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An unusual homoisoflavanone and a structurally-related dihydrochalcone from *Polygonum ferrugineum* (Polygonaceae)

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Abstract

The homoisoflavanone 5,7-dihydroxy-6-methoxy-3-(9-hydroxy-phenylmethyl)-chroman-4-one (**1**) and its structurally related 2′,4′,6′-trihydroxy-3′-methoxy-α-hydroxymethyl-β-hydroxy-dihydrochalcone (**2**) along with the known pashanone (**3**), flavokawin B (**4**) and cardamonin or alpinetin chalcone (**5**) pinostrobin (**6**) and 5,8-dimethoxy-7-hydroxychroman-4-one (**7**) were isolated from dry leaves of *Polygonum ferrugineum* (Polygonaceae). To our knowledge, this is the first report of the isolation of a homoisoflavanone from the *Polygonum* genus and the Polygonaceae family, and could be an important chemotaxonomic finding. In addition, the pattern of substitution of this homoisoflavanone is different from others previously reported.

Keywords: *Polygonum ferrugineum*; Polygonaceae; Homoisoflavanone; Dihydrochalcone; Flavanones; Chalcones; 5,7-Dihydroxy-6-methoxy-3-(9-hydroxy-phenylmethyl)-chroman-4-one; 2′,4′,6′-Trihydroxy-3′-methoxy-α-hydroxymethyl-β-hydroxydihydrochalcone

1. Introduction

The genus *Polygonum* (Polygonaceae), comprising about 300 species (Wang et al., 2005), is distributed worldwide in temperate climates, and is widely found in South America. It is well known for producing a variety of secondary metabolites including flavonoids (Sartor et al., 1999; Peng et al., 2003), triterpenoids (Duwiejua et al., 1999), anthraquinones (Matsuda et al., 2001; Yim et al., 1998), coumarins (Sun and Sneden, 1999), phenylpropanoids (Murai et al., 2001; Takasaki et al., 2001), lignans (Kim et al., 1994), sesquiterpenoids (Datta et al., 2000), stilbenoids (Nonaka et al., 1982), and tannins (Wang et al., 2005). Amongst them, flavonoids are the most common components found in *Polygonum* spp. and have previously been used as chemotaxonomic markers of the genus, playing an important role in the systematics of Polygonaceae species (Datta et al., 2002).

Within the 21 *Polygonum* species growing in Argentina (Gattuso, 1998), *Polygonum ferrugineum* Wedd. common name *caatay guazú*, a native shrub found in swampy grounds in the north and center of the country, has not previously been studied for its chemical composition. As a part of our ongoing project devoted to the phytochemical study of the *Polygonum* genus, we have isolated a novel homoisoflavanone (**1**) and a novel dihydrochalcone derivative (**2**) structurally related to (**1**), along with five known compounds, three chalcones (**3**–**5**) and two flavanones (**6** and **7**).

The present paper describes the isolation of compounds **1**–**7** and the structural characterization of the two new compounds **1** and **2**.

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The finding of a homoisoflavanone in *P. ferrugineum* has a special significance since homoisoflavanones are a class of naturally occurring secondary metabolites with a basic structure consisting of a 3-benzyl or 3-benzylidene chromanone (Du Toit et al., 2005) with limited occurrence in nature. They possess great chemotaxonomic value particularly within the Hyacinthaceae family, considering that homoisoflavanones have been found only in the sub-families Hyancinthoidea and Chlorogaloidea and recently in Ornithogaloidea (Mulholland et al., 2004) and Urginoidea (Koorbanally et al., 2005). They have also been found in two species of Liliaceae and in only one species of Agavaceae (Likhitwitayawuid et al., 2002).

2. Results and discussion

Two new compounds **1** and **2** were isolated from dry leaves of *P. ferrugineum*, along with five known compounds, three chalcones: 2′,6′-dihydroxy-3′,4′-dimethoxychalcone (pashanone) (**3**) (Ramakrishnan et al., 1974), 2′-hydroxy-4′,6′-dimethoxychalcone (flavokawin B) (**4**) (Ahmed et al., 1981), and 2′,4′-dihydroxy-6′-methoxychalcone (cardamonin or alpinetin chalcone) (**5**) (Krishna and Chaganty, 1972); and two flavanones: 5-hydroxy-7-methoxyflavanone (pinostrobin) (**6**) (Burke and Nair, 1986) and 5,8-dimethoxy-7-hydroxyflavanone (**7**) (Bratoeff and Pérez-Amador, 1994).

The HRMS obtained with MALDI of compound **1** gave a $[M + Na^+]$ at 339.0844 corresponding to $C_{17}H_{16}O_6Na$ (calcd. 339.0839). The IR spectrum showed characteristic absorption bands for an α,β-unsaturated carbonyl system at 1638 cm^{-1}, bridge ethers at 1285 cm^{-1} and a broad hydroxyl stretching band at 3300–3450 cm^{-1}. The UV maximal absorptions at 240 and 301 nm were suggestive of a flavonoid skeleton (Langlois et al., 2005). Bathochromic shifts with NaOMe (+28 nm), NaOAc (+28 nm) and $AlCl_3$ (+12 nm) (Mabry et al., 1970) indicated the presence of an –OH group on C-5 and C-7 (or C-4′). The ^1H NMR spectrum (Table 1) showed the presence of two aromatic moieties: a five-proton multiplet between δ 7.34–7.41 and a one-proton singlet at δ 6.01. The first group of signals indicated the presence of an unsubstituted aromatic ring B, confirming that the –OH was not at C-4′. The second resonance at δ 6.01 was typical for a methine proton of a polysubstituted aromatic ring A. The aliphatic proton signals at δ 2.94 (1H, *ddd*, $J = 7.8$, 4.3 and 7.4 Hz, H-3), δ 3.93 (1H, *dd*, $J = 11.5$ and 7.4 Hz, H-2β), δ 4.14 (1H, *dd*, $J = 11.5$ and 4.3 Hz, H-2α) and δ 5.11 (1H, *d*, $J = 7.8$ Hz, H-9) were indicative of the presence of a system (2)CH_2–(3)CH–(9)CH– in the structure, which was corroborated by the HH-COSY correlations observed (not shown). Singlets at δ 3.82 and δ 6.54 and a broad singlet at δ 12.02 were assigned to the protons of an aromatic –OCH_3, an aliphatic –OH and to an aromatic –OH, respectively.

Based on spectroscopic data, a homoisoflavanone skeleton with a pentasubstituted ring A and an unsubstituted ring B was proposed.

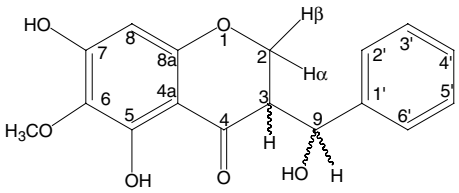
^{13}C NMR and DEPT experiments indicated the presence of 17 carbon atoms including six quaternary aromatic carbons and one carbonyl carbon (δ 198.9, C-4) involved in a intramolecular hydrogen bond interaction with the –OH group of C-5 (Agrawal et al., 1989). The rest of the resonances were: δ 60.7 (–O<u>C</u>H_3), δ 67.8 (–<u>C</u>H_2O–), δ 51.0 and δ 72.4 (aliphatic –<u>C</u>H), δ 94.1 (=<u>C</u>H) and a set of three characteristic signals at δ 126.5, 128.2 and 128.4 for the =<u>C</u>H groups of a monosubstituted aromatic system.

The positions of ring A substituents were confirmed from the HH-COSY (not shown) and COLOC (Fig. 1) correlations: –OH groups were assigned to C-5 and C-7 and –OCH_3 to C-6; the last position was assigned on the basis of a correlation observed between the –OCH_3 singlet (δ 3.82) and C-6 (δ 128.6) in addition to the correlations of the H-8 singlet (δ 6.01) with C-6 (δ 128.6), C-7 (δ 158.7), C-8a (δ 157.8) and C-4a (δ 102.5). In addition, the long-range correlation observed between the H-2β signal and C-8a was crucial to assign C-2 at δ 67.8 and to confirm the position of the aliphatic –OH group at δ 6.54 on C-9.

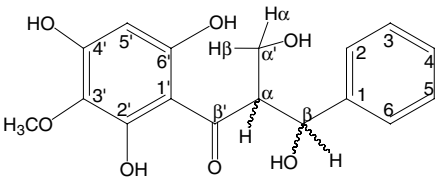
In order to confirm the number of –OH groups present in **1**, we considered the preparation of a peracetylated derivative by the usual Ac_2O/pyridine procedure. Besides acetylation, the reaction produced also the $\Delta^{3,9}$-dehydrated product **1Ac** with a molecular ion at *m*/*z* 382 observed by GC–MS and corroborated by HRFABMS ($[MH^+]$ at 383). The ^1H NMR spectrum, compared to that of **1** showed: (a) two singlets at δ 2.33 and δ 2.45 corresponding to –CH_3 of acetyl groups, (b) a lack of signals corresponding to a –(2)CH_2–(3)CH–(9)CH– moiety and (c) the appearance of both, a singlet at δ 7.80 due to the vinyl proton on C-9 and two singlets at δ 5.26 and δ 5.27 due to the 2H-2. ^{13}C NMR signals were in agreement. In order to deduce the configuration of C-3(9) double bond of **1Ac**, a series of NOE experiments were performed. Irradiation of the H-9 resonance gave an enhanced signal for the H-2′/H-6′ system but not for the H-2 signal. Irradiation of the H-2 resonance led to enhancement of the H-2′/H-6′ system, but no effect was observed for the H-9 signal. So, the configuration of the C-3(9) double bond was deduced to be (*E*) which is in agreement with literature for benzylidene-homoisoflavanones (Corsaro et al., 1992; Bangani et al., 1999). The generation of a 3-benzylidene-4-chromanone during the acetylation process was additional evidence for the presence of the –OH group on C-9 of **1** and would be useful for the determination of the relative configuration of C-9 as will be explained below. Based on all previous spectroscopic data, structure **1** was unequivocally established as 5,7-dihydroxy-6-methoxy-3-(9-hydroxy-phenylmethyl)-chroman-4-one and it represents the first example of a homisoflavanone bearing a hydroxyl group on C-9.

The CD spectrum of homoisoflavanone **1** showed a negative Cotton effect at 315 nm. This value is in the region of the negative Cotton effect produced by a series

Table 1
NMR spectroscopic data for compounds **1** and **2**



1



2

	Compound **1**		Compound **2**	
	^{13}C	^{1}H	^{13}C	^{1}H
1	–	–	140.6	–
2	67.8	3.93 (*dd*, 11.5, 7.4), Hβ 4.14 (*dd*, 11.5, 4.3), Hα	125.5	7.26–7.39 (*m*)
3	51.0	2.94 (*ddd*, 7.8, 4.3, 7.4)	128.5	7.26–7.39 (*m*)
4	198.9	–	127.8	7.26–7.39 (*m*)
4a	102.5	–	–	–
5	154.5	–	128.5	7.26–7.39 (*m*)
6	128.6	–	125.5	7.26–7.39 (*m*)
7	158.7	–	–	–
8	94.1	6.01 (*s*)	–	–
8a	157.8	–	–	–
9	72.4	5.11 (*d*, 7.8)	–	–
1′	139.6	–	103.5	–
2′	126.5	7.34–7.41 (*m*)	157.5	–
3′	128.4	7.34–7.41 (*m*)	128.1	–
4′	128.2	7.34–7.41 (*m*)	158.9	–
5′	128.4	7.34–7.41 (*m*)	94.2	6.01 (*s*)
6′	126.5	7.34–7.41 (*m*)	154.4	–
α	–	–	51.4	3.07 (*ddd*, 4.0, 5.1, 10.7)
α′	–	–	66.6	4.49 (*dd*, 10.7, 11.2) (Ha) 4.23 (*dd*, 11.4, 5.1) (Hb)
β	–	–	69.7	5.61 (*bs*)
β′	–	–	197.3	–
O<u>H</u>–C$_5$	–	12.02 (*s*)	–	–
O<u>H</u>–C$_9$	–	6.54 (*s*)	–	–
OCH$_3$–C$_6$	60.7	3.82 (*s*)	–	–
O<u>H</u>–C$_{\alpha'}$	–	–	–	6.50 (*bs*)
O<u>H</u>–C$_\beta$	–	–	–	2.53 (*d*, 4.1)
O<u>H</u>–C$_{2'}$	–	–	–	12.14 (*s*)
OCH$_3$–C$_{3'}$	–	–	60.9	3.93 (*s*)

200 MHz (^{1}H), 50 MHz (^{13}C) in CDCl$_3$–DMSO-d_6; δ values are given in ppm and J values (in parentheses) in Hz.

of homoisoflavanones (285–297 nm for 3-benzylchroman-4-one skeleton and 333–337 nm for benzocyclobutenehomoisoflavanones) (Adinolfi et al., 1988, 1990; Corsaro et al., 1992) although it does not fully coincide with the reported values. This difference, probably due to the structural differences between them and compound **1** (an extra chiral carbon in C-9 due to the presence of an extra –OH), prevent us from drawing any definitive conclusion about the absolute configuration of chiral centers.

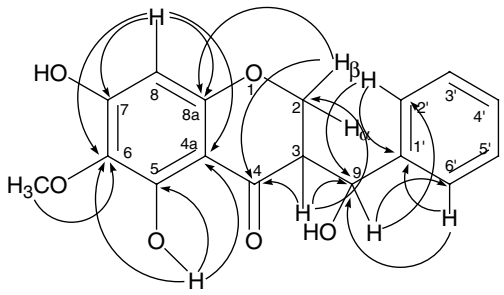


Fig. 1. Correlations obtained with COLOC experiments for 5,7-dihydroxy-6-methoxy-3-(9-hydroxy-phenylmethyl)-chroman-4-one (**1**) isolated from *Polygonum ferrugineum*.

So, additional studies including theoretical calculations of the electronic CD spectra for all four diastereoisomers are necessary to compare with the experimental data presented here.

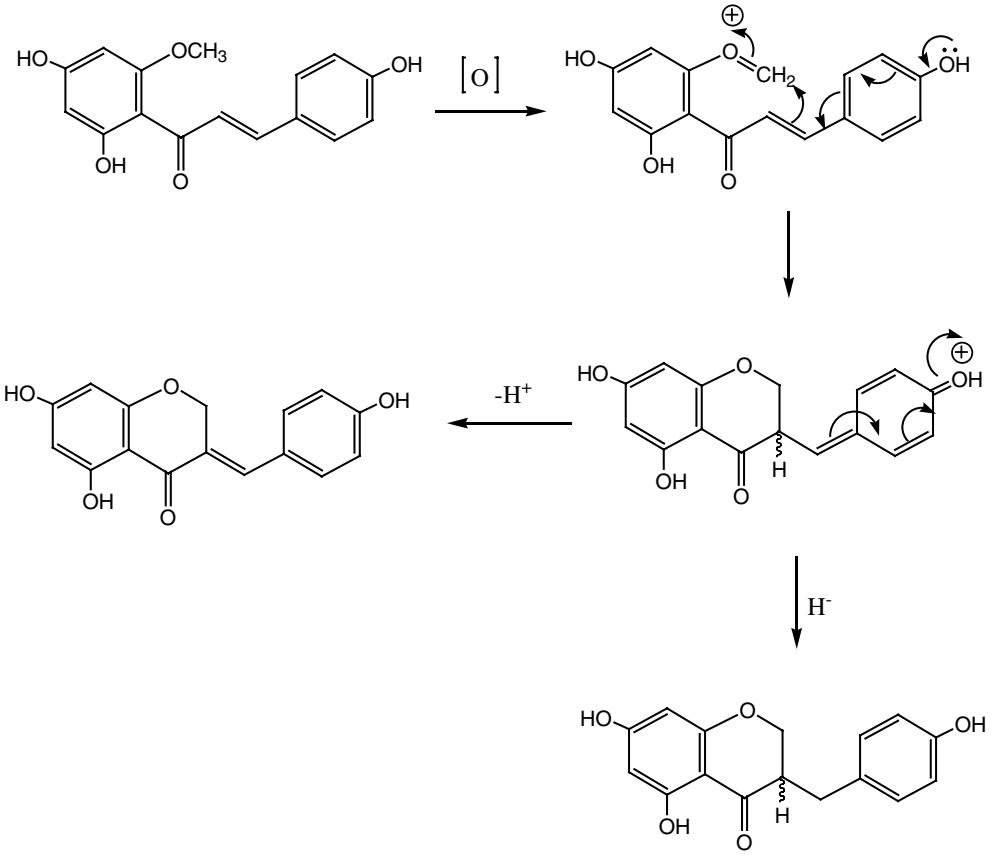
Once the C-3 absolute configuration is determined, the configuration of C-9 could be deduced on the basis of the ease of elimination of its –OH under the very mild conditions (pyr. r.t., E_2 mechanism) to yield **1Ac** as the *E*-configuration product.

Compound **2** was obtained as a white powder. The IR spectrum demonstrated the presence of hydroxyl groups (3465 and 3300 cm^{-1}) and a carbonyl (1638 cm^{-1}) group. The UV absorption at 290 nm was suggestive of a dihydrochalcone skeleton. ^{1}H NMR, ^{13}C NMR, DEPT, HH-COSY and HC-COSY experiments indicated the presence of a structure with the same substitution pattern on rings A and B as compound **1** (Table 1). The ^{1}H NMR spectrum showed a broad singlet at δ 6.50 (OH–Cα') and a set of signals at δ 4.49 (1H, *dd*, $J = 10.7$ and 11.2 Hz), δ 4.23 (1H, *dd*, $J = 11.4$ and 5.1 Hz), δ 3.07 (1H, *ddd*, $J = 4.0$, 5.1 and 10.7 Hz) and $\delta = 5.61$ (1H, *bs*), which strongly suggested that this structure contained a $–CH_2–CH–CH–$ system, as for compound **1**. The doublet at δ 2.53 (1H, $J = 4.1$ Hz) was suggestive of a proton from an aliphatic –OH group. Selective irradiation at δ 2.53 produced a change in the broad singlet at δ 5.61 to give a doublet, and the irradiation at δ 5.61 resulted in a singlet at δ 2.53 and a double doublet at δ 3.07. These results suggest geminal protons at δ 4.49 and δ 4.23 with a coupling constant = 11.4 Hz. Both protons are additionally coupled to the proton at δ 3.07 ($J = 10.7$ and 5.1 Hz, respectively), which in turn is coupled ($J = 4$ Hz) with the proton at δ 5.61, indicating that this broad singlet was actually a triplet. All correlations were confirmed with the HH-COSY experiment.

The ^{13}C NMR and DEPT spectra indicated the presence of 17 carbons (similar to compound **1**) of which six were quaternary aromatic carbons and one was a carbonyl carbon (δ 197.2, C-β') involved in intramolecular hydrogen bond interactions with –OH group. The rest of the signals were: δ 60.8 for $–O\underline{C}H_3$, δ 66.6 for $–\underline{C}H_2O–$, δ 51.4 and δ 69.7 for aliphatic $–\underline{C}H$, δ 94.2 for $=\underline{C}H$ and a set of three characteristic signals for the $=\underline{C}H$ groups of a monosubstituted aromatic system (δ 125.4, 127.8 and 128.5). Correlations obtained with HH-COSY allowed us to build a dihydrochalcone structure with the same substitution pattern than compound **1**, hydroxylated on C-β and hydroxymethylated on C-α. It was identified as 2′,4′,6′-trihydroxy-3′-methoxy-α-hydroxymethyl-β-hydroxy-dihydrochalcone (**2**). The HRFABMS gave a molecular ion at 316, corresponding to the $[M–H_2O]^{+}$.

The isolation of a homoisoflavanone in *P. ferrugineum* constitutes the first report on this type of compound in *Polygonum* genus and Polygonaceae family and could be an important chemotaxonomic finding.

In addition, the structural characteristics of this homoisoflavanone such as the presence of an –OH on C-9 and the lack of substitution on ring B constitute unusual features among the previously reported 3-benzylchroman-4-ones. The latter always bear oxygenated substituents at 3′ or/



Scheme 1a. Proposed biosynthetic routes from 2′-methoxy-4′,6′-dihydroxy-4-alcoxy chalcones to 3-benzyl or 3-benzylidene chroman-4-one derivatives (Dewick, 1975).

and 4′ positions (Likhitwitayawuid et al., 2002; Corsaro et al., 1992; Silayo et al., 1999), and do not possess a OH group at C-9 but sometimes have one at C-3 (Likhitwitayawuid et al., 2002; Corsaro et al., 1992; Crouch et al., 1999).

The appearance of a different substitution pattern of 3-benzylchroman-4-one skeleton is interesting from the point of view of the biosynthesis of homoisoflavanones.

It is clear that the compound reported here do not proceed via the same biosynthetic pathway previously proposed for 4′ oxygenated homoisoflavanones (Dewick, 1975), since the hydroxyl group (or its methoxy derivative) is postulated to take part in the formation of the C-ring (Scheme 1a). Without the C-4′-OR, a different mechanism would be required. Based on the whole mechanism proposed by Dewick, that necessarily involves a 2′-OMe chalcone for homoisoflavonoid biosynthesis, a possible alternative mechanism, which accounts for the presence of a 9-OH group when ring B is unsubstituted, is showed in Scheme 1b. This biosynthetic route is supported by the fact that the flavanone derivative of the precursor 2′,4′-dihydroxy-3′,6′-dimethoxychalcone (5,8-dimethoxy-7-hydroxyflavanone **7**), was found in the same extract from which the homoisoflavanone was isolated (see Section 4).

3. Concluding remarks

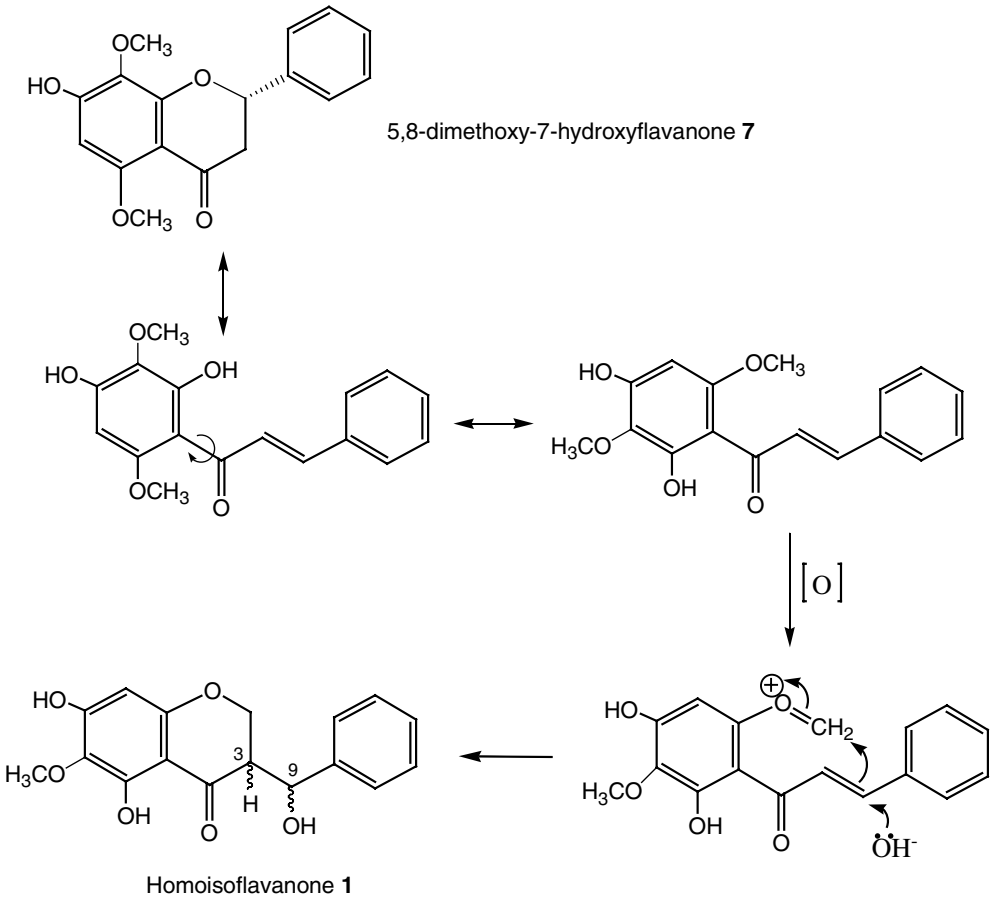
It is interesting to note that the lack of substituents on ring B in homoisoflavanone **1** and compound **2** is in contrast with the oxygenated ring B which is always found in the homoisoflavanones previously described in Hyacinthaceae. Nevertheless, this feature appears to be characteristic of *P. ferrugineum*, since all other related compounds isolated along with compounds **1** and **2** have a non-substituted ring B. This is in agreement with the isolation of flavonoid-type compounds without substituents on ring B from *Polygonum* genus, for example 6,7-dimethoxy-5-hydroxyflavanone (onysilin) from *P. stagninum* (Datta et al., 2002).

Since flavonoids have been previously used as chemotaxonomic markers in *Polygonum* genus, the appearance of homoisoflavanones added to their structural characteristics, opens a new field for future research.

4. Experimental

4.1. General experimental procedures

Plant material was powdered in a Fristzch Pulverisette 15 mill (Germany). Extraction was performed with a Hei-



Scheme 1b. Possible biosynthetic route for homoisoflavanone **1** from the 2′,4′-dihydroxy-3′,6′-dimethoxychalcone, structure in equilibrium with 5,8-dimethoxy-7-hydroxyflavanone **7**.

dolph RZR 50 shaker (Germany). Solvents were purchased from Ciccarelli (San Lorenzo, Argentina) or Anedra (San Fernando, Argentina) and purified before use. Dimethyl amino pyridine (DMAP) was from Fluka (USA). Extracts were concentrated in a rotary evaporator Büchi R-205 (Flawil, Switzerland). The aqueous sub-extract was lyophilized in a Labconco Freeze Dry System 79340 (Kansas, MO). Silica-gel 60 H for CC and pre-coated silica gel 60 F_{254} used for TLC were from Merck (Buenos Aires). NMR spectra were recorded in $CDCl_3$ (Aldrich, St Louis, MO) or in $CDCl_3$+DMSO-d_6 and TMS (Aldrich) as internal standard on a Bruker 200. IR spectra were run on a Hitachi (FT-IR) apparatus. Optical rotations were recorded on a Jasco DIP-1000 2.3.00 using the 589 nm line of sodium lamp. Melting points were obtained in an Ernst Leitz Wetzlar apparatus and are uncorr. All gas chromatograms associated with the mass detector were obtained in a CG-MS Turbo Mass Perkin–Elmer, column PE1 30 m × 0.25 mm of inner diameter, film 0.1 μ, ionization energy 70 eV. HRFAB mass spectra were recorded in a VG-ZAB (100–2600) apparatus, MALDI were recorded in a DE-STR in the Mass Spectrometry Laboratory from University of California, Riverside. UV–Vis spectra were recorded on a Beckman DU-640 spectrophotometer. CD spectra were recorded on a Jasco Model J-700 Spectropolarimeter.

4.2. Plant material

P. ferrugineum Wedd. (Polygonaceae) was collected at Puerto Gaboto, Santa Fe province, Argentina in December 2002. A *voucher specimen* was deposited in the Herbarium of the Vegetal Biology Area of the National University of Rosario (UNR 99).

4.3. Extraction and isolation

Air-dried powdered leaves of *P. ferrugineum* (1 kg) were extracted with MeOH (3 × 1.5 l) at room temp. After evaporation in vacuo, the MeOH residue (101.22 g) was re-suspended in MeOH/H_2O (70:30) and successively partitioned with *n*-hexane (Hex), CH_2Cl_2, EtOAc and *n*-BuOH, yielding after evaporation a Hex (25.25 g), a CH_2Cl_2 (17.84 g), an EtOAc (6.12 g), a *n*-BuOH (46.13 g) and an aqueous (5.00 g) extract, respectively.

The Hex extract was initially subjected to vacuum liquid chromatography (VLC) on silica gel ($CHCl_3$–EtOAc gradient/Me_2CO/MeOH) to give 13 fractions (1–13). Fraction 1 was fractionated by repeated column chromatography (CC) over silica gel (*n*-Hex–EtOAc gradient) to afford 5-hydroxy-7-methoxyflavanone (pinostrobin) (**6**) (Burke and Nair, 1986) and 2′-hydroxy-4′,6′-dimethoxychalcone (flavokawin B) (**4**) (Ahmed et al., 1981) (100 and 40 mg, respectively).

The CH_2Cl_2 extract was also subjected to VLC on silica gel ($CHCl_3$–EtOAc gradient/Me_2CO/MeOH) to give 13 fractions (I–XIII). Fraction IV was separated by CC (Hex–EtOAc and EtOAc–MeOH gradient) to give Iva–IVn fractions. Fraction IVd was subjected to repeated CC (Hex–EtOAc/MeOH) to afford 2′,6′-dihydroxy-3′,4′-dimethoxychalcone (pashanone) (**3**) (90 mg) (Ramakrishnan et al., 1974). Fraction IVg was purified by CC under identical conditions yielding 2′,4′-dihydroxy-6′-methoxychalcone (cardamonin or alpinetin chalcone) (30 mg) (**5**) (Krishna and Chaganty, 1972). Fraction IVh was subjected to CC (Hex–EtOAc/EtOAc–MeOH/MeOH) and prep. TLC over silica gel (Cl_3CH–EtOAc–HCO_2H 90:10:1 as eluent × 3) to obtain 5,7-dihydroxy-6-methoxy-3-(9-hydroxyphenylmethyl)-chroman-4-one (33 mg) (**1**), a new compound which was named homoferrugenone and 2′,4′,6′-trihydroxy-3′-methoxy-α-hydroxymethyl-β-hydroxy-dihydrochalcone (8 mg) (**2**), also a new compound named homoferrugendihydrochalcone. In addition, fraction IV was dissolved in Et_2O and cooled overnight to give 5,8-dimethoxy-7-hydroxyflavanone (11 mg) (**7**) (Bratoeff and Pérez-Amador, 1994).

4.3.1. (−)-5,7-Dihydroxy-6-methoxy-3-(9-hydroxyphenylmethyl)-chroman-4-one (*1*)

White needles; mp 157–159 °C; $[\alpha]_D$ −8.67 (CH_2Cl_2; *c* 0.365); IR (KBr) ν_{max} 3465, 3300, 2991, 2946, 1638, 1443, 743 cm^{-1}; UV (MeOH) λ_{max} (log ε): 301 (4.24), (+$AlCl_3$) 313, (+NaOMe) 329, (+NaOAc) 329; CD (MeOH, $c = 3.95 \times 10^{-5}$ M) $[\theta]_{327}$ 0, $[\theta]_{315}$ − 4136, $[\theta]_{307}$ 0, $[\theta]_{290}$ + 23,604, $[\theta]_{260}$ + 1640, $[\theta]_{255}$ 0; for ^{1}H NMR (200 MHz, $CDCl_3$–DMSO-d_6/TMS) and ^{13}C NMR (50 MHz, $CDCl_3$–DMSO-d_6/TMS), see Table 1; HRMS MALDI *m*/*z* 339.0844 (calcd. for $C_{17}H_{16}O_6Na$, 339.0839).

4.3.2. (−)-2′,4′,6′-Trihydroxy-3′-methoxy-α-hydroxymethyl-β-hydroxy-dihydrochalcone (*2*)

White powder; $C_{17}H_{18}O_7$, $[\alpha]_D$ −6.1 ($CHCl_3$; *c* 0.250); UV (MeOH) λ_{max} (log ε): 290 (4.54); IR (KBr) ν_{max} 3465, 3300, 2991, 2946, 1638, 1443, 743 cm^{-1}; for ^{1}H NMR (200 MHz, $CDCl_3$–DMSO-d_6/TMS) and ^{13}C NMR (50 MHz, $CDCl_3$–DMSO-d_6/TMS), see Table 1. HRFABMS *m*/*z* 316.0941 (calcd. for $C_{17}H_{16}O_6$, 316.0947), $[M–H_2O]^+$.

4.4. Acetylation of (*1*)

Compound **1** (11.5 mg), dry pyridine (0.35 ml), Ac_2O (56 μl, 0.6 mmol) and DMAP (0.87 mg, 0.007 mmol) were stirred at room temperature for 48 h under N_2. The mixture was then diluted in EtOAc and successively washed with HCl 0.1 N, $NaHCO_3$ 10% P/V and brine. 5,7-Diacetyl-6-methoxy-3-benzylidene-chroman-4-one (**1Ac**) (8.5 mg) was obtained after crystallization from Hex:EtOAc (87.5:12.5)..

4.4.1. (E)-5,7-diacetyl-6-methoxy-3-benzylidene-chroman-4-one (*1Ac*)

White needles; mp 139–141 °C; ^{1}H NMR (200 MHz, $CDCl_3$–DMSO-d_6/TMS): δ 2.33 (3H, *s*, –CH_3), δ 2.45 (3H, *s*, –CH_3), δ 3.78 (3H, *s*, –OCH_3), δ 5.26 (1H, *s*, H-2), δ 5.27 (1H, *s*, H-2), δ 6.68 (1H, *s*, H-8),δ 7.27–7.49

(5H, *m*, H-2′, H-3′, H-4′, H-5′, H-6′), δ 7.80 (1H, *s*, H-9); ^{13}C NMR (50 MHz, $CDCl_3$–DMSO-d_6/TMS): δ 20.4 (–CH_3), δ 20.8 (–CH_3), δ 61.4 (–OCH_3), δ 67.4 (C-2), δ 109.9 (C-8), δ 113.7 (C-4a), δ 128.5 (C-2′, C-6′), δ 129.4 (C-3′, C-5′), δ 129.7 (C-4′), δ 130.4 (C-3), δ 134.0 (C-1′), δ 137.7 (C-9), δ 139.8 (C-6), δ 144.3 (C-7), δ 149.6 (C-5), δ 157.6 (C-8a), δ 167.7 (C═O), δ 168.8 (C═O), δ 179.7 (C═O). HRFABMS [MH^+] at 383.1115 (calcd. for $C_{21}H_{19}O_7$, 383.1130).

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