] The genus consists of 32 species, three of which are endemic to central Chile, including *G. sphacelata*.[2] No chemical investigations have previously been reported for this genus.

This study is part of continuing phytochemical investigations of plants from arid environments in Chile for potential biomedical uses. We report here on the isolation and structure elucidation of two novel flavanones, 5,7,3′-trihydroxy-6,4′,5′-trimethoxyflavanone (**1**) and 5,3′-dihydroxy-6,7,4′,5′-tetramethoxyflavanone (**2**), as well as eight known compounds—including 1,3-*O*-di-*trans*-*p*-coumaroylglycerol (**3**),[3] 1-*O*-*trans*-*p*-coumaroylglycerol (**4**),[4,5] a mixture of 1-(ω-feruloyldocosanoyl)glycerol (**5**) and 1-(ω-feruloyltetracosanoyl)glycerol (**6**),[6] *trans*-ferulic acid 22-hydroxydocosanoic acid ester (**7**),[6] arborinone (**8**),[7,8] arborinol (**9**),[7,9] and isoarborinol (**10**)[7,8]—from the dichloromethane−methanol extract of *G. sphacelata*.



1 R = OH
2 R = OCH_3

Compound **1** was isolated as a yellow solid by HPLC on a Diol Si gel phase. Positive HRFABMS showed the $[M]^+$ ion at *m/z* 362.0999 corresponding to the molecular formula $C_{18}H_{18}O_8$ (calcd 362.1002). ^1H NMR data indicated the presence of one methylene (δ 2.81 and 3.10) and one oxygen-substituted methine group at δ 5.29. Three methoxy groups signals appeared at δ 3.90, 3.92, and 3.96. The proton spectrum also showed three aromatic protons, two of which appeared as doublets ($J = 2.0$) at δ 6.58 and 6.69, suggesting meta coupling, and one as a singlet at δ 6.15. The ^{13}C NMR spectrum showed 12 aromatic carbons between δ 94.8 and 158.8, a carbonyl carbon at δ 196.7, one oxygen-substituted methine at δ 78.6, and one methylene in the aliphatic region. All of these data are consistent with a trihydroxy-trimethoxyflavanone (Table 1).

The substitution pattern was determined both by comparison with chemical-shift data compiled by Agrawal *et al.*[10] for differing flavanone substitution patterns and by 2D NMR, namely HSQC and HMBC. According to Agrawal *et al.*,[10] the position of C-2 at δ 78.6 in compound **1** indicates that both the C-2′ and C-6′ positions are unsubstituted; the chemical shift of C-4 at δ 196.7 indicates the presence of a 5-OH.

These assignments are supported by the HMBC spectrum (Table 1). Lack of substitution at the C-2′ and C-6′ positions is shown by the H-2′ correlation to C-2, C-4′, and C-6′, while H-6′ correlates to C-2, C-2′, and C-4′. The methoxy group at δ_H 3.92−δ_C 60.4 shows an HMBC correlation to C-4′ allowing placement in the para position on the B-ring. The proton of 5-OH at δ 12.2 displays HMBC correlations to C-5, C-6, and C-10. The proton at δ 6.15 was placed at the 8 position due to HMBC correlations to C-6, C-7, and C-10. The methoxy group at δ 3.96 was assigned to the 6 position as it correlates to C-6. The third methoxy group at δ 3.90 was assigned to the 5′ position because of chemical-shift effects at the C-6′ carbon and the lack of symmetry in the B-ring. Comparison with spectral data available for similar B-ring structures confirmed this assignment.[11] Based on these observations, we assigned **1** the structure of 5,7,3′-trihydroxy-6,4′,5′-trimethoxyflavanone (see Figure 1).

Compound **2** was isolated by HPLC on Diol Si gel as a yellow amorphous solid. Positive HRFABMS showed the $[M]^+$ ion at *m/z* 376.1154 corresponding to the molecular formula $C_{19}H_{20}O_8$ (calcd 376.1158). The ^1H and ^{13}C NMR spectra for **2** were almost identical to those of **1**, with the exception of an additional methoxy group appearing in both the proton and carbon spectra. The chemical shift of the carbonyl group at C-4 was essentially unchanged when compared to compound **1**, excluding the possibility of a methoxy group at position 5. Due to the lack of symmetry in the B-ring, which would appear if the C-3′ position of **1** were methoxylated, the methoxy group at δ_H 3.90/δ_C 56.3 was placed at C-7. HMBC correlations supported this conclusion (Table 1), and compound **2** was determined to be 5,3′-dihydroxy-6,7,4′,5′-tetramethoxyflavanone.

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Table 1. ^{1}H and ^{13}C NMR and HMBC Correlations for 5,7,3′-Trihydroxy-6,4′,5′-trimethoxyflavanone (**1**) and 5,3′-Dihydroxy-6,7,4′,5′-tetramethoxyflavanone (**2**)

	1 ($CDCl_3$)			**2** ($CDCl_3$)		
position	δ_C	δ_H	HMBC	δ_C	δ_H	HMBC
2	78.6d	5.29dd (3,13)	C2′	79.6d	5.30dd (3,13)	C2′
3a	42.8t	2.81dd (3,17)		43.4t	2.81dd (3,17)	
3b		3.10dd(13,17)	C2, C1′		3.10dd (13,17)	C2
4	196.7s			196.6s		
5	154.6s			155.2s[a]		
5-O*H*		12.2	C5, C6, C10		11.9	C6, C10
6	128.7s			131.0s		
7	158.8s			161.3s		
8	94.8d	6.15	C6, C7, C10	91.6d	6.15	C6, C7, C10
9	157.5s			158.8s[a]		
10	103.2s			103.5s		
1′	134.6s			134.5s		
2′	106.2d	6.69d (2)	C2, C3′, C4′, C6′	106.2d	6.72d (2)	C2, C4′, C6′
3′	149.9s			149.9s		
4′	136.0s			136.1s		
5′	152.8s			152.9s		
6′	102.2d	6.58d (2)	C2, C2′, C4′	102.2d	6.59d (2)	C2, C2′, C4′
6-O*CH*$_3$	60.4q	3.96	C6	61.1q	3.87	C6
7-O*CH*$_3$				56.3q	3.90	C7
4′-O*CH*$_3$	60.4q	3.92	C4′	61.1q	3.94	C4′
5′-O*CH*$_3$	55.4q	3.90	C5′	56.1q	3.92	C5′

[a] Interchangeable.

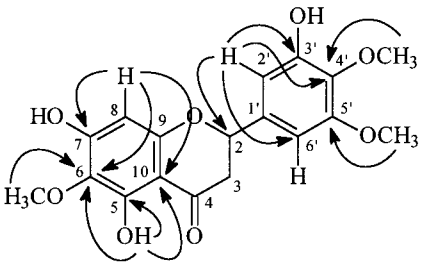

Figure 1. Selected HMBC correlations for 5,7,3′-trihydroxy-6,4′,5′-trimethoxyflavanone (**1**).

Experimental Section

General Experimental Procedures. Optical rotations were measured in CH_2Cl_2 on a JASCO P1020 polarimeter. UV spectra were recorded on a Beckman DU-600 spectrophotometer. HPLC was performed with a Varian 9002 pump equipped with a Varian Star 9040 refractive index detector and a 4.6 × 250 mm Lichrosphere 100 Diol Si gel (5 μm) or a 10 × 250 Econosphere Si gel (10 μm) column. Si gel 60 (32−63 μm and 63−200 μm, Scientific Adsorbents, Inc.) was utilized for column chromatography. 1D and 2D NMR spectra were acquired in $CDCl_3$ on a Bruker DRX-600 at 600 MHz (^{1}H) and 150 MHz (^{13}C). HMBC spectra were acquired with J = 10 Hz. Spectra were referenced to residual chloroform at δ_H = 7.24 and to $CDCl_3$ at δ_C = 77.0. HRFABMS were recorded on a JEOL JMS−HX 110 with a resolution of 5000 and a mixed matrix containing 50% glycerol, 25% *m*-NBA, 25% thioglycerol, plus 0.1% TFA. LREIMS were obtained with a Hewlett-Packard 5988A (70 eV).

Plant Material. The aerial parts of *G. sphacelata* were collected in Chile during April 1994 (35°56′ S, 72°43′ W, Trehualemu, Departamento Parral, Region del Maule) and identified by Gloria Montenegro and Liliana Iturriaga. A voucher specimen (no. 0310) was deposited in the Herbarium of the Pontificia Universidad Católica de Chile, Santiago, Chile. Intellectual Property Rights Agreements for plant collections and collaborative research have been fully executed between The University of Arizona and the Pontificia Universidad Católica de Chile.

Extraction and Isolation. Air-dried, ground material of *G. sphacelata* (1 kg) was extracted with CH_2Cl_2−MeOH (1:1). The dried extract (15.7 g) was subjected to normal-phase Si gel column chromatography (CC, 7.5 × 70 cm column), and eluted with an acetone−hexane gradient followed by a MeOH wash to yield 14 fractions. From Fraction 1 a precipitate was collected (100 mg) that showed three distinct spots on TLC when sprayed with an anisaldehyde−sulfuric acid spray reagent.[12] HPLC on Si gel with 4% EtOAc in hexane was used to obtain arborinone (**7**, 4.0 mg), arborinol (**8**, 4.5 mg), and 3β-isoarborinol (**9**, 3.5 mg) from 20 mg of this mixture. Fraction 9 (1.45 g) underwent Si gel CC (4 × 34 cm) with a EtOAc−hexane gradient followed by a MeOH wash to yield 15 fractions. Fraction 9-6 (116.5 mg) was resubjected to CC (2 × 35 cm) followed by HPLC on Diol Si gel with 30% EtOAc in hexane to yield 5,7,3′-trihydroxy-6,4′,5′-trimethoxyflavanone (**1**, 1.8 mg); 5,3′-dihydroxy-6,7,4′,5′-tetramethoxyflavanone (**2**, 2.5 mg); and *trans*-ferulic acid 22-hydroxydocosanoic acid ester (**7**, 4.7 mg). Fraction 9-12 (65.0 mg) gave a precipitate (20.0 mg) that was identified as a mixture of 1-(ω-feruloyldocosanoyl)glycerol (**5**) and 1-(ω-feruloyltetracosanoyl)glycerol (**6**). Fraction 10 was chromatographed on Si gel (2 × 35 cm) and eluted with an EtOAc−hexane gradient and subsequent MeOH wash to give 17 fractions. A white precipitate was separated from the combined fractions 10-8 and 10-9 (40.7 mg) and was identified as 1,3-*O*-di-*trans*-*p*-coumaroylglycerol (**3**, 5.0 mg). Fraction 10-14 (69.9 mg) was subjected to HPLC on Diol Si gel with 20% acetone in CH_2Cl_2 to yield 1-*O*-*trans*-*p*-coumaroylglycerol (**4**, 1.5 mg).

5,7,3′-Trihydroxy-6,4′,5′-trimethoxyflavanone (1): yellow solid; $[\alpha]^{25}_D$ −14.0 ($CHCl_3$, *c* 1.0); UV (CH_2Cl_2) λ_{max} (log ϵ) 288 (4.07); ^{1}H, ^{13}C, and 2D NMR, see Table 1; EIMS *m*/*z* (rel int): 362 $[M]^+$ (57), 344 (5), 329 (6), 319 (2), 313 (2), 259 (1), 247 (2), 209 (6), 182 (91), 167 (91), 154 (21), 137 (18), 122 (10), 77 (20), 69 (100); positive HRFABMS *m*/*z* 362.3372 (calcd for $C_{18}H_{18}O_8$, 362.3361).

5,3′-Dihydroxy-6,7,4′,5′-tetramethoxyflavanone (2): yellow solid; $[\alpha]^{25}_D$ −4.8 ($CHCl_3$; *c* 1.5); UV (CH_2Cl_2) λ_{max} 291 (log ϵ 4.04); ^{1}H, ^{13}C, and 2D NMR, see Table 1; EIMS *m*/*z* (rel int): 376 $[M]^+$ (59), 358 (5), 343 (8), 223 (6), 196 (95), 181 (100), 165 (36), 153 (23), 137 (14), 69 (30); positive HRFABMS *m*/*z* 376.3648 (calcd for $C_{19}H_{20}O_8$, 376.3630).

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