PERGAMON

Phytochemistry 55 (2000) 823–832

PHYTOCHEMISTRY

www.elsevier.com/locate/phytochem

Bi- and tetraflavonoids from *Aristolochia ridicula*

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Received 3 January 2000; received in revised form 5 April 2000

Abstract

From stems of *Aristolochia ridicula*, two biflavones, four unusual chalcone-flavone dimers and one tetraflavonoid were isolated. The structures of the seven compounds were elucidated by spectroscopic methods.

Keywords: *Aristolochia ridicula*; Aristolochiaceae; Flavonoids; Biflavonoid, tetraflavonoid

1. Introduction

In our continuing research on the Aristolochiacea family, particularly on *Aristolochia genus* (Bomm, Zukerman-Schpector and Lopes, 1999; Nascimento and Lopes, 1999), we have examined the constituents of stems of *Aristolochia ridicula* Brown. To our knowledge, no phytochemical investigation has been carried out on this species. This paper deals with the isolation and structure determination of two biflavones, four rearranged chalcone-flavone dimers and one tetraflavonoid from the acetone extract.

Although several flavonoids, such as flavonols and dihydroflavonols, have been isolated from species belonging to the Aristolochiaceae family (Lopes et al., 1991), this is the first report of bi- and tetraflavonoids in this family.

2. Results and discussion

Compounds **1–7** were isolated by repeated precipitation procedures from the acetone extract of stems and purified by preparative HPLC.

The ^{13}C NMR spectra of compounds **1** and **2** showed a total of 31 signals, two of which were clearly twice the intensity of the others, giving 33 carbons in each molecule. The ESI-MS of both compounds displayed a *quasi*-molecular ion $[M+H]^+$ at m/z 597, consistent with the molecular formula $C_{33}H_{24}O_{11}$. The ^1H and ^{13}C NMR spectra of **1** and **2** (Tables 1 and 2) were very similar. They showed the presence of three aromatic methoxyl groups, and 1,4-disubstituted (B ring) and 1,3,4-trisubstituted aromatic rings (B' ring). In addition, tetrasubstituted (A ring) and pentasubstituted (A′ ring) rings with alternating oxygenation patterns were observed. The ^1H and ^{13}C NMR spectra of **1** showed signals for two hydrogen-bonded hydroxy groups at δ 13.22 (OH-5 and OH-5″) and two carbonyl carbons at δ 182.7 (C-4 and C-4″), whereas the spectra of **2** showed only one hydrogen-bonded hydroxy group (OH-5″ at δ 12.90) and two carbonyl carbons at δ 182.0 (C-4″) and 174.5 (C-4). Both compounds showed additional signals reminiscent of three tetrasubstituted sp^2 carbons and one trisubstituted sp^2 carbon (**1**: δ 103.9, **2**: δ 103.6, CH-3″). These results suggested biflavone structures for these compounds in which the monomer units should be linked through positions C-3 and C-6″ or C-8″, since the pentasubstituted aromatic ring contains two non-oxygenated quaternary carbons (Harborne and Mabry, 1982; Harborne, 1993). The analyses of NOESY, ^1H-^1H COSY, HMQC, and HMBC spectra of both compounds allowed the precise assignments of all hydrogens and carbons (Tables 1 and 2). The HMBC spectra of these compounds showed correlations between C-2″ (**1**: δ 164.4 and **2**: δ 163.4) and H-2‴ (**1**: δ 7.60, **2**: δ 7.24), H-6‴ (**1**: δ 7.57, **2**: δ 7.30) and H-3″ (**1**: δ 6.64, **2**: δ 6.42), showing that the dioxygenated aromatic ring is located on unit II of these biflavones. In addition, the correlations observed between hydrogen-bonded OH-5″ and C-5″, C-6″ and C-10″, and that between C-10″ and H-3″, allowed us to establish that the two flavonoid units were

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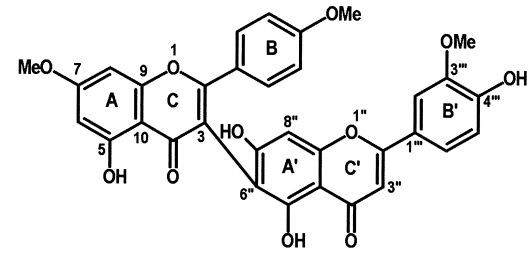
0031-9422/00/$ - see front matter
PII: S0031-9422(00)00293-4



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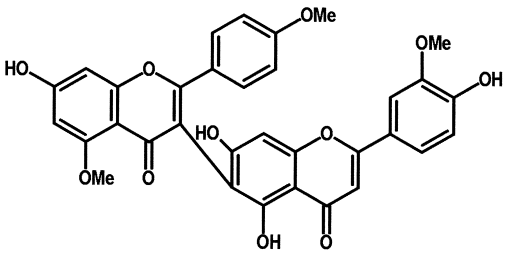

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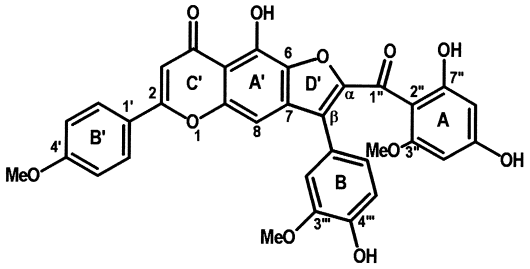

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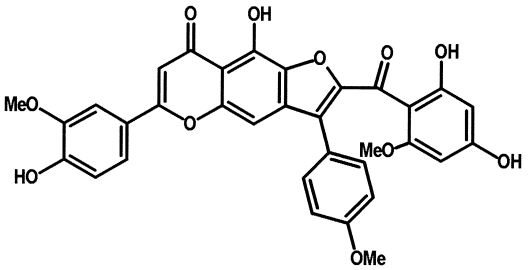

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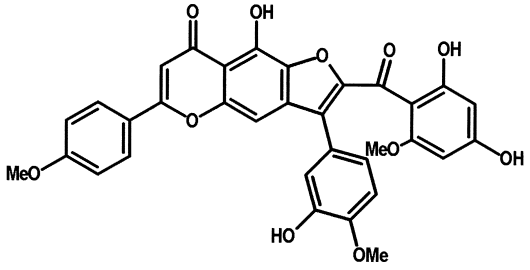

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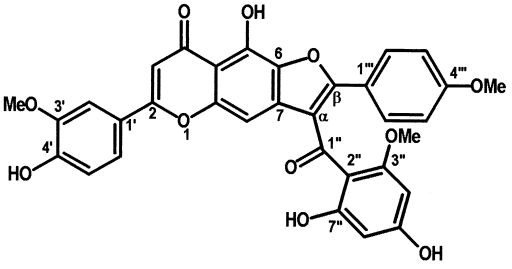

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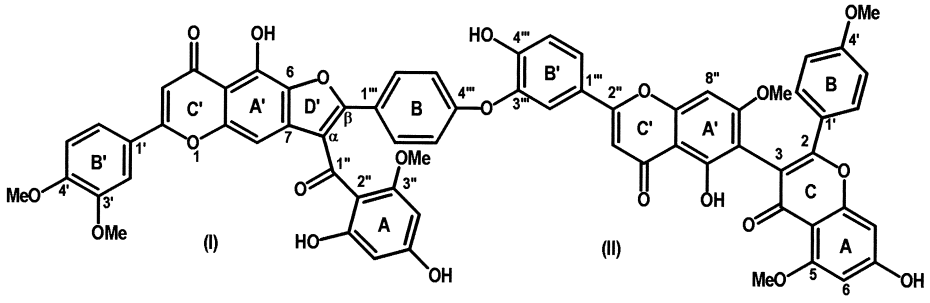

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linked by a bond involving C-3 and C-6″. Thus, C-5″ carried the only hydrogen-bonded hydroxy group of biflavone **2** and C-5 carried a methoxy group (δ 3.65), which was confirmed through observation of its NOE interaction with H-6 (δ 6.27). NOESY and gNOESY experiments confirmed the position of the other methoxy groups, leading to the conclusion that compounds **1** and **2** differ by the position of the substituents at C-5 and C-7 in the A ring. The biflavone structures were corroborated by their mass spectra, which displayed ions at m/z 189 and 167, characterizing the A ring, and at m/z 430 arising from retro Diels–Alder rearrangements involving the C ring.

Isomers **3–6** were also suggested to be biflavonoids ($C_{33}H_{24}O_{11}$) by ESI-MS spectra, since they displayed a *quasi*-molecular ion $[M+H]^+$ at m/z 597, and their ^{13}C NMR spectra showed a total of 31 signals. Two of these signals were clearly twice the intensity of the others, suggesting the presence of a 1,4-disubstituted aromatic ring. The ^{1}H-^{13}C COSY and DEPT spectra showed 30

Table 1
^{13}C NMR spectral data for compounds **1** and **2**[a,b]

C	**1**[c,e]		**2**[d,e]	
	^{13}C (δ)	HMBC	^{13}C (δ)	HMBC
2	160.8 (*s*)	H-2′, H-6′	160.8 (*s*)	H-2′, H-6′
3	114.8 (*s*)		113.5 (*s*)	
4	182.7 (*s*)		174.5 (*s*)	
5	160.2 (*s*)	H-6, OH-5	160.7 (*s*)	OMe-5
6	95.5 (*d*)	H-8	95.1 (*d*)	H-8
7	162.0 (*s*)	OMe-7	162.3 (*s*)	H-6
8	96.8 (*d*)	H-6	96.7 (*d*)	H-6
9	160.5 (*s*)		162.7 (*s*)	
10	107.9 (*s*)	H-6, H-8	106.7 (*s*)	H-8
1′	126.3 (*s*)	H-3′, H-5′	125.4 (*s*)	H-3′, H-5′
2′	130.1 (*d*)	H-6′	129.5 (*d*)	H-6′
3′	113.9 (*d*)	H-5′	113.9 (*d*)	H-5′
4′	161.6 (*s*)	H-2′, H-6′	160.3 (*s*)	H-2′, H-6′
5′	113.9 (*d*)	H-3′	113.9 (*d*)	H-3′
6′	130.1 (*d*)	H-2′	129.5 (*d*)	H-2′
2″	164.4 (*s*)	H-2‴, H-3″,H-6‴	163.4 (*s*)	H-2‴, H-3″, H-6‴
3′	103.9 (*d*)		103.6 (*d*)	
4″	182.7 (*s*)		182.0 (*s*)	
5″	160.8 (*s*)	OH-5″	159.6 (*s*)	OH-5″
6″	106.9 (*s*)	OH-5″, H-8″	106.2 (*s*)	OH-5″, H-8″
7″	163.1[e] (*s*)	H-8″	163.9[e] (*s*)	H-8″
8″	94.4 (*d*)		93.8 (*d*)	
9″	157.2[e] (*s*)	H-8″	156.8[e] (*s*)	H-8″
10″	104.0 (*s*)	H-3″, OH-5″, H-8″	103.5 (*s*)	H-3″, OH-5″, H-8″
1‴	123.2 (*s*)	H-5‴	120.7 (*s*)	H-5‴
2‴	110.1 (*d*)	H-6‴	110.3 (*d*)	
3‴	148.5 (*s*)	H-5‴	148.3 (*s*)	OMe-3‴, H-5‴
4‴	151.0 (*s*)	H-6‴, H-2‴	151.0 (*s*)	H-6‴, H-2‴
5‴	115.9 (*d*)		116.1 (*d*)	
6‴	120.9 (*d*)	H-2‴	121.8 (*d*)	H-2‴
OMe-3‴	56.2 (*q*)		56.2 (*q*)	
OMe-4′	55.3 (*q*)		56.1 (*q*)	
OMe-5			55.5 (*q*)	
OMe-7	56.0 (*q*)			

[a] Multiplicities were established by DEPT experiments.
[b] Signals assigned by means of 2D-NMR experiments.
[c] Recorded in acetone-d_6, 126 MHz.
[d] Recorded in DMSO-d_6–$CDCl_3$ (1:1), 50 MHz.
[e] Assignments may be interchangeable within the same column.

sp^2 carbons with 13 carbons bearing an oxygen atom, three of which were linked to *O*-methyl groups while two were carbonyl groups. Except for three *O*-methyl groups, there were no signals below δ 90.0, confirming the absence of aliphatic carbons (Table 3).

The ^1H and ^{13}C NMR spectra showed that the four compounds have two hydrogen-bonded hydroxyl-carbonyl groups (**3**: δ_H 13.53, δ_{CO} 192.2, 183.4; **4**: δ_H 13.47, 13.50, δ_{CO} 192.2, 183.7; **5**: δ_H 13.51, 13.54, δ_{CO} 192.0, 183.3; **6**: δ_H 13.51, 13.70, δ_{CO} 192.2, 183.7) (Tables 3 and 4). These chemical shifts are characteristic of chalcone and flavone (Harborne and Mabry, 1982; Drewes et al., 1987; Tih et al., 1989; Harborne, 1993). However, no signals reminiscent of the vinyl system (C-α, C-β) of chalcones were observed, which suggested the formation of an additional ring involving the chalcone moiety.

One of the main ions observed by ESI-MS of compounds **3–6**, at *m*/*z* 457, should originate from the loss of the fragment ion at *m*/*z* 139 (A ring of the chalcone moiety). In addition, the ions at *m*/*z* 123 and 107 characterized the 3-methoxy-4-hydroxy trisubstituted and 4-methoxy disubstituted rings, respectively.

From a detailed analysis of ^1H-^1H COSY, ^1H-^{13}C COSY, long-range ^1H-^{13}C COSY and HMBC spectra of each compound, the aromatic protons were shown to belong to one mono-oxygenated *p*-disubstituted, one 3,4-dioxygenated trisubstituted, and one trioxygenated tetrasubstituted ring with alternating oxy-substituents, as expected for the A ring of flavonoids. Moreover, these compounds presented a pentasubstituted aromatic ring with the unusual 5,6-dioxygenation pattern for the A ring of flavonoids in which the only aromatic proton

Table 2
^{1}H NMR spectral data for compounds **1** and **2**

H	**1**[a]		**2**[b]	
	(δ H, *J* in Hz)	gNOESY	(δ H, *J* in Hz)	NOESY
6	6.54 (*d*, *J*=2.0)	OH-5	6.27 (*d*, *J*=1.5)	OMe-5
8	6.46 (*d*, *J*=2.0)	OMe-7	6.40 (*d*, *J*=1.5)	
2′	7.55 (*d*, *J*=9.0)	H-3′	7.41 (*d*, *J*=7.9)	H-3′
3′	6.85 (*d*, *J*=9.0)	H-2′, H-8″, OMe-4′	6.68(*d*, *J*=7.9)	H-2′, H-8″, OMe-4′
5′	6.85 (*d*, *J*=9.0)	H-6′, H-8″, OMe-4′	6.68(*d*, *J*=7.9)	H-6′, H-8″, OMe-4′
6′	7.55 (*d*, *J*=9.0)	H-5′	7.41 (*d*, *J*=7.9)	H-5′
3″	6.64 (*s*)	H-2‴, H-6‴	6.42 (*s*)	H-2‴, H-6‴
8″	6.58 (*s*)	H-3′, H-5′	6.42 (*s*)	H-3′, H-5′
2‴	7.60 (*d*, *J*=1.5)	H-3″, OMe-3‴	7.24 (*d*, *J*=2.1)	H-3″, OMe-3‴
5‴	7.00 (*d*, *J*=8.0)	H-6‴	6.88 (*d*, *J*=8.6)	H-6‴
6‴	7.57 (*dd*, *J*=8.0, 1.5)	H-5‴	7.30 (*dd*, *J*=8.6, 2.1)	H-5‴
OMe-3‴	3.97 (*s*)	H-2‴	3.85 (*s*)	H-2‴
OMe-4′	3.75 (*s*)	H-3′, H-5′	3.75 (*s*)	H-3′, H-5′
OMe-5			3.65 (*s*)	H-6
OMe-7	3.82 (*s*)	H-8		
OH-5	13.22 (*s*)	H-6		
OH-5″	13.22 (*s*)		12.90 (*s*)	

[a] Recorded in acetone-d_6, 500 MHz.
[b] Recorded in DMSO-d_6–$CDCl_3$ (1:1), 200 MHz.

(**3**: δ 7.22, **4**: δ 7.14; **5**: 7.22; **6**: 7.53) appeared at a lower field than those observed for flavones **1** and **2**, as a result of the lack of the oxy-substituent on C-7. The two remaining sp^2 carbons were assigned to C-α and C-β of the benzofuran ring (A′–D′) in each compound. Analysis of ^{1}H-^{13}C COSY, HMBC and NOESY spectra allowed us to establish the substituents on these rings. The HMBC spectra showed correlations of C-10 with H-3, and with the hydrogen-bonded hydroxy group (OH-5). In addition, the correlations between C-1′ and H-3, as well as between C-2 and H-2′, H-6′ indicated that the mono-oxygenated aromatic ring was linked to the flavone moiety in compounds **3** and **5**. In compounds **4** and **6**, analogous correlations led us to place the dioxygenated aromatic ring at the flavone moiety. These observations allowed us to locate the remaining dioxygenated aromatic ring of compounds **3** and **5** and the monooxygenated aromatic ring of compounds **4** and **6** on the chalcone unit of these biflavonoids. The hydrogen-bonded hydroxy proton (OH-5) also showed correlations with C-5, C-10 and C-6. H-8 showed correlations with C-6, C-7, C-9 and C-10 in the spectra of compounds **3**–**6**, establishing that the two flavonoid units of these compounds were linked by C-6, C-7, C-α and C-β.

The correlation between H-8 and H-2‴, H-6‴ shown by NOESY experiment confirmed the structural moiety C-8–C-7–C-β–C-1″ for biflavonoids **3**–**5**. The NOESY spectra of **3**–**5** were consistent with the methoxy groups at C-3″, C-3‴, and C-4′, at C-3′, C-3″, and C-4‴, and at C-3″, C-4′, and C-4‴, respectively (Figs. 1 and 2). The methoxy groups resonating at ∼δ 3.20 were placed at C-3″, i.e. under anisotropic shielding from the B ring of the chalcone moiety. These assignments were confirmed by weak NOEs observed between the methoxy protons OMe-3″ and H-2‴, H-6‴. Furthermore, the HMBC correlation between H-8 and C-β observed for compounds **3**–**5** corroborated the unusual oxygenation pattern of the A' ring of these compounds and established the linkages C-β–C-7 and C-α–O–C-6 between the chalcone and flavone moieties for these compounds.

The ^{1}H and ^{13}C NMR spectral data for compounds **3**–**5** were very similar. Compound **3** differed from **5** only by substituent positions at C-3‴ and C-4‴, whereas **4** differed from **3** by aromatic substituents at C-2 (B′ ring) and C-β (B ring), which were reversed.

The ^{1}H and ^{13}C NMR spectra of compound **6** proved to be similar to those of compound **4** with the exception of a singlet at δ 7.53, which showed a correlation with the signal at δ 91.0, as depicted on the HMQC spectrum. These signals were therefore assigned to H-8 and C-8, respectively. The deshielding of the H-8 signal in compound **6**, compared to those in compounds **3**–**5**, was assumed to be due to its location within the deshielding cone of the carbonyl group of the chalcone unit. This assumption led us to propose an inverse mode of linkage between the flavone and chalcone moieties for compound **6**, i.e. through bonds C-α–C-7 and C-β–O–C-6. The observation of the signal for MeO-3″ (δ 3.18) at a higher field than expected for an aromatic methoxyl group was due to anisotropic shielding by the B ring of the chalcone moiety, as in compounds **3**–**5**.

Comparing the effect of the addition of shift reagents on UV absorption bands of dimeric compounds **1**–**6** with those reported for chalcone and flavone monomers (Markham, 1982), we could infer that compounds **3**–**6** did not react as chalcones. This is consistent with the

Table 3
^{13}C NMR spectral data for compounds **3**–**6**[a]

C	**3**[b,d,e]		**4**[c,d,e]		**5**[b,d,e]		**6**[b]
	^{13}C (δ)	HMBC	^{13}C (δ)	HMBC	^{13}C (δ)	HMBC	^{13}C (δ)
2	164.7 (*s*)	H-3, H-2′, H-6′	164.9 (*s*)	H-3, H-2′, H-6′	164.7 (*s*)	H-3, H-2′, H-6′	164.9 (*s*)
3	102.5 (*d*)		102.8 (*d*)		102.5 (*d*)		102.8 (*d*)
4	183.4 (*s*)	H-3	183.7 (*s*)	H-3	183.3 (*s*)	H-3	183.7 (*s*)
5	153.5 (*s*)	OH-5	153.7 (*s*)	OH-5	153.5 (*s*)	OH-5	153.7 (*s*)
6	156.6[e] (*s*)	H-8	156.6[e] (*s*)	H-8	156.6[e] (*s*)	H-8	156.6[e] (*s*)
7	113.1 (*s*)	H-8	113.5 (*s*)	H-8	113.1 (*s*)	H-8	113.0 (*s*)
8	90.2 (*d*)		91.0 (*d*)		90.1 (*d*)		91.0 (*d*)
9	154.2[e] (*s*)	H-8	154.5[e] (*s*)	H-8	154.2[e] (*s*)	H-8	154.0[e] (*s*)
10	105.5 (*s*)	H-8, OH-5, H-3	105.6 (*s*)	H-8, OH-5, H-3	105.5 (*s*)	H-8, OH-5, H-3	105.6 (*s*)
1′	121.5 (*s*)	H-3′, H-5′	122.5 (*s*)	H-5′	121.5 (*s*)	H-3′, H-5′	121.3 (*s*)
2′	127.6 (*d*)	H-6′	110.6 (*d*)	H-6′	127.5 (*d*)	H-6′	110.6 (*d*)
3′	114.1 (*d*)	H-5′	147.8 (*s*)	H-5′, OMe-3′	114.1 (*d*)	H-5′	148.3 (*s*)
4′	159.9 (*s*)	H-2′, H-6′, OMe-4′	151.3 (*s*)	H-2′, H-6′	159.9 (*s*)	H-2′, H-6′, OMe-4′	150.9 (*s*)
5′	114.1 (*d*)	H-3′	116.1 (*d*)		114.1 (*d*)	H-3′	116.1 (*d*)
6′	127.6 (*d*)	H-2′	120.6 (*d*)	H-2′	127.5 (*d*)	H-2′	121.6 (*d*)
α	163.3 (*s*)		160.0 (*s*)		164.0 (*s*)	H-2‴	151.3 (*s*)
β	151.0 (*s*)	H-8	151.8 (*s*)	H-8, H-2‴, H-6‴	151.0 (*s*)	H-8, H-2‴	160.0 (*s*)
1″	192.2 (*s*)		192.2 (*s*)		192.0 (*s*)		192.2 (*s*)
2″	106.4 (*s*)	H-4″, H-6″, OH-7″	106.4 (*s*)	H-4″, H-6″, OH-7″	106.4 (*s*)	H-4″, H-6″, OH-7″	106.5 (*s*)
3″	163.6 (*s*)	OMe-3″	163.9 (*s*)	OMe-3″	163.5 (*s*)	OMe-3″	163.9 (*s*)
4″	91.3 (*d*)	H-6″	92.0 (*d*)	H-6″	91.3 (*d*)	H-6″	92.0 (*d*)
5″	166.6 (*s*)		166.6 (*s*)		166.6 (*s*)		166.8 (*s*)
6″	95.9 (*d*)	H-4″, OH-7″	96.2 (*d*)	H-4″, OH-7″	95.9 (*d*)	H-4″, OH-7″	96.0 (*d*)
7″	167.1 (*s*)		168.0 (*s*)	OH-7″	167.0 (*s*)	OH-7″	167.0 (*s*)
1‴	121.7 (*s*)	H-5‴	121.6 (*s*)	H-3‴, H-5‴	121.7 (*s*)	H-5‴	120.2 (*s*)
2‴	109.5 (*d*)	H-6‴	128.0 (*d*)	H-6‴	109.6 (*d*)	H-6‴	127.8 (*d*)
3‴	147.6 (*s*)	OMe-3‴	115.1 (*d*)	H-5‴	147.8 (*s*)	H-5‴	114.9 (*d*)
4‴	150.7 (*s*)	H-2‴, H-6‴	161.0 (*s*)	H-2‴, H-6‴	150.7 (*s*)	H-2‴, H-6‴	160.4 (*s*)
5‴	115.7 (*d*)		115.1 (*d*)	H-3‴	115.8 (*d*)		114.9 (*d*)
6‴	120.5 (*d*)	H-2‴	128.0 (*d*)	H-2‴	120.4 (*d*)	H-2‴	127.8 (*d*)
OMe-3‴	55.8 (*q*)		55.5 (*q*)				
OMe-3″	55.2 (*q*)		56.2 (*q*)		55.3 (*q*)		56.2 (*q*)
OMe-3′							56.0 (*q*)
OMe-4′	55.0 (*q*)		55.0 (*q*)		55.0 (*q*)		
OMe-4‴					55.8 (*q*)		55.5 (*q*)

[a] Multiplicities were established by DEPT experiments.
[b] Recorded in DMSO-d_6–$CDCl_3$ (1:1), 50 MHz.
[c] Recorded in DMSO-d_6–$CDCl_3$ (1:1), 100 MHz.
[d] Signals assigned by means of 2D NMR experiments.
[e] Assignments may be interchangeable within the same column.

NMR spectral data (mainly NOESY) previously discussed. However, compounds **1**–**6** presented characteristics of flavone monomers. Compounds **1** and **3**–**6** showed UV absorption shifts of band I ($\lambda \cong 345$ nm) with the addition of $AlCl_3$/HCl, indicating 5-OH flavone units. After addition of NaOAc to standard solutions, the UV curves of compounds **1**, **2**, **4** and **6** exhibited shifts of bands I due to the presence of a 4′-OH on the flavone unit (B ring). The presence of a 7-OH flavone moiety in **2** was confirmed by an extra absorption band ($\lambda \cong 385$ nm), as well as by a shift of the absorption band II ($\lambda = 206$ nm, $\Delta\lambda = +6$ nm) upon the addition of NaOAc to standard solution.

All six dimers exhibited optical activity. The rotation around the carbon–carbon interflavonyl bond between C-3 and C-6″ could be sterically hindered for compounds **1** and **2**, leading to atropisomerism (Seeger et al., 1995). The same effect could explain the optical activity of **3** and **5**, since the rotations of the substituents at C-α, C-β were not free, as previously indicated by strong NOESY interactions of MeO-3″ with H-6‴ and of H-8 with H-2‴ (Figs. 1 and 2).

Compound **7** was suggested to be a tetraflavonoid, since its ESI-MS displayed an $[M+H]^+$ at *m*/*z* 1175, and ions at *m*/*z* 599 $[M+H+Na]^{++}$ and *m*/*z* 595 (dimer units). In addition, its ^1H and ^{13}C NMR spectra as well as HMQC showed almost the same signals as those observed in the spectra of compounds **2** and **6**.

Opening of the C′ ring by β H-3 transfer (McLafferty, 1980) to O–C-9 could explain the formation of a base

Table 4
^{1}H NMR spectral data for compounds **3**–**6** (δ, J in Hz)

H	**3**[a]	**4**[b]	**5**[a]	**6**[a]
3	6.65 (*s*)	6.56 (*s*)	6.65 (*s*)	6.96 (*s*)
8	7.22 (*s*)	7.14 (*s*)	7.22 (*s*)	7.53 (*s*)
2′	7.59 (*d*, J=8.3)	7.37 (*d*, J=2.0)	7.57 (*d*, J=8.3)	7.60 (*d*, J=2.0)
3′	6.89 (*d*, J=8.3)		6.89 (*d*, J=8.3)	
5′	6.89 (*d*, J=8.3)	6.96 (*d*, J=8.3)	6.89 (*d*, J=8.3)	6.94 (*d*, J=8.0)
6′	7.59 (*d*, J=8.3)	7.43 (*dd*, J=8.3, 2.0)	7.57 (*d*, J=8.3)	7.63 (*dd*, J=8.0, 2.0)
4″	5.76 (*d*, J=2.0)	5.73 (*d*, J=2.0)	5.76 (*d*, J=2.0)	5.83 (*d*, J=2.0)
6″	6.00 (*d*, J=2.0)	6.00 (*d*, J=2.0)	5.99 (*d*, J=2.0)	5.97 (*d*, J=2.0)
2‴	7.43 (*d*, J=2.0)	7.58 (*d*, J=8.3)	7.43 (*d*, J=2.0)	7.55 (*d*, J=8.3)
3‴		6.86 (*d*, J=8.3)		7.00 (*d*, J=8.3)
5‴	6.96 (*d*, J=8.3)	6.86 (*d*, J=8.3)	6.96 (*d*, J=8.3)	7.00 (*d*, J=8.3)
6‴	7.48 (*dd*, J=8.3, 2.0)	7.58 (*d*, J=8.3)	7.49 (*dd*, J=8.3, 2.0)	7.55 (*d*, J=8.3)
OMe-3‴	3.94 (*s*)			
OMe-3″	3.18 (*s*)	3.19 (*s*)	3.22 (*s*)	3.18 (*s*)
OMe-3′		3.93 (*s*)		3.91 (*s*)
OMe-4′	3.78 (*s*)		3.75 (*s*)	
OMe-4‴		3.76 (*s*)	3.90 (*s*)	3.78 (*s*)
OH-5	13.53 (*s*)	13.47 (*s*)	13.54[a] (*s*)	13.70[a] (*s*)
OH-7″	13.53 (*s*)	13.50 (*s*)	13.51[a] (*s*)	13.51[a] (*s*)

[a] Recorded in DMSO-d_6–$CDCl_3$ (1:1), 200 MHz.
[b] Recorded in DMSO-d_6, 400 MHz.

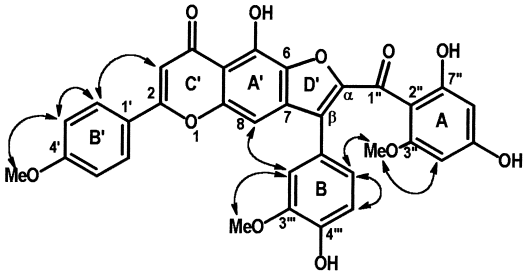

Fig. 1. Selected NOESY interactions for compound **3**.

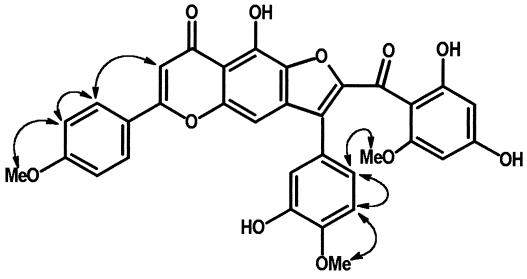

Fig. 2. Selected NOESY interactions for compound **5**.

peak, at m/z 161, and of the ion $[M-161]^+$ at m/z 1013. Further fragmentation of this ion could lead to the majority of the ions observed in the spectrum, including those which originated from retro Diels–Alder rearrangements involving the I-C′ and II-C′ rings.

The HMBC spectrum of compound **7** was used to obtain the assignments of quaternary carbons and to independently confirm the connectivities. All expected correlations via ^{3}J(C,H) according to the proposed structure were observed (Tables 5 and 6). Analysis of the HMBC and NOESY (Fig. 3) spectra revealed characteristic correlations that defined the positions of the mono-, di- and trioxygenated aromatic rings and their substitution patterns. The correlations between C-2 (δ 166.0) of the flavone-chalcone moiety (unit I) and H-2′ (δ 7.65) and H-3 (δ 7.02) showed the presence of the 3,4-dimethoxy aromatic B ring on the flavone moiety. The NOESY spectrum showed interactions of H-I-6′ (δ 7.68) with H-3 (δ 7.02) and of H-5′ (δ 6.94) with H-I-6′ and MeO-I-4′ (δ 3.80), which confirmed the presence of the dimethoxy aromatic ring on unit I. HMBC correlations between C-I-β (δ 160.0) and H-I-2‴, H-I-6‴ (δ 7.46) established a *p*-disubstituted aromatic ring on the chalcone moiety of unit I. The correlations of C-II-2″ (δ 165.0) with H-II-2‴ (δ 7.56), H-II-6‴ (δ 7.58), and H-II-3″ (δ 6.90) defined the position of the remaining dioxygenated aromatic ring, which was involved in the linkage between the two units of the tetraflavonoid. The NOESY correlation between H-II-2‴ and H-I-3‴, H-I-5‴ corroborated the suggestion that the two biflavonoid units (I–II) were linked through C-I-4‴→O→C-II-3‴. The NOESY correlations observed between the methoxy and aromatic protons led not only to the placement of the methoxy groups at the aromatic rings, but also to establishment of the spatial arrangements of the flavonoyl units in II, showing the proximity between OCH_3-7″ and H-2′, H-6′.

The biosynthesis of compounds **1** and **2** could involve an oxidative coupling of two flavone units, leading to linkage of these units through C-3 and C-6″ bonds. Compounds **3** to **6** may arise from oxidative coupling

Table 5
^{13}C NMR spectral data for compound **7**[a–c,e,f]

C	7-I		7-II	
	^{13}C (δ)	HMBC	^{13}C (δ)	HMBC
2	166.0 (*s*)	H-3, H-2′	f	
3	103.5 (*s*)		f	
4	184.0 (*s*)	H-3	f	
5	f		161.5 (*s*)	OMe-5, H-6
6	157.5[e] (*s*)	H-8	97.3 (*d*)	H-8
7	113.8 (*s*)	H-8	164.0 (*s*)	H-6, H-8
8	91.7 (*d*)		96.0 (*d*)	H-6, H-7
9	154.0[e] (*s*)	H-8	160.0 (*s*)	
10	106.0 (*s*)	H-8, OH-5, H-3	107.0 (*s*)	H-6, H-8
1′	121.8 (*s*)	H-3′, H-5′	121.8 (*s*)	H-3′, H-5′
2′	111.0 (*d*)	H-6′	128.2 (*d*)	H-6′
3′	149.0 (*s*)	H-5′, OMe-3′	115.5 (*d*)	H-5′
4′	152.0 (*s*)	H-2′, H-6′, OMe-4′	161.0 (*s*)	H-2′, H-6′, OMe-4′
5′	116.7 (*d*)		115.5 (*d*)	H-3′
6′	121.8 (*d*)	H-2′	128.2 (*d*)	H-2′
α	149.0 (*s*)	H-8		
β	160.0 (*s*)	H-2‴, H-6‴		
2″	107.0 (*s*)	H-4″, H-6″, OH-7″	165.0 (*s*)	H-2‴, H-3″, H-6‴
3″	164.8 (*s*)	OMe-3″	104.1 (*d*)	
4″	92.8 (*d*)	H-6″	182.5 (*s*)	H-3″
5″	168.0 (*s*)		160.0 (*s*)	OH-5″
6″	96.5 (*d*)	H-4″	104.0 (*s*)	OH-5″, H-8″
7″	168.0 (*s*)	H-4″, H-6″	164.5[e] (*s*)	H-8″, OMe-7″
8″			94.8 (*d*)	
9″			157.5[e] (*s*)	H-8″
10″			106.5 (*s*)	H-3″, OH-5″, H-8″
1‴	126.0 (*s*)	H-3‴, H-5‴	122.0 (*s*)	H-5‴, H-3″
2‴	130.0 (*d*)	H-6‴	111.5 (*d*)	H-6‴
3‴	114.7 (*d*)	H-5‴	149.0 (*s*)	H-5‴
4‴	161.5 (*s*)	H-6‴, H-2‴	151.9 (*s*)	H-6‴, H-2‴
5‴	114.7 (*d*)	H-3‴	116.7 (*d*)	
6‴	130.0 (*d*)	H-2‴	121.0 (*d*)	H-2‴
OMe-3′	56.5 (*q*)			
OMe-3″	56.2 (*q*)			
OMe-4′	56.3 (*q*)		55.9 (*q*)	
OMe-5			56.0 (*q*)	
OMe-7″			56.5 (*q*)	

[a] Multiplicities were established by DEPT experiments.
[b] Signals assigned by means of 2D-NMR experiments.
[c] Recorded in acetone-d_6, 126 MHz.
[e] Assignments may be interchangeable within the same column.
[f] Signal not observed.

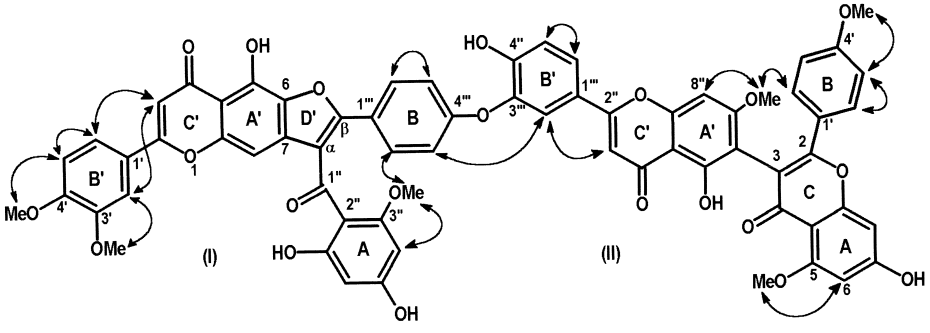

Fig. 3. Selected NOESY interactions for compound **7**.

Table 6
^{1}H NMR spectral data for compound **7** (acetone-d_6, 500 MHz)

H	**7**-I (δ H, J in Hz)	**7**-II (δ H, J in Hz)
3	7.02 (*s*)	
6		6.40 (*d*, $J=2.2$)
8	7.57 (*s*)	6.48 (*d*, $J=2.2$)
2′	7.65 (*d*, $J=2.1$)	7.60 (*d*, $J=8.1$)
3′		7.06 (*d*, $J=8.1$)
5′	6.94 (*d*, $J=8.1$)	7.06 (*d*, $J=8.1$)
6′	7.68 (*dd*, $J=8.1$, 2.1)	7.60 (*d*, $J=8.1$)
3″		6.90 (*s*)
4″	5.85 (*d*, $J=2.0$)	
6″	5.98 (d, $J=2.0$)	
8″		6.54 (*s*)
2‴	7.46 (*d*, $J=8.0$)	7.56 (*d*, $J=2.3$)
3‴	6.90 (*d*, $J=8.0$)	
5‴	6.90 (*d*, $J=8.0$)	6.97 (*d*, $J=8.2$)
6‴	7.46 (*d*, $J=8.0$)	7.58 (*dd*, $J=8.2$, 2.3)
OMe-3″	3.22 (*s*)	
OMe-3′	3.92 (*s*)	
OMe-4′	3.80 (*s*)	3.52 (*s*)
OMe-5		3.80 (*s*)
OMe-7″		3.90 (*s*)
OH-5	13.08 (*s*)	
OH-5″		13.10
OH-7″	13.42 (*s*)	

between 6-hydroxyflavone and chalcone derivatives, followed by further elaboration, which could lead to rearranged benzofuran derivatives. Compound **7**, in turn, could be formed by oxidative coupling of a biflavone and a benzofuran derivative through a C-I-4‴→O→C-II-3‴ linkage.

The structures presented for compounds **1**–**7** have new carbon skeletons. Although triflavonoids have already been isolated, mainly from ferns (*Polypodium decumanum*) and mosses (*Batramia* spp) (Harborne, 1993; Geiger et al., 1995; Seeger et al., 1995), to our knowledge the occurrence of tetraflavonoids has been limited to *Lophira alata* (Ochnaceae) (Murakami et al., 1991; Tih et al., 1992).

3. Experimental

3.1. General

The 1D- (^{1}H, ^{13}C, DEPT, and gNOESY) and 2D- (^{1}H-^{1}H COSY, ^{1}H-^{13}C COSY optimized for $J=7$ Hz and 145 Hz, HMQC, HMBC and NOESY) NMR spectra were recorded on a Bruker AC200, Varian Inova 400 or Varian Inova 500. The mass spectra were obtained on an HP 5985 spectrometer (EIMS) and on a Fisons Platform II by flow injection into the electrospray source (ESI/MS). HPLC analyses were carried out using a Shimadzu liquid chromatograph 10 Avp equipped with a UV–vis detector and automatic fraction collector. The columns were RP 18 (Waters) and chromatograms were acquired at 254 and 280 nm. The IR spectra were obtained on a Nicolet-730 FT–R spectrometer using KBr discs. UV absorption was measured in a Hewlett Packard 8452 A Diode array spectrophotometer. Optical rotations were measured on a Polamat A Carl Zeiss Jena.

3.2. Plant material

The plant material was collected in São Joaquim da Barra, SP, Brazil, and identified as *Aristolochia ridicula* Brown by Dr. Condorcet Aranha. A voucher specimen was deposited at the herbarium of the Instituto Agronômico de Campinas, Campinas, SP, Brazil. The material was separated by plant parts, dried (~45°) and ground.

3.3. Isolation

Ground stems (850 g) were extracted exhaustively at room temperature with hexane, Me_2CO and EtOH, successively, and the extracts were then individually concentrated. The crude acetone extract (4.0 g) was washed with hexane and $CHCl_3$ and dissolved in hot Me_2CO. After freezing, a precipitate was separated (3.5 g). The solution was concentrated and dissolved in hot MeOH (ca. 70 ml) to give a bright yellow solution and a precipitate. Further precipitation by re-heating and cooling the methanol solution (3× ca. 60 ml) gave compounds **4**, **5** and **7**, successively, which were individually purified by repetitive precipitation procedures (3× ca. 12 ml, from MeOH). Yellow precipitates of different tonalities indicated selective precipitation of compounds. The mother liquors from the methanol solutions were combined, concentrated and submitted to precipitation procedures from acetone (ca. 70 ml) as described previously, leading to the isolation of **1**–**3** and **6**, successively. Further purification of these compounds was carried out by HPLC (C-18, MeOH: H_2O–2:3 for **1**, **2** and **4**, MeOH: H_2O–3:7 for **3**, **5**–**7**) to give **1** (12 mg), **2** (105 mg), **3** (9 mg), **4** (13 mg), **5** (14 mg), **6** (9 mg) and **7** (15 mg).

3.4. 4‴,5,5″,7″-Tetrahydroxy-3‴,4′,7-trimethoxy-3,6″-biflavone (*1*)

Yellow solid, mp: 230–232°C (Me_2CO). $[\alpha]_D^{25}$ −33.7° (Me_2CO; *c* 0.6100). (Found: C, 66.44; H, 4.06. $C_{33}H_{24}O_{11}$ requires: C, 66.43; H 4.05, %). EIMS (probe) 70 eV, *m*/*z* (rel. int.): 596 $[M]^+$ (8), 457 (6), 430 $[M\text{-}166]^+$ (9), 340 (8), 325 (19), 189 (100), 167 (30), 151 (78). UV λ_{max}^{MeOH} nm (log ε): 308 (3.7), 345 (3.6). UV $\lambda_{max}^{MeOH+NaOMe}$ nm (log ε): 300 (3.5), 325 (3.5), 410 (3.6). $\lambda_{max}^{MeOH+NaOMe+HCl}$ nm (log ε): 308 (3.7), 345 (3.6). UV

$\lambda_{max}^{MeOH+NaOAc}$ nm (log (ε): 300 (3.4), 330 (3.4), 410 (3.4). $\lambda_{max}^{MeOH+NaOAc+H_3BO_3}$ nm (log ε): 308 (3.5), 325 (3.5), 345 (3.5). $\lambda_{max}^{MeOH+AlCl_3}$ nm (log ε): 320 (3.6), 375 (3.5). $\lambda_{max}^{MeOH+AlCl_3+HCl}$ nm (log ε): 320 (3.6), 360 (3.5). IR ν_{max}^{KBr} cm^{-1}: 3445, 1650, 1633, 1621, 1594, 1573, 1559, 1505, 1472, 1460. ^{13}C NMR: see Table 1. ^{1}H NMR: see Table 2.

3.5. 4‴,5″,7,7″-Tetrahydroxy-3‴,4′,7-trimethoxy-3,6″-biflavone (**2**)

Yellow solid, mp: 224–226°C (Me_2CO). $[\alpha]_D^{25}$ +30.1° (Me_2CO; *c* 2.000). (Found: C, 66.54; H, 4.08. $C_{33}H_{24}O_{11}$ requires: C, 66.43; H 4.05, %). Positive ESI-MS (probe) 70 eV, *m*/*z* (rel. int.): 597 $[M+H]^+$ (100%), 430[M-166]$^+$ (20), 190 (5), 189 (80), 167 (8). UV λ_{max}^{MeOH} nm (log ε): 260 (3.4), 323 (3.3), 337 sh (3.3), 385 (3.2). UV $\lambda_{max}^{MeOH+NaOMe}$ nm (log ε): 267 (3.4), 324 (3.3), 402 (3.3). $\lambda_{max}^{MeOH+NaOMe+HCl}$ nm (log ε): 262 (3.3), 323 (3.3), 343 (3.3), 385 (3.2). UV $\lambda_{max}^{MeOH+NaOAc}$ nm (log ε): 264 (2.7), 339 sh (2.7), 383 (2.7). UV $\lambda_{max}^{MeOH+NaOAc+H_3BO_3}$ nm (log ε): 264 (2.7), 339 sh (2.7), 386 (2.7). UV $\lambda_{max}^{MeOH+AlCl_3}$ nm (log ε): 263 (3.3), 285 (3.3), 324 sh (3.3), 386 (3.3). $\lambda_{max}^{MeOH+AlC_3+HCl}$ nm (log ε): 261 (3.3), 287 (3.3), 323 (3.3), 352 (3.2), 385 (3.2). IR ν_{max}^{KBr} cm^{-1}: 3444, 1649, 1630, 1621, 1578, 1511, 1459. ^{13}C NMR: see Table 1. ^{1}H NMR: see Table 2.

3.6. 4‴,5,5″,7″-Tetrahydroxy-3″,3‴,4′-trimethoxy-6-O-α,7-β-flavone-chalcone (**3**)

Yellow solid, mp: 253–255°C (Me_2CO). $[\alpha]_D^{25}$ +15.5° (Me_2CO *c* 1.0000). (Found: C, 66.84; H, 4.01. $C_{33}H_{24}O_{11}$ requires: C, 66.43; H 4.05, %). Positive ESI-MS (probe) 70 eV, *m*/*z* (rel. int.): 597 $[M+H]^+$ (70%), 596 $[M]^+$ (94), 457 [M-139]$^+$ (100), 442 (57), 254 (75), 186 (56), 137 (44). UV λ_{max}^{MeOH} nm (log ε): 307 (3.7), 345 (3.6). UV $\lambda_{max}^{MeOH+NaOMe}$ nm (log ε): 300 (3.5), 335 (3.6). $\lambda_{max}^{MeOH+NaOMe+HCl}$ nm (log ε): 307 (3.7), 345 (3.6). UV $\lambda_{max}^{MeOH+NaOAc}$ nm (log ε): 300 (3.7), 325 (3.7), 345 sh (3.7), 408 (3.7). $\lambda_{max}^{MeOH+NaOAc+H_3BO_3}$ nm (log ε): 307 (3.4), 345 (3.3). $\lambda_{max}^{MeOH+AlCl_3}$ nm (log ε): 320 (3.7), 370 (3.7). $\lambda_{max}^{MeOH+AlCl_3+HCl}$ nm (log ε): 320 (3.7), 370 (3.7). IR μ_{max}^{KBr} cm^{-1}: 3408, 1624, 1589, 1508, 1481. ^{13}C NMR: see Table 3. ^{1}H NMR: see Table 4.

3.7. 4′,5,5″,7″-Tetrahydroxy-3′,3″,4‴-trimethoxy-6-O-α,7-β-flavone-chalcone (**4**)

Yellow solid, mp: 236–238°C (Me_2CO). $[\alpha]_D^{25}$ +23.1° (Me_2CO *c* 0.8002). (Found: C, 66.50; H, 4.12. $C_{33}H_{24}O_{11}$ requires: C, 66.43; H 4.05, %). Positive ESI-MS (probe) 70 eV, *m*/*z* (rel. int.): 597 $[M+H]^+$ (100%), 457 [M-139]$^+$ (46). UV λ_{max}^{MeOH} nm (log ε): 307 (3.5), 346 (3.4). UV $\lambda_{max}^{MeOH+NaOMe}$ nm (log ε): 298 (3.3), 328 (3.3), 409 (3.4). $\lambda_{max}^{MeOH+NaOMe+HCl}$ nm (log ε): 307 (3.4), 348 (3.4). UV $\lambda_{max}^{MeOH+NaOAc}$ nm (log ε): 300 (3.5), 326 sh (3.5), 412 (3.5). $\lambda_{max}^{MeOH+NaOAc+H_3BO_3}$ nm (log ε): 302 (3.7), 332 (3.7), 350 sh (3.7), 384 (3.7). $\lambda_{max}^{MeOH+AlCl_3}$ nm (log ε): 313 (4.0), 370 (4.0). $\lambda_{max}^{MeOH+AlCl_3+HCl}$ nm (log ε): 313 (3.5), 367 (3.4). IR ν_{max}^{KBr} cm^{-1}: 3566, 3446, 1650, 1632, 1621, 1594, 1509, 1472. ^{13}C NMR: see Table 3. ^{1}H NMR: see Table 4.

3.8. 3‴,5,5″,7″-Tetrahydroxy-3″,4′,4‴-trimethoxy-6-O-α,7-β-flavone-chalcone (**5**)

Yellow solid. mp: 254–256°C (Me_2CO). $[\alpha]_D^{25}$ +17.4° (Me_2CO *c* 0.9000). (Found: C, 66.94; H, 4.09. $C_{33}H_{24}O_{11}$ requires: C, 66.43; H 4.05, %). Positive ESI-MS (probe) 70 eV, *m*/*z* (rel. int.): 597 $[M+H]^+$ (70 %), 596 $[M]^+$ (94), 457 [M-139]$^+$ (100), 442 (57), 254 (75), 186 (50), 137 (40). UV λ_{max}^{MeOH} nm (log ε): 307 (3.7), 345 (3.6). UV $\lambda_{max}^{MeOH+NaOMe}$ nm (log ε): 300 (3.5), 335 (3.6). $\lambda_{max}^{MeOH+NaOMe+HCl}$ nm (log ε): 307 (3.7), 345 (3.6). UV $\lambda_{max}^{MeOH+NaOAc}$ nm (log ε): 300 (3.7), 325 (3.7), 345 sh (3.7), 408 (3.7). $\lambda_{max}^{MeOH+NaOAc+H_3BO_3}$ nm (log ε): 307 (3.4), 345 (3.3). $\lambda_{max}^{MeOH+AlCl_3}$ nm (log ε): 320 (3.7), 370 (3.7). $\lambda_{max}^{MeOH+AlCl_3+HCl}$ nm (log ε): 320 (3.7), 370 (3.7). IR ν_{max}^{KBr} cm^{-1}: 3418, 1623, 1590, 1508, 1481. ^{13}C NMR: see Table 3. ^{1}H NMR: see Table 4.

3.9. 4′,5,5″,7″-Tetrahydroxy-3′,3″,4‴-trimethoxy-6-O-β,7-α-flavone-chalcone (**6**)

Yellow solid. mp: 250–253°C (Me_2CO). $[\alpha]_D^{25}$ –30.1° (Me_2CO *c* 0.9000). (Found: C, 66.00; H, 4.00. $C_{33}H_{24}O_{11}$ requires: C, 66.43; H 4.05, %). Positive ESI-MS (probe) 70 eV, *m*/*z* (rel. int.): 597 $[M+H]^+$ (70%), 596 $[M]^+$ (100), 457 [M-139]$^+$ (40). UV λ_{max}^{MeOH} nm (log ε): 290 (3.7), 365 (3.6). IR ν_{max}^{KBr} cm^{-1}: 3421, 1651, 1634, 1621, 1580, 1560, 1510, 1459. ^{13}C NMR: see Table 3. ^{1}H NMR: see Table 4.

3.10. (4‴,5″,7-Trihydroxy-4′,5,7″-trimethoxy-3,6″-biflavone)-3‴-O-4‴-(5,5″,7″-trihydroxy-3′,3″,4′-trimethoxy-6-O-β,7-α-flavone-chalcone) (**7**)

Yellow solid. mp: 243–246°C (Me_2CO). (Found: C, 67.57; H, 3.90. $C_{66}H_{46}O_{21}$ requires C, 67.46; H, 3.95%). Positive ESI-MS (probe) 70 eV, *m*/*z* (rel. int.): 1175 $[M+H]^+$ (<1%), 1067 [M-107]$^+$ (2), 1060 [M-137+23]$^+$ (4), 1013 [M-161]$^+$ (3), 985 [1013-CO]+ (5), 874 [1013-139]$^+$ (7), 815 [1013-167-OMe]$^+$ (24), 633 (31), 619 [596+Na]$^+$ (34), 599 [M+H+Na]$^{++}$ (51),

597 (62), 595 (41), 551 [1013-462]$^{+}$ (39), 507 [1013 + H]$^{++}$ (49), 463 (57), 413 (97), 284 (57), 161 (100). UV λ_{max}^{MeOH} nm (log ε): 307 (3.7), 345 (3.6). IR ν_{max}^{KBr} cm^{-1}: 3556, 3446, 1650, 1632, 1622, 1592, 1509, 1472. ^{13}C NMR: see Table 5. ^{1}H NMR: see Table 6.

Acknowledgements

The authors thank the Fundação de Amparo à Pesquisa do Estado de São Paulo (FAPESP) for financial support, Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq) for the fellowship to F.J.C.Carneiro.

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