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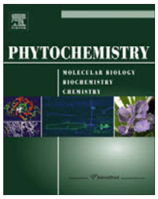

Tetraflavonoid and biflavonoids from *Aristolochia ridicula*

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ABSTRACT

Biflavones, a chalcone–flavone, and a tetraflavonoid with a new carbon skeleton were isolated from the leaves of *Aristolochia ridicula*. Their structures were determined by chemical derivatizations and spectrometric analyses.

1. Introduction

Species belonging to the Aristolochiaceae family have been used in Brazilian traditional medicine as stomachic, antiophidian, anti-inflammatory, antiasthmatic, and abortifacient agents (Lopes et al., 2001), and more recently, in slimming therapy as a substitute for, or in addition to, medicinal plants (Francisco et al., 2003). Flavonols, dihydroflavonols, and isoflavonols have also been isolated from these species (Lopes et al., 2001). However, within this family, bi- and tetraflavonoids have only been found in *Aristolochia ridicula* Brown (Carneiro et al., 2000; Lopes et al., 2001; Machado and Lopes, 2005). Previous studies on this species led to isolation of four biflavones, four chalcone–flavones, and two diverse tetraflavonoid types (Carneiro et al., 2000; Machado and Lopes, 2005). As part of our continuing studies on *A. ridicula*, we have examined further constituents of its leaves. From the acetone extract, three new flavonoids: a biflavone (ridiculuflavone D, **1**), a chalcone–flavone (ridiculuflavonylchalcone B, **2**), and a tetraflavonoid with a new carbon skeleton (ridiculuflavonylchalcone C, **3**), together with a known biflavone (ridiculuflavone C, **4**) were isolated (Fig. 1).

2. Results and discussion

Compounds **1**–**4** were isolated by chromatography and partition procedures from the acetone extract of the leaves and analyzed by HPLC-UV-MS and spectrometric methods (IR, UV, NMR, and MS).

The HRMS spectra of compound **1** showed *quasi*-molecular ions $[M-H]^-$ at m/z 567.0921 ($C_{31}H_{20}O_{11}-H$). The ^1H and ^{13}C NMR spectra of **1** (Table 1) in CD_3OD suggested the presence of 1,4-disubstituted (B ring) and 1,3,4-trisubstituted aromatic rings (B′ ring), as well as a tetrasubstituted (A ring) and a pentasubstituted (A′ ring) aromatic rings with alternating oxygenation patterns. In addition, these spectra showed signals for a methoxyl group (δ_C 56.4, δ_H 3.90), two signals for carbonyl groups (δ_C 178.6, 183.8), three quaternary carbons (δ_C 114.8, 163.1, and 166.3), and a CH (δ_C 103.9, δ_H 6.55). Moreover, the ^1H NMR spectra in CD_3CN showed two singlets at δ 13.58 and 13.80 which are characteristic of hydrogen-bonded hydroxyl groups (OH-5 and OH-5″). The ^1H and ^{13}C NMR, IR, and UV spectroscopic data of **1** were very similar to those reported in the literature for ridiculuflavone B (**5**) (Machado and Lopes, 2005). The main difference between the spectroscopic data of **1** and **5** is due to interchange of the substituents at C-5 and C-7. The linkage of the methoxyl group to C-7 in **1** was corroborated by gNOESY experiments that showed interaction between OCH_3 (δ_H 3.90) and H-8 (δ_H 6.45). These experiments together with gHMBC experiments established that the flavone units were linked through C-3 and C-6″, and also helped us to determine the substituents on the B and B′ rings. This new biflavone was named ridiculuflavone D.

A molecular formula of $C_{33}H_{24}O_{11}$ was determined for compound **2** based on its HRMS spectra, which showed *quasi*-molecular ions at m/z 595.1235 ($C_{33}H_{24}O_{11}-H$). The ^1H and ^{13}C NMR spectra

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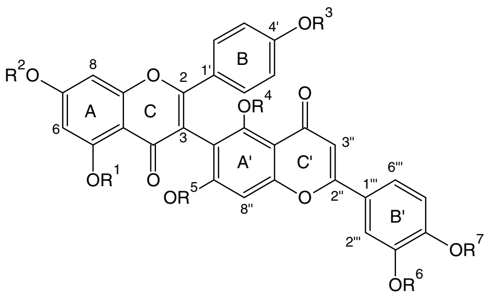
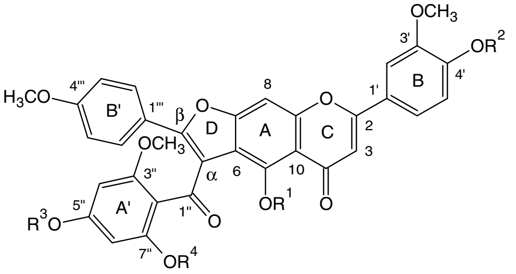


	R¹	R²	R³	R⁴	R⁵	R⁶	R⁷
1	H	CH_3	H	H	H	H	H
4	CH_3	H	CH_3	H	H	CH_3	H
4a	CH_3	CH_3	CH_3	CH_3	CH_3	CH_3	CH_3
5	CH_3	H	H	H	H	H	H



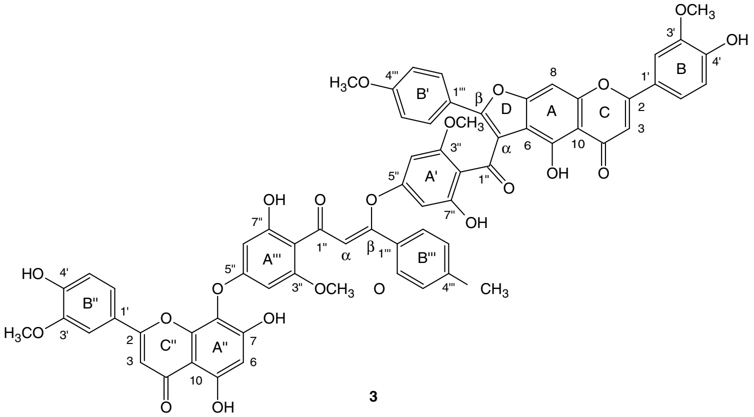
	R¹	R²	R³	R⁴
2	H	H	H	H
2a	CH_3	CH_3	CH_3	H
2b	CH_3	CH_3	CH_3	CH_3
7	H	H	CH_3	H

Fig. 1. Chemical structures for compounds **1**–**7**.

of **2** (Tables 2 and 3) suggested the presence of two hydrogen-bonded hydroxyl group (δ_H 13.51 and 13.78), 1,4-disubstituted (B′ ring) and 1,3,4-trisubstituted (B ring), tetrasubstituted (A′ ring), and pentasubstituted (A ring) aromatic rings. Furthermore, these spectra showed signals for three methoxyl groups (δ_C 56.2, 56.5, and 57.1; δ_H 3.29, 3.86, and 4.03), two carbonyl groups (δ_C 185.1 and 194.2), three quaternary carbons (δ_C 114.1, 152.5, and 166.0), and a CH (δ_C 104.0, δ_H 6.77). These results, particularly the chemical shifts of the carbonyl carbon and methoxyl hydrogens at δ 194.2 and δ 3.29, respectively, suggested a chalcone unit, which should be linked to a flavone unit. Correlations between OH-5 (δ_H 13.78) and C-5 (δ_C 155.7), C-6 (δ_C 114.3), and C-10 (δ_C 107.0) were observed by *g*HMBC experiments, and they helped us to determine the substituents on the pentasubstituted (A ring) aromatic ring. All of the correlations observed by *g*HMBC (Table 2) and *g*NOESY (Table 3) experiments constituted evidence that the linkages between chalcone and flavone moieties could be either through C-α-C-6, C-β-*O*-C-7 or C-α-*O*-C-7, C-β-C-6. In both cases, these linkages should lead to the formation of an additional furanic ring (D ring) in the structure of **2** (Fig. 2). Unfortunately, no correlation between C-α or C-β (δ_C 114.1 and 152.5) and H-2‴ and H-6‴, which could help to determine the carbon skeleton of **2**, was observed by *g*HMBC experiments. Except for MS, the spectrometric data obtained for **2** were very similar to those obtained for tetramer **6**, which was previously isolated from this species (Machado and Lopes, 2005). To confirm that **2** is a biflavonoid, and therefore different from **6** (symmetrical tetramer), and to determine the substitution patterns of the aromatic rings, **2** was subjected to methylations and gave **2a** and **2b** derivatives. The ^{1}H and ^{13}C NMR spectra of both derivatives showed signals for methoxyl groups; six for **2a** and seven for **2b**. The methylation of hydroxyl groups on the A, A′, and B′ rings observed for both derivatives excluded the possibility that **2** was a symmetrical tetramer similar to that isolated previously (**6**). Moreover, the correlation observed by *g*HMBC between the carbons at δ 156.0 for **2a** and 154.8 for **2b** (C-β) and the hydrogens H-2‴ and H-6‴ led to determination of the carbon skeleton, in which a chalcone was linked to a flavone through C-α-C-6 and C-β-*O*-C-7. The spectral data for **2** are quite similar to those of cissampeloflavone (**7**) (Ramírez et al., 2003a,b) and agree with the same carbon skeleton determined for both compounds. Biflavonoid **2** was named ridiculuflavonylchalcone B.

The negative and positive-ion HRMS spectra of **3** showed *quasi*-molecular ions $[M-H]^-$ at *m*/*z* 1191.2527 ($C_{66}H_{48}O_{22}-H$) and $[M+Na]^+$ at *m*/*z* 1215.2535 ($C_{66}H_{48}O_{22}+Na$), as well as base peaks at *m*/*z* 595.1242 and *m*/*z* 611.1549, respectively. Under negative-ion conditions, the ESI-MS/MS of the ions at *m*/*z* 595.12 gave rise to a series of spectra identical to those obtained from **2**. Moreover, except for C-5″ (δ_C 163.6), all of the signals for carbons and hydrogens assigned for **2** were also observed in the ^{1}H and ^{13}C NMR spectra (Tables 4 and 5) of **3** with the assistance of *g*HMQC and *g*HMBC experiments. Hence, a partial structure I was suggested for **3** (Fig. 3). A detailed analysis of these spectra and correlations observed by *g*HMQC, *g*HMBC, *g*COSY, and *g*NOESY experiments provided evidence for two additional units for **3**: a flavone containing pentasubstitued A″ and trisubstituted B″ rings (partial structure II), and a chalcone containing tetrasubstituted A‴ and disubstituted B‴ rings (partial structure III) (Fig. 3). Both units contained a hydrogen-bonded hydroxyl group (OH-II-5 and OH-III-7″). The correlations between H-III-α and C-III-1‴ and C-III-β established the B‴ ring substitutions in unit III. The loss of H-III-α together with the B‴ ring (M−108) could give rise to important fragmentary ions by MS. Indeed, these fragmentations together with those due to retro Diels–Alder rearrangements involving the C and C″ rings accounted for most of the fragmentations evidenced by the MS spectra. Therefore, these fragmentations excluded the possibility that linkages between the three units were through B or B″ rings. Furthermore, the positive-ion ESI-MS spectra of **3** showed ions at *m*/*z* 728 and 437, which suggested α-carbonyl bond fragmentations of III (C-III-1″-C-III-α and C-III-1″-C-III-2″, respectively), a base peak at *m*/*z* 421, which may have arisen from breakage of the bond C-I-5″-*O*-C-III-β and a retro Diels–Alder rearrangement involving the C″ ring ($[M-579-192]^+$), and ions at *m*/*z* 507 $[M+H-579-107]^+$. An analysis of all of these data established the unit sequence (I–III–II). Units III and II could be linked through either C-III-5″-*O*-C-II-7 or C-III-5″-*O*-C-II-8. To choose between these two structural alternatives, we compared the chemical shifts of hydrogens and carbons on the A″ aromatic ring to those reported for flavones, such as 5,8,3′,4′,5′-pentahydroxy-3,7-dimethoxyflavone and limocitrin 3-*O*-β-D-glucopyranoside (Bennini et al., 1992; Chung et al., 1999). In addition, the effects on the chemical shifts due to the substitution of a methoxyl for a "phenoxyl" group on the aromatic rings (Crews et al., 1998) of these model compounds were also considered to estimate the carbon chemical shifts for both structural alternatives. Therefore, this analysis points to the C-III-5″-*O*-C-II-8 linkage and as a result structure **3** proposed for the tetramer (named riciculuflavonylchalcone C) seems to be the most feasible.

Compound **4** was identified to be ridiculuflavone C, previously isolated from *A. ridicula*, based on a comparison of their spectrometric data (MS, IR, UV, ^{1}H and ^{13}C NMR), as well as by a detailed analysis of the spectrometric data of its methyl derivative (**4a**) (Table 6).



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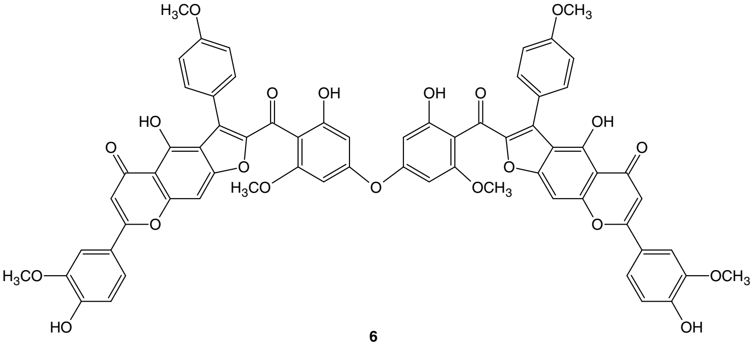

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Fig. 1 (continued)

Table 1
^{13}C and ^{1}H NMR spectroscopic data for compound **1**

C and H	δ ^{13}C[a,b]	gHMBC	δ ^{1}H[c,d]
2	163.1 *s*	H-2′, H-6′	
3	114.8 *s*		
4	178.6 *s*		
5	161.4 *s*	H-6	
6	96.3 *d*	H-8	6.53 (*d*, 2.0)
7	162.7 *s*	H-6, OCH_3-7, H-8	
8	97.7 *d*	H-6	6.45 (*d*, 2.0)
9	165.4 *s*	H-8	
10	107.8 *s*	H-6, H-8	
1′	125.7 *s*	H-3′, H-5′	
2′	130.9 *d*	H-6′	7.43 (*d*, 9.0)
3′	115.9 *d*	H-5′	6.70 (*d*, 9.0)
4′	160.7 *s*	H-2′, H-3′, H-5′, H-6′	
5′	115.9 *d*	H-3′	6.70 (*d*, 9.0)
6′	130.9 *d*	H-2′	7.43 (*d*, 9.0)
2″	166.3 *s*	H-3″	
3″	103.9 *d*		6.55 (*s*)
4″	183.8 *s*	H-3″	
5″	161.8 *s*		
6″	108.0 *s*	H-8″	
7″	164.7 *s*[e]	H-8″	
8″	94.8 *d*		6.51 (*s*)
9″	159.0 *s*[e]	H-8″	
10″	105.1 *s*	H-3″, H-8″	
1‴	123.8 *s*	H-3″, H-5‴	
2‴	114.2 *d*	H-6‴	7.40 (*d*, 1.0)
3‴	147.1 *s*	H-2‴, H-5‴	
4‴	151.2 *s*	H-2‴, H-5‴, H-6‴	
5‴	116.8 *d*		6.92 (*d*, 9.0)
6‴	120.3 *d*	H-2‴	7.41 (*dd*, 9.0, 1.0)
OCH_3-7	56.4 *q*		3.90 (*s*)

[a] Recorded in CD_3OD, 126 MHz.
[b] The ^{13}C NMR data were assigned with the assistance of *g*HMQC and *g*HMBC experiments.
[c] Recorded in CD_3OD, 500 MHz.
[d] Multiplicities were determined with the assistance of ^{1}H–^{1}H COSY experiments, 500 MHz.
[e] Assignments may be interchangeable within the same column.

Tetramer **3** and biflavone **1** showed complex CD curves from 220 to 420 nm. Similarly, chalcone–flavones **2** and **2a** showed comparable and complex CD curves from 220 to 370 nm, whereas derivatives **2b** and **4a**, which did not have hydrogen-bonded hydroxyl group (OH-5″ and/or OH-7″), did not show any significant Cotton effect. From these observations, we can infer that hydrogen-bonded hydroxyl groups, OH-5″-CO-4″ in compound **1** and OH-7″-CO-1″ in **2** and **2a**, are essential for axial chiralities impose by the rotors at C-3-C-6″ and C-α-CO-1″, respectively. These remarks are in agreement with the magnetic equivalence observed for carbons and hydrogens on the A′ ring (CH-4″/CH-6″, C-3″/C-7″, and OCH_3-3″/OCH_3-7″) of **2b**, and the free rotation of this ring about the CO-1″–C-2″ bond.

Tetraflavonoids are rare in nature and have only been found in *A. ridicula* (Carneiro et al., 2000; Machado and Lopes, 2005), *Lophira alata* (Ochnaceae) (Murakami et al., 1992a,b; Tih et al., 1992a,b), *Lophira lanceolata* (Tih et al., 1989), and *Cephalotaxus wilsoniana* (Cephalotaxaceae) (Wang et al., 2004).

3. Conclusions

To date, five biflavones and five chalcone–flavones, including **1** and **2**, with unusual carbon skeletons, have been isolated from *A. ridicula*. Only 10 tetraflavonoids have been described in the literature, two of these were isolated from this species. We described here an additional tetramer (**3**) that has a new carbon skeleton.

4. Experimental

4.1. General experimental procedures

One-dimensional (^{1}H, ^{13}C, DEPT, and *g*NOESY) and two-dimensional (^{1}H–^{1}H *g*COSY, *g*HMQC, *g*HMBC, and *g*NOESY) NMR experiments were performed on a Varian INOVA 500 spectrometer

Table 2
^{13}C NMR spectroscopic data for compounds **2**, **2a**, and **2b**

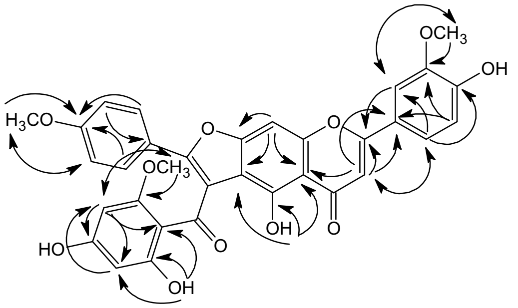
C	2[a]		**2a**[b]		**2b**[c]	
	δ[d]	gHMBC	δ[d]	gHMBC	δ[d]	gHMBC
2	166.0 *s*	H-2′, H-3, H-6′	161.4 *s*	H-3, H-6′	161.6 *s*	H-3
3	104.0 *d*		105.8 *d*		106.5 *d*	
4	185.1 *s*		[g]		[g]	
5	155.7 *s*	OH-5	152.8 *s*	OCH$_3$-5	154.0 *s*	OCH$_3$-5
6	114.3 *s*	OH-5, H-8	[g]		[g]	
7	155.3 *s*[e]	H-8	[g]		[g]	
8	91.5 *d*		96.0 *d*		96.0 *d*	
9	158.2 *s*[e]		[g]		[g]	
10	107.0 *s*	H-3, OH-5, H-8	[g]		114.4 *s*	H-3
1′	123.8 *s*	H-3, H-5′	124.0 *s*	H-5′	124.4 *s*	H-5′
2′	110.8 *d*	H-6′	109.0 *d*		108.5 *d*	
3′	148.8 *s*	H-2′, OCH$_3$-3′, H-5′	150.2 *s*	OCH$_3$-3′, H-5′	149.5 *s*	OCH$_3$-3′, H-5′
4′	151.3 *s*	H-2′, H-5′, H-6′	152.6 *s*	OCH$_3$-4′, H-2′	152.0 *s*	H-2′, OCH$_3$-4′
5′	116.3 *d*		111.0 *d*		111.5 *d*	
6′	121.8 *d*	H-2′	119.8 *d*	H-2′	119.5 *d*	H-2′
α	114.1 *s*		[g]		[g]	
β	152.5 *s*		156.0 *s*	H-2‴, H-6‴	154.8 *s*	H-2‴, H-6‴
1″	194.2 *s*		[g]		[g]	
2″	108.2 *s*	H-4″, OH-7″	[g]		113.0 *s*	H-4″, H-6″
3″	165.2 *s*	OCH$_3$-3″	163.5 *s*	OCH$_3$-3″	161.4 *s*	OCH$_3$-3″, H-4″
4″	92.5 *d*	H-6″	90.5 *d*		91.0 *d*	H-6″
5″	167.2 *s*	H-4″	168.2 *s*	OCH$_3$-5″	164.1 *s*	H-4″, OCH$_3$-5″, H-6″
6″	96.7 *d*	H-4″, OH-7″	93.5 *d*		91.0 *d*	H-4″
7″	168.5 *s*	OH-7″	[g]		161.4 *s*	H-6″, OCH$_3$-7″
1‴	122.7 *s*	H-3‴, H-5‴	122.0 *s*	H-3‴, H-5‴	122.2 *s*	H-3‴, H-5‴
2‴	129.0 *d*	H-6‴	127.5 *d*		129.0 *d*	
3‴	115.5 *d*	H-5‴	114.0 *d*		114.0 *d*	
4‴	161.7 *s*	H-2‴, OCH$_3$-4‴, H-6‴	161.0 *s*	H-2‴, OCH$_3$-4‴, H-6‴	160.5 *s*	H-2‴, OCH$_3$-4‴, H-6‴
5‴	115.5 *d*	H-3‴	114.0 *d*		114.0 *d*	
6‴	129.0 *d*	H-2‴	127.5 *d*		129.0 *d*	
OCH$_3$-3′	57.1 *q*		55.2 *q*[e]		56.0 *q*[e]	
OCH$_3$-3″	56.5 *q*		55.0 *q*[e]		55.6 *q*	
OCH$_3$-4′			54.8 *q*[f]		55.8 *q*[e]	
OCH$_3$-4‴	56.2 *q*		54.6 *q*[f]		55.0 *q*	
OCH$_3$-5			61.6 *q*		62.8 *q*	
OCH$_3$-5″			55.0 *q*		55.0 *q*	
OCH$_3$-7″					55.6 *q*	

[a] Recorded in CD$_3$CN, 126 MHz.
[b] Recorded in (CD$_3$)$_2$CO, 126 MHz.
[c] Recorded in CDCl$_3$, 126 MHz.
[d] The ^{13}C NMR data were assigned with the assistance of DEPT 90° and 135°, *g*HMQC, and *g*HMBC experiments.
[e,f] Assignments may be interchangeable within the same column.
[g] Signal not observed.

Table 3
^{1}H NMR spectroscopic data for compounds **2**, **2a**, and **2b** (500 MHz, *J* in Hz)[a]

H	2[b]		2a[c]		2b[d]	
	δ	1D- and 2D-gNOESY	δ	2D-gNOESY	δ	2D-gNOESY
3	6.77 (*s*)	H-2′, H-6′	6.68 (*s*)	H-2′, H-6′	6.62 (*s*)	H-2′
8	7.37 (*s*)		7.38 (*s*)		7.44 (*s*)	
2′	7.62 (*d*, 2.3)	H-3, OCH$_3$-3′	7.67 (*d*, 2.0)	H-3, OCH$_3$-3′	7.39 (*d*, 2.0)	H-3, OCH$_3$-3′
5′	7.05 (*d*, 8.3)	H-6′	7.18 (*d*, 8.5)	OCH$_3$-4′, H-6′	7.00 (*d*, 8.8)	OCH$_3$-4′
6′	7.66 (*dd*, 8.3, 2.3)	H-3, H-5′	7.72 (*dd*, 8.5, 2.0)	H-5′	7.57 (*dd*, 8.8, 2.0)	H-5′
4″	5.90 (*d*, 2.3)	OCH$_3$-3″	6.00 (*d*, 2.5)	OCH$_3$-3″	6.01 (*s*)	OCH$_3$-3″, OCH$_3$-5″
6″	6.09 (*d*, 2.3)		6.23 (*d*, 2.5)	OCH$_3$-5″	6.01 (*s*)	OCH$_3$-5″, OCH$_3$-7″
2‴	7.68 (*d*, 9.0)	H-3‴	7.73 (*d*, 8.3)	H-3‴	7.73 (*d*, 9.3)	H-3‴
3‴	7.02 (*d*, 9.0)	H-2‴, OCH$_3$-4‴	7.05 (*d*, 8.3)	H-2‴, OCH$_3$-4‴	6.87 (*d*, 9.3)	H-2‴, OCH$_3$-4‴
5‴	7.02 (*d*, 9.0)	OCH$_3$-4‴, H-6‴	7.05 (*d*, 8.3)	OCH$_3$-4‴, H-6‴	6.87 (*d*, 9.3)	OCH$_3$-4‴, H-6‴
6‴	7.68 (*d*, 9.0)	H-5‴	7.73 (*d*, 8.3)	H-5‴	7.73 (*d*, 9.3)	H-5‴
OCH$_3$-3′	4.03 (*s*)	H-2′	4.02 (*s*)	H-2′	4.00 (*s*)	H-2′
OCH$_3$-3″	3.29 (*s*)	H-4″	3.37 (*s*)	H-4″	3.63 (*s*)	H-4″
OCH$_3$-4′			3.97 (*s*)	H-5′	3.98 (*s*)	H-5′
OCH$_3$-4‴	3.86 (*s*)	H-3‴, H-5‴	3.88 (*s*)	H-3‴, H-5‴	3.83 (*s*)	H-3‴, H-5‴
OCH$_3$-5			3.77 (*s*)		3.90 (*s*)	
OCH$_3$-5″			3.93 (*s*)		3.79 (*s*)	H-4″, H-6″
OCH$_3$-7″					3.63 (*s*)	H-6″
OH-5	13.78 (*s*)					
OH-7″	13.51 (*s*)		13.64 (*s*)			

[a] Multiplicities were determined with the assistance of ^{1}H–^{1}H COSY experiments.
[b] Recorded in CD$_3$CN.
[c] Recorded in (CD$_3$)$_2$CO.
[d] Recorded in CDCl$_3$.



2

Fig. 2. Selected HMBC correlations (→) and nOe interactions (↔) for compound **2**.

(11.7 T) at 500 MHz (^{1}H) and 126 MHz (^{13}C), using deuterium solvents (CD_3OD, CD_3CN, $(CD_3)_2CO$, and $CDCl_3$) ($\geqslant$99.9% D) as internal standards for ^{13}C NMR chemical shifts, and residual solvents as internal standards for ^{1}H NMR. Mass spectra (ESI-MS) were obtained on a Fisons Platform II, and flow injection into the electrospray source was used for LC-ESI-MS. High-resolution mass spectra (HRMS) were obtained on a Bruker Daltonics ultrOTOFq (ESI-TOFMS). IR spectra were obtained on a Perkin–Elmer 1600 FT-IR spectrometer using KBr discs. UV absorptions were measured on a Perkin–Elmer UV–vis Lambda 14P spectrophotometer. Optical rotations were measured on a Perkin Elmer 341-LC polarimeter. HPLC analyses were performed using a Shimadzu liquid chromatograph (SPD-10Avp) equipped with a UV–vis detector. The columns were RP-18 (Varian, C_{18}, 250 × 4.6 mm) and phenyl (Shimadzu, 150 × 4.6 mm) with a particle size of 5 μm, flow rate: 1.0 ml min^{-1}, and chromatograms were acquired at 254 and 336 nm. TLC: Silica gel 60 PF_{254}. Melting points were recorded on a Microquímica MQAPF-302 melting point apparatus and are uncorr.

4.2. Plant materials

The plant materials were collected in São Joaquim da Barra, SP, Brazil, in February 2000, and identified as *Aristolochia ridicula* Brown by Dr. Condorcet Aranha and as *c. s. Aristolochia ridicula* H.B.K. by Dr. Lindolpho Capellari Júnior. A voucher specimen (ESA88276) was deposited at the herbarium of the Escola Superior de Agricultura, Luiz de Queiroz (ESALQ), Piracicaba, SP, Brazil. The material was separated according to the plant parts, dried (~45 °C), and ground.

4.3. Extraction, isolation, and chemical transformation

The plant material from the leaves was extracted exhaustively at room temperature with hexane, acetone, and ethanol, successively, and the extracts were individually concentrated (Machado and Lopes, 2005).

The crude acetone extract (3.25 g) was washed with hexane, $CHCl_3$, and acetone, successively. The soluble fraction in acetone

Table 4
^{13}C NMR spectroscopic data for compound **3**[a,b]

C	Unity I		Unity II		Unity III	
	δ	gHMBC	*δ*	gHMBC	*δ*	gHMBC
2	165.2 *s*	H-2′, H-3, H-6′	164.5 *s*	H-2′, H-3, H-6′		
3	103.2 *d*		104.0 *d*			
4	184.4 *s*	H-3	182.7 *s*			
5	154.3 *s*	OH-5	157.2 *s*	H-6		
6	113.5 *s*	OH-5, H-8	95.5 *d*			
7	154.9 *s*[c]	H-8	162.1 *s*	H-6		
8	90.7 *d*		125.0 *s*			
9	157.4 *s*[c]	H-8	153.7 *s*			
10	106.3 *s*	H-3, OH-5, H-8	105.0 *s*	H-3, OH-5, H-6		
1′	122.7 *s*	H-3	123.2 *s*	H-3		
2′	110.1 *d*	H-6′	110.0 *d*	H-6′		
3′	148.1 *s*	H-2′, OCH_3-3′, H-5′	148.0 *s*	H-2′, OCH_3-3′, H-5′		
4′	150.7 *s*	H-2′, H-6′	150.4 *s*	H-2′, H-5′, H-6′		
5′	115.7 *d*		115.6 *d*			
6′	121.0 *d*	H-2′	120.8 *d*	H-2′		
α	113.3 *s*				115.2 *d*	
β	151.8 *s*	H-2‴, H-6‴			156.1 *s*	H-α
1″	193.4 *s*				190.2 *s*	
2″	107.0 *s*	H-4″, H-6″, OH-7″			107.5 *s*	H-4″
3″	164.5 *s*	OCH_3-3″			161.6 *s*	OCH_3-3″
4″	91.8 *d*	H-6″			96.8 *d*	H-6″
5″	163.6 *s*	H-4″			162.7 *s*	H-4″, H-6″
6″	96.0 *d*	H-4″, OH-7″			94.2 *d*	H-4″
7″	167.7 *s*	H-6″, OH-7″			159.9 *s*	H-6″
1‴	122.0 *s*	H-3‴, H-5‴			125.6 *s*	H-α, H-3‴, H-5‴
2‴	128.2 *d*	H-6‴			129.9 *d*	H-6‴
3‴	114.7 *d*	H-5‴			113.9 *d*	H-5‴
4‴	160.9 *s*	H-2‴, H-3‴, OCH_3-4‴, H-5‴, H-6‴			161.4 *s*	H-2‴, H-3‴, OCH_3-4‴, H-5‴, H-6‴
5‴	114.7 *d*	H-3‴			113.9 *d*	H-3‴
6‴	128.2 *d*	H-2‴			129.9 *d*	H-2‴
OCH_3-3′	56.32 *q*		56.28 *q*			
OCH_3-3″	55.7 *q*				56.0 *q*	
OCH_3-4‴	55.4 *q*				55.3 *q*	

[a] Recorded in CD_3CN, 126 MHz.
[b] The ^{13}C NMR data were assigned with the assistance of DEPT 90° and 135°, *g*HMQC, and *g*HMBC experiments.
[c] Assignments may be interchangeable within the same column.

Table 5
^{1}H NMR spectroscopic data for compound **3** (500 MHz, CD_3CN, *J* in Hz)[a,b]

H	Unity I		Unity II		Unity III	
	δ	1D-gNOESY	δ	1D-gNOESY		1D-gNOESY
3	6.78 (*s*)	H-2′	6.67 (*s*)	H-2′		
6			6.60 (*s*)			
8	7.38 (*s*)					
2′	7.62 (*d*, 2.5)		7.53 (*br d*, 2.0)	H-3, OCH_3-3′		
5′	7.05 (*d*, 8.0)		7.00 (*d*, 8.0)			
6′	7.66 (*dd*, 8.0, 2.5)		7.56 (*d*, 8.0, 2.0)			
α					6.78 (*s*)	
4″	5.90 (*d*, 2.3)				6.48 (*d*, 2.3)	
6″	6.09 (*d*, 2.3)				6.59 (*d*, 2.3)	
2‴	7.69 (*d*, 9.3)				7.54 (*d*, 9.0)	H-3‴
3‴	7.03 (*d*, 9.3)				6.89 (*d*, 9.0)	
5‴	7.03 (*d*, 9.3)				6.89 (*d*, 9.0)	
6‴	7.69 (*d*, 9.3)				7.54 (*d*, 9.0)	H-5‴
OCH_3-3′	4.03 (*s*)	H-2′	4.00 (*s*)	H-2′		
OCH_3-3″	3.30 (*s*)	H-4″			3.91 (*s*)	H-4″
OCH_3-4‴	3.87 (*s*)	H-3‴, H-5‴			3.81 (*s*)	H-3‴, H-5‴
OH-5	13.75 (*s*)		13.19 (*s*)			
OH-7″	13.51 (*s*)				13.51 (*s*)	

[a] Recorded in CD_3CN, 500 MHz.

[b] Multiplicities were determined with the assistance of ^{1}H–^{1}H COSY experiment.

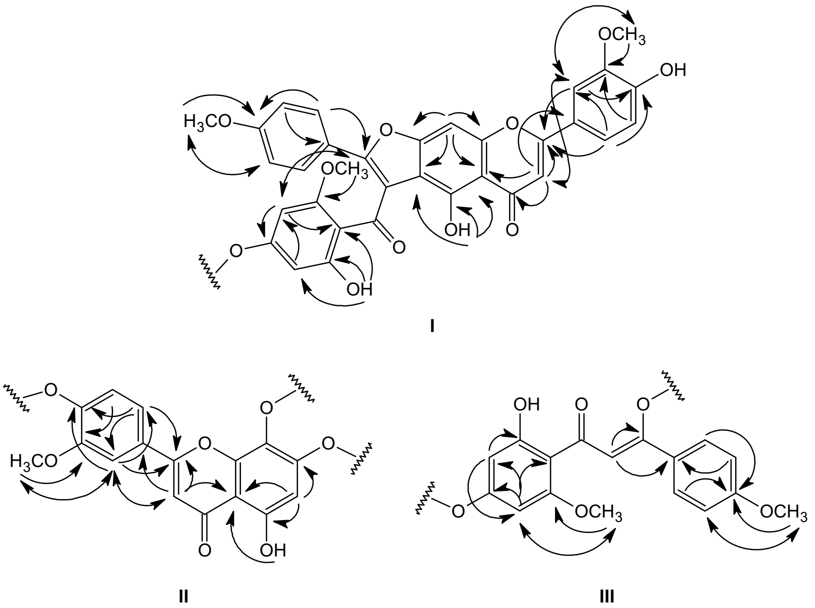


Fig. 3. Selected HMBC correlations (→) and nOe interactions (↔) for substructures I, II, and III of **3**.

was concentrated (680.0 mg) and fractionated over Sephadex LH-20 (MeOH) to give five fractions. Fractions 4 (63.0 mg) and 5 (23.1 mg) were washed with CH_3CN and concentrated. The soluble CH_3CN fraction from fraction 4 was then washed with EtOH to give **3** (6.2 mg), as an insoluble portion, and the EtOH solution remaining was subjected to CC (ODS, MeOH–H_2O+0.5% HOAc, gradient, 40–100% MeOH) to give **1** (3.3 mg). The CH_3CN solution from fraction 5 was subjected to CC (ODS, CH_3CN–H_2O, gradient, 5–100% CH_3CN) to give **4** (4.2 mg) and **2** (6.0 mg).

Compounds **2** (5.3 mg) and **4** (2.4 mg) were dissolved in CH_3CN and individually subjected to methylation (CH_2N_2, 10 days at −10 °C). The products were subjected to TLC (SiO_2, hexane–EtOAc 3:7) to give **2a** (2.8 mg), **2b** (2.2 mg), and **4a** (2.3 mg).

Fractions, derivatives, and all of the isolated compounds were analyzed by HPLC-UV-MS (phenyl, CH_3CN–H_2O 7:3, v/v) to establish their identity, composition, and purity, and to obtain their MS spectra.

*4.3.1. 3‴,4′,4‴,5,5″,7″-Hexahydroxy-7-methoxy-3,6″-biflavone (ridiculuflavone D, **1**)*

Yellow solid; m.p. 238.3–239.3 °C; $[\alpha]_D^{25}$ −6.0 (*c* 0.055, MeOH); CD (MeOH; *c* 0.040) $[\theta]_{230}$ +2.115, $[\theta]_{238}$ +2.148, $[\theta]_{260}$ +1.679 sh, $[\theta]_{270}$ +2.171, $[\theta]_{324}$ +458 sh, $[\theta]_{336}$ +767, $[\theta]_{346}$ +465 sh, $[\theta]_{365}$ 0, $[\theta]_{382}$ −227, $[\theta]_{403}$ 0; UV λ_{max}^{MeOH} nm (log ε): 262 (4.3), 334 (4.2); IR ν_{max}^{KBr} cm^{-1}: 3345, 1630, 1097, 1038; for ^{1}H and ^{13}C NMR (CD_3OD) spectra, see Table 1; HR-ESI-MS (probe) 4500 V, *m*/*z* (rel. int.): 567.0921 $[M-H]^-$ (100) (calculated for $C_{31}H_{20}O_{11}$–H = 567.0922); ESI-MS/MS (probe) 4500 V from ions at *m*/*z* 567.09, *m*/*z* (rel. int.): 401.07 $[M-H-166]^-$.

*4.3.2. 4′,5,5″,7″-Tetrahydroxy-3′,3″,4‴-trimethoxy-7-O-β:6-α-flavone-chalcone (ridiculuflavonychalcone B, **2**)*

Yellow solid; m.p. 160.5–162.5 °C; $[\alpha]_D^{25}$ −9.0 (*c* 0.046, MeOH); CD (MeOH; *c* 0.035) $[\theta]_{220}$ +3.501, $[\theta]_{260}$ +188, $[\theta]_{290}$ +770, $[\theta]_{308}$

Table 6
^{13}C and ^{1}H NMR spectroscopic data for compound **4a**

C and H	δ ^{13}C[a,b,c]	gHMBC[a]	δ ^{1}H[d,e]	2D-gNOESY
2	160.2 *s*	H-2′, H-6′		
5	161.6 *s*	OCH_3-5		
6	96.0 *d*[f]		6.33 (*d*, 2.3)[f]	OCH_3-5 or OCH_3-7
7	163.4 *s*	OCH_3-7		
8	92.2 *d*[f]		6.50 (*d*, 2.3)[f]	OCH_3-7 or OCH_3-5
10	108.4 *s*	H-6		
1′	125.4 *s*	H-3′, H-5′		
2′	129.2 *d*		7.38 (*d*, 9.0)	H-3′
3′	113.4 *d*		6.69 (*d*, 9.0)	H-2′, OCH_3-4′
4′	162.0 *s*	OCH_3-4′		
5′	113.4 *d*		6.69 (*d*, 9.0)	OCH_3-4′, H-6′
6′	129.2 *d*		7.38 (*d*, 9.0)	H-5′
2″	160.8 *s*	H-3″		
3″	107.8 *d*		6.52 (*s*)	
5″	161.2 *s*	OCH_3-5″ or OCH_3-7″		
6″	116.2 *s*	H-8″		
7″	158.2 *s*	OCH_3-7″ or OCH_3-5″		
8″	95.0 *d*		6.70 (*s*)	
9″	159.2 *s*	H-8″		
10″	112.6 *s*	H-3″, H-8″		
1‴	124.0 *s*	H-5‴		
2‴	108.5 *d*		7.28 (*d*, 2.3)	OCH_3-3‴
3‴	149.2 *s*	OCH_3-3‴, H-5‴		
4‴	151.6 *s*	OCH_3-4‴		
5‴	111.0 *d*		6.91 (*d*, 8.5)	OCH_3-4‴
6‴	119.4 *d*	H-2‴	7.45 (*dd*, 8.5, 2.3)	
OCH_3-3‴	55.8 *q*		3.92 (*s*)	H-2‴
OCH_3-4′	55.8 *q*		3.75 (*s*)	H-3′, H-5′
OCH_3-4‴	55.8 *q*		3.90 (*s*)	H-5‴
OCH_3-5	55.8 *q*		3.86 (*s*)[g]	H-6
OCH_3-5″	62.0 *q*		3.687 (*s*)[h]	
OCH_3-7	55.8 *q*		3.85 (*s*)[g]	H-8
OCH_3-7″	54.6 *q*		3.689 (*s*)[h]	H-8″

[a] Recorded in $CDCl_3$, 126 MHz.
[b] The ^{13}C NMR data were assigned with the assistance of *g*HMQC and *g*HMBC experiments.
[c] Signal not observed for C-3, C-4, C-9, and C-4″.
[d] Recorded in $CDCl_3$, 500 MHz.
[e] Multiplicities were determined with the assistance of ^{1}H–^{1}H COSY experiments, 500 MHz.
[f,g,h] Assignments may be interchangeable within the same column.

+546, $[\theta]_{328}$ +995 sh, $[\theta]_{344}$ +2.094, $[\theta]_{365}$ 0, $[\theta]_{372}$ −108 sh, $[\theta]_{388}$ −242, $[\theta]_{399}$ 0; UV λ_{max}^{MeOH} nm (logε): 308 (4.6), 342 (4.5); IR ν_{max}^{KBr} cm^{-1}: 3437, 1624, 1032; for ^{1}H and ^{13}C NMR (CD_3CN) spectra, see Tables 3 and 2, respectively; HR-ESI-MS (probe) 4500 V, *m*/*z* (rel. int.): 595.1235 $[M-H]^-$ (100) (calculated for $C_{33}H_{24}O_{11}$–H = 595.1235); ESI-MS/MS (probe) 4500 V from ions at *m*/*z* 595.12, *m*/*z* (rel. int.): 580.10 $[M-H-CH_3]^{-\cdot}$, 429.10 $[M-H-166]^-$, 414.08 $[M-H-CH_3-166]^{-\cdot}$.

4.3.3. 7″-Hydroxy-3′,3″,4′,4‴,5,5″-hexamethoxy-7-O-β:6-α-flavone-chalcone (ridiculuflavonychalcone D, 2a)

Yellow solid; m.p. 149.3–151.0 °C (Me_2CO); $[\alpha]_D^{25}$ −1.0 (*c* 0.070, MeOH); CD (MeOH; *c* 0.300) $[\theta]_{220}$ +4.244, $[\theta]_{260}$ +599, $[\theta]_{278}$ +696 sh, $[\theta]_{290}$ +954, $[\theta]_{328}$ +1.107, $[\theta]_{342}$ +1.323, $[\theta]_{365}$ 0, $[\theta]_{372}$ −78, $[\theta]_{381}$ 0; UV λ_{max}^{MeOH} nm (logε): 301 (4.2), 340 (4.1); IR ν_{max}^{KBr} cm^{-1}: 3439, 1624; for ^{1}H and ^{13}C NMR ($CDCl_3$) spectra, see Tables 3 and 2, respectively; ESI-MS (probe) 2170 V, *m*/*z* (rel. int.): 677 $[M+K]^+$ (85), 639 $[M+H]^+$ (100).

4.3.4. 3′,3″,4′,4‴,5,5″,7″-Heptamethoxy-7-O-β:6-α-flavone-chalcone (ridiculuflavonychalcone E, 2b)

Yellow solid; m.p. 148.1–149.3 °C (Me_2CO); $[\alpha]_D^{25}$ −3.0 (*c* 0.065, MeOH); CD (MeOH; *c* 0.200) $[\theta]_{220}$ +359, $[\theta]_{256}$ +47, $[\theta]_{276}$ +30, $[\theta]_{284}$ +18 sh, $[\theta]_{290}$ 0, $[\theta]_{294}$ −19, $[\theta]_{304}$ 0, $[\theta]_{346}$ +91, $[\theta]_{355}$ 0, $[\theta]_{358}$ −25, $[\theta]_{366}$ 0, $[\theta]_{372}$ +24, $[\theta]_{388}$ +135, $[\theta]_{418}$ +130; UV λ_{max}^{MeOH} nm (logε): 286 (4.0), 335 (4.0); IR ν_{max}^{KBr} cm^{-1}: 1632, 1117; for ^{1}H and ^{13}C NMR [$(CD_3)_2CO$] spectra, see Tables 3 and 2, respectively; ESI-MS (probe) 2030 V, *m*/*z* (rel. int.): 691 $[M+K]^+$ (27), 675 $[M+Na]^+$ (75), 653 $[M+H]^+$ (100).

4.3.5. (4′,5,7″-Trihydroxy-3′,3″,4‴-trimethoxy-7-O-β:6-α-flavone-chalcone)-5″-O-β-[(7″-hydroxy-3″,4‴-dimethoxy)-5″-O-8-(4′,5,7-trihydroxy-3′-methoxy-flavone)]-chalcone (ridiculuflavonylchalcone C, 3)

Yellow solid; m.p. 166.1–168.5 °C (Me_2CO); $[\alpha]_D^{25}$ −2.0 (*c* 0.050, MeOH); CD (MeOH; *c* 0.200) $[\theta]_{234}$ +808, $[\theta]_{258}$ +391 sh, $[\theta]_{284}$ +175 sh, $[\theta]_{311}$ 0, $[\theta]_{318}$ −41, $[\theta]_{323}$ 0, $[\theta]_{332}$ +105, $[\theta]_{342}$ +122, $[\theta]_{352}$ +110, $[\theta]_{363}$ 0, $[\theta]_{370}$ −58, $[\theta]_{388}$ −69, $[\theta]_{401}$ 0, $[\theta]_{420}$ −12; UV λ_{max}^{MeOH} nm (logε): 326 (3.9), 337 (3.9); IR ν_{max}^{KBr} cm^{-1}: 3444, 1636, 1097; for ^{1}H and ^{13}C NMR (CD_3CN) spectra, see Tables 5 and 4, respectively; HR-ESI-MS (probe) 4500 V, *m*/*z* (rel. int.): 1191.2527 $[M-H]^-$ (14) (calculated for $C_{66}H_{48}O_{22}$–H = 1191.2528), 595.1242 (100); ESI-MS/MS (probe) 4500 V from ions at *m*/*z* 595.12, *m*/*z* (rel. int.): 580.11 $[M-H-CH_3]^{-\cdot}$ (**7**), 429.10 $[M-H-166]^-$ (41), 414.08 $[M-H-CH_3-166]^{-\cdot}$ (50); HR-ESI-MS (probe) 4500 V, *m*/*z* (rel. int.): 1215.2535 $[M+Na]^+$ (1) (calculated for $C_{66}H_{48}O_{22}$+Na = 1215.2529), 963.18 (5), 919.15 (11), 875.16 (20), 831.17 (34), 787.17 (58), 743.18 (65), 699.41 (65), 655.11 (94), 611.15 (100); ESI-MS (probe) 2660 V, *m*/*z* (rel. int.): 876 (5), 788 (16), 772 (16), 728 (23), 611 (24), 597 (43), 595 (63), 552 (24), 507 (34), 463 (48), 437 (33), 421 (100).

4.3.6. 3‴,4′,4‴,5,5″,7,7″-Heptamethoxy-3,6″-biflavone (ridiculuflavone E, 4a)

Yellow solid; m.p. 193.3–196.1 °C (Me_2CO); $[\alpha]_D^{25}$ −1.0 (*c* 0.060, MeOH); CD (MeOH; *c* 0.300) $[\theta]_{220}$ +1.659, $[\theta]_{260}$ +199, $[\theta]_{272}$ +161 sh, $[\theta]_{288}$ +37 sh, $[\theta]_{310}$ 0, $[\theta]_{318}$ −60, $[\theta]_{321}$ 0, $[\theta]_{330}$ +229, $[\theta]_{344}$ +231, $[\theta]_{363}$ 0; UV λ_{max}^{MeOH} nm (logε): 264 (4.1), 326 (4.1); IR ν_{max}^{KBr} cm^{-1}: 1630; for ^{1}H and ^{13}C NMR ($CDCl_3$) spectra, see Table 6; ESI-MS (probe) 2030 V, *m*/*z* (rel. int.): 691 $[M+K]^+$ (39), 675 $[M+Na]^+$ (100), 653 $[M+H]^+$ (25).

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