http://dx.doi.org/10.5935/0103-5053.20150101

J. Braz. Chem. Soc., Vol. 26, No. 7, 1527-1531, 2015.
Printed in Brazil - ©2015 Sociedade Brasileira de Química
0103 - 5053 $6.00+0.00

Short Report

A New Biflavonoid from *Schinopsis brasiliensis* (Anacardiaceae)

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Phytochemical investigation of the MeOH extract of *Schinopsis brasiliensis* Engl. stems led to the isolation of the new biflavanone (2*R**,3*R**,2''*R**,3''*R**)-7-hydroxy-4'-methoxy-flavanone-(3→3'')-3''',7''-dihydroxy-4'''-methoxy-flavanone and 4,2',4'-trihydroxychalcone-(3→*O*→4'')-2''',4'''-dihydroxychalcone, whereas methyl gallate, gallic acid, (6*R*,9*R*)-megastigma-4-en-3-one-9-*O*-β-glucopyranoside, quercetin-3-*O*-β-D-xylopyranoside and tricetin-3'-*O*-β-D-glucopyranoside were isolated from the MeOH extract of leaves. Their chemical structures were elucidated using spectroscopic methods and comparison with the literature data. Both biflavonoids showed weak inhibition activities of acetyl and butyrylcholinesterase enzymes when compared with eserine.

Keywords: biflavanone, bichalcone, *Schinopsis brasiliensis*, Anacardiaceae

Introduction

The genus *Schinopsis* (Anacardiaceae) is composed of fourteen species, and their woods are mainly used in the leather tanning and wood industries due their resistance to degradation by humidity, attack by insects and ultraviolet radiation.[1] *Schinopsis brasiliensis* Engl. is a large tree that is popularly known in the Brazilian northeastern region as "baraúna". The local population employs its leaves and bark in the treatment of inflammation, sexual impotence, diarrhea, and cattle intestinal worms.[2] Unfortunately, it is presently considered an endangered species due to the extensive use of its wood in house construction.

Species of Anacardiaceae are known to produce toxic or allergenic alkylphenols, especially those present in *Rhus*, *Lannea* and *Anacardium* species. Biflavonoids are another class of compounds present in different genera of this family such as *Anacardium*, *Cotinus*, *Gomphrena*, *Myracrodruon*, *Rhus*, *Schinopsis*, *Schinus* and *Semecarpus*.[3] Thus, these two classes of compounds seem to be chemotaxonomic markers for this family. To date, there is just one previous work describing the presence of *n*-alkylphenols in *S. brasiliensis*.[4]

The present work describes the results of a phytochemical investigation, including acetylcholinesterase (AChE) assays, of active chloroform soluble fraction of the MeOH extracts of the stems and leaves obtained after partition of the crude methanol extract of *S. brasiliensis*. Usual chromatographic methods applied to the stem chloroform fraction led to isolation of the biflavonoids (2*R**,3*R**,2''*R**,3''*R**)-7-hydroxy-4'-methoxy-flavanone-(3→3'')-3''',7''-dihydroxy-4'''-methoxy-flavanone (**1**) and 4,2',4'-trihydroxychalcone-(3→*O*→4'')-2''',4'''-dihydroxychalcone (**2**) (Figure 1). The dichloromethane-soluble fraction of the MeOH extract of the leaves that also showed inhibition of the AChE in TLC (thin layer chromatography) monitoring test furnished two flavonoids (**3** and **4**), megastigmane (**5**), gallic acid (**6**) and methyl gallate (**7**) (Figure 1). Compound **1** is a newly discovered compound, and compounds **2**, **4** and **5** were isolated here for the first time in the Anacardiaceae family. Finally, this is the first report of the isolation of compound **3** in the *Schinopsis* genus.

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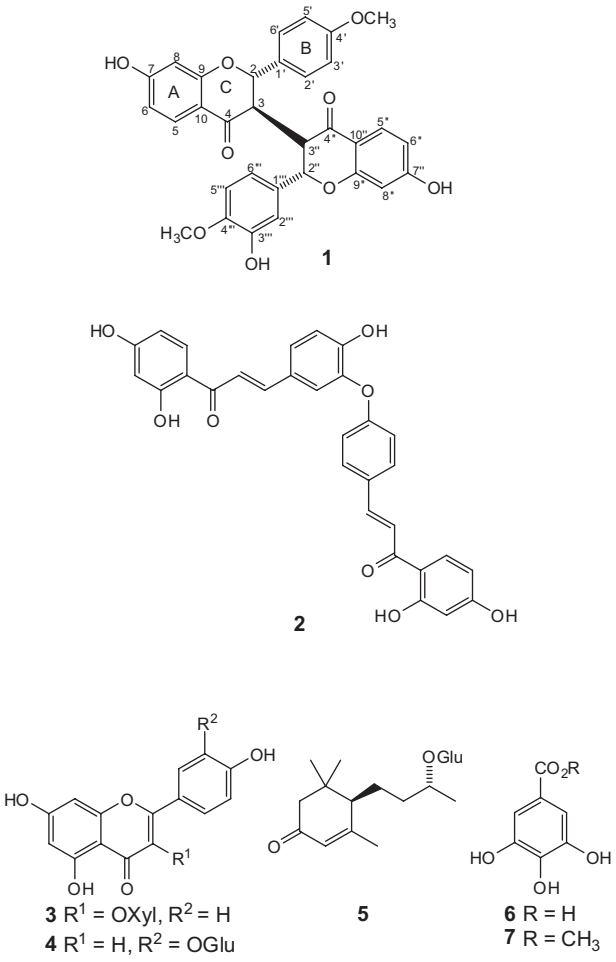

Figure 1. Compounds isolated from *Schinopsis brasiliensis*.

Experimental

General

^{1}H NMR, NOESY (nuclear Overhauser effect spectroscopy) and HMBC (heteronuclear multiple bond correlation) spectra were obtained using a Bruker AC-500 instrument operating at 500 MHz for ^{1}H and 125 MHz for ^{13}C. ^{13}C (PND and DEPT) NMR spectra were obtained on a Varian Gemini 2000 instrument operating at 300 MHz for ^{1}H and 75 MHz for ^{13}C. In all spectra were employed $CO(CD_3)_2$, C_5D_5N, and CD_3OD as the solvent and reference. High-resolution electrospray ionisation mass spectrometry (HRESIMS) spectra were recorded in negative mode using a micrOTOF system from Bruker Daltonics. Optical rotation was performed using a microcell (Perkin-Elmer, model 343 polarimeter). The inhibition of AChE was measured using a microplate reader (Biotek, model EL 800). Column chromatography (CC) was conducted on silica gel 60 (Acros) or Sephadex LH-20 (Sigma). The fractions were monitored using TLC on silica gel, and the spots were visualized with iodine fumes and UV light (254/366 nm). All reagents and enzymes were purchased from Sigma.

Plant material

The stems and leaves of *S. brasiliensis* were collected in the surroundings of Valente-BA, Brazil. The plant was identified by Prof Maria L. S. Guedes, and a voucher was deposited at the Herbarium Alexandre Leal Costa at the Universidade Federal da Bahia (UFBA) under number 038056.

Extraction and isolation

The stems (2 kg) and leaves (579 g) were separated, dried and powdered. Then, they were exhaustively extracted with MeOH at room temperature, furnishing crude extracts (56.3 and 79.2 g, respectively).

The methanol extract of the stems was subjected to solvent-partition using hexane, chloroform, ethyl acetate and butanol. The resulting $CHCl_3$ soluble fraction (6.65 g) was subjected to CC over silica gel 60 eluted with different mixtures of $CHCl_3$:EtOAc (9:1 → 1:1). The column fractions (50 mL each) obtained were further combined into 7 fractions based on the TLC profile. The sixth fraction (1054.0 mg) eluted with $CHCl_3$:EtOAc (6:4) was subjected to another CC over silica gel 60 and was eluted with a mixture of CH_2Cl_2:acetone:acetic acid (9:1:0.01), fractions of 11 mL each to afford a yellow solid composed of a mixture of compounds **1** and **2**. This mixture (24.4 mg) was subjected to CC over Sephadex LH-20 and sequentially eluted with 50 mL of CH_2Cl_2:hexane (1:1), CH_2Cl_2:acetone (9:1), CH_2Cl_2:MeOH (9:1; 4:1; 1:1) and methanol. Using this procedure, biflavonoids **1** (10.5 mg) and **2** (12.4 mg) were isolated by elution with CH_2Cl_2-MeOH (4:1) and (1:1), respectively.

The crude methanolic extract of the leaves (79.2 g) was dissolved in MeOH:H_2O (7:3) and subjected to liquid-liquid extraction with CH_2Cl_2, yielding 6.71 g of CH_2Cl_2 soluble compounds. The CH_2Cl_2 fraction was subjected to CC on silica gel using $CHCl_3$ and mixtures of $CHCl_3$:MeOH (95:5→7:3, fractions of 50 mL). From the fraction eluted with $CHCl_3$:MeOH 95:5 and 8:2, methyl gallate **7** (2 g) and gallic acid **6** (33 mg) were obtained. The fraction eluted with $CHCl_3$:MeOH (7:3) furnished tricetin-3'-*O*-β-D-glucopyranoside **4** (35 mg) after recrystallization in $CHCl_3$:MeOH (1:1). The fraction eluted with $CHCl_3$:MeOH (85:15) was subjected to Sephadex LH-20 gel permeation, eluting with CH_2Cl_2:MeOH (1:1), and this procedure afforded quercetin-3-*O*-β-D-

xylopyranoside **3** (46.8 mg) and (6*R*,9*R*)-megastigma-4-en-3-one 9-*O*-β-glucopyranoside **5** (21.5 mg).

(2*R**,3*R**,2"*R**,3"*R**)-7-Hydroxy-4'-methoxy-flavanone-(3→3")-3"',7"-dihydroxy-4"'-methoxy-flavanone (**1**)

Yellow amorphous solid; $[\alpha]_D^{25}$ = +12 (*c* 0.3, MeOH); NMR data: see Table 1. HRESIMS *m/z* $[M - H]^-$ 553.1494 (calc. for $C_{32}H_{26}O_9$, 553.1499).

Acetylcholinesterase (AChE) and butyrylcholinesterase (BuChE) inhibition tests

The compounds and $CHCl_3$ soluble fraction of the MeOH extract of stems of *S. brasiliensis* were evaluated using bioautographic TLC test for acetylcholinesterase activity, which showed positive spots using a methodology based in the colorimetry, described by Marston *et al*.[5] The *in vitro* quantification of AChE and BuChE inhibition by compounds **1** and **2** were determined by spectrophotometry using a colorimetric method adapted from Ellman *et al*.[6] Briefly, 15 µL of acetylcholine iodide or butylcholine and 62 µL of DTNB (5,5'-dithiobis-(2-nitrobenzoic acid) (3 mmol L^{-1}) were incubated with 5 µL of pure compound (500-4 µmol L^{-1}), eserine (positive control), or buffer for 15 min in a 96-well microplate. The reaction was then started by adding 12 µL of enzyme in buffer (0.22 U mL^{-1}). The change in absorbance was recorded at 405 nm using the microplate reader. DTNB, AChE or BuChE and the substrate were dissolved in 0.1 mol L^{-1} sodium phosphate buffer (pH 7.4). All samples were analyzed in triplicate.

Results and Discussion

The active chloroform soluble fraction obtained from the MeOH crude extract partition of *S. brasiliensis* stems, was submitted to various chromatographic procedures in silica gel and Sephadex LH-20 to afford compounds **1** and **2**, and their structures were elucidated by HRMS (high resolution mass spectrometry) and mono- and bi-dimensional NMR experiments. The molecular formula of **1** ($C_{32}H_{26}O_9$) was obtained from the peak $[M - H]^-$ at *m/z* 553.1494 observed in the negative HRESIMS (calc. 553.1499). The ^{13}C NMR and DEPT (distortionless enhancement by polarization transfer) spectra indicated the presence of two methoxyl groups, thirteen aromatic methine carbons, six oxygenated aromatic carbons, two methine sp^3 carbons and two similar acyl groups (δ 191.6 and 191.5). These data were consistent with the biflavanone skeletal units, which are linked by the C-ring flavonoid. The ^1H NMR spectrum showed signals corresponding to two sets of methine [δ_H 2.63 (dd; 1.4, 12.2 Hz) and 2.76 (dd; 1.4, 12.2 Hz)] and oxybenzylic [δ_H 5.98 (d; 12.2 Hz) and 5.92 (d; 12.2 Hz)] hydrogens. These observations were corroborated by the four signals in the ^{13}C NMR spectra at δ_C 51.6, 51.8, 84.3 and 85.0. These resonances and the coupling constants observed in the ^1H NMR spectrum suggested the presence of two cyclic systems bonded by the two C-rings of the biflavanone units. The coupling constants displayed by H-2 and H-2'' indicated that these hydrogens are in the pseudo-axial position with the vicinal hydrogens H-3 and H-3'', respectively. This finding and the similarities of the ^{13}C chemical shifts of C-2/C-2'' and C-3/C-3'' indicated that both units show the same relative configurations. The ^1H NMR and the ^1H-^1H COSY (correlation spectroscopy) spectra permitted the assignment of the four aromatic rings with two AMX, one ABX, and one AA'BB' set of hydrogen coupling systems. The substitution pattern of the A-rings of the two units of compound **1** was shown by the peaks, integrating for 2 H each, displayed as doublets at δ_H 6.32 (2.3 Hz) and 7.71 (8.7 Hz) and a double doublet at δ_H 6.58 (2.3 and 8.7 Hz). These signals indicated that the A-rings of the biflavanone moieties are substituted in C-7 and C-7'' by the hydroxyl groups. The B-rings of this compound were identified as being 1,4-disubstituted and 1,3,4-trisubstituted aromatic rings based on the ^1H and ^{13}C NMR data. The doublets at δ_H 7.07 and 6.94 (2H each) and the methines at δ_C 130.0 and 114.8 were indicative of a 1,4-disubstituted aromatic system. The set of signals in the range of δ_H 6.62-6.95, in addition to the characteristic resonances observed in the ^{13}C NMR (δ_C 131.0, 115.1, 147.7, 149.2, 112.2 and 120.2), indicated the presence of a 1,3,4-trisubstituted aromatic ring with oxygenated substitution in positions 3 and 4.

The assignment of all of the hydrogenated carbons was made possible by observing the heteronuclear correlations plotted in the HMQC (heteronuclear multiple quantum correlation) experiment. The location of each substituent in the flavonoid skeleton was assigned by correlations observed in the HMBC spectrum and corroborated by the NOESY experiment. The correlations of the methoxy hydrogens at δ_H 3.84 and C-4', besides the peaks at δ_H 5.98 (H-2) and δ_H 6.94 (H-3'/H-5'), and C-1' (δ_C 130.1) permitted the identification of the B-ring of unit I as a 1,4-disubstituted aromatic ring and unequivocal assignment of all of the ^1H and ^{13}C resonances. The correlations observed in the NOESY spectrum between the methoxyl hydrogens, H-3'/H-5', H-2'/-6' and H-2 corroborated the above proposition. The long range correlations also allowed to confirm that the C-4"' and C-3"' of the B-ring of unit II bore a methoxyl and hydroxyl group, respectively. These findings were possible mainly due to the two key correlations observed in the HMBC spectra. Firstly, the

correlation of methoxyl hydrogens at δ_H 3.84 and the peak at δ_C 161.2 permitted to assign C-4' of unit I. The singlet at δ_H 3.89 (4'''-OCH_3), the peaks centered near δ_H 6.63 (H-6'''/H-2'''), the doublet at δ_H 6.95 (H-5''') correlating with the carbon at δ_C 149.2 (C-4''') corroborated with the proposed substitution on unit II. The correct position of the methoxyl group bearing the C-4''' was unequivocally attributed by the NOESY interaction of its hydrogens and H-5''' displayed as a doublet. The correlations observed in the NOESY and HMBC spectra also corroborated the unusual C-3→C-3'' connection between the biflavanone moieties (Figure 2), permitting the identification of **1** as the new (2*R**,3*R**,2''*R**,3''*R**)-7-hydroxy-4'-methoxy-flavanone-(3→3'')-3''',7'''-dihydroxy-4'''-methoxy-flavanone.

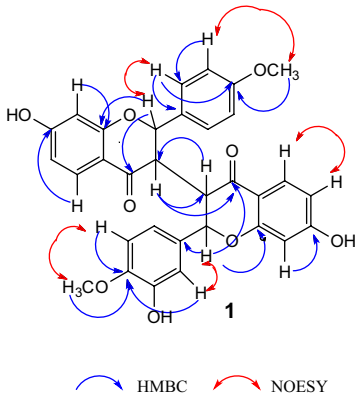

Figure 2. Key HMBC and NOESY correlations of compound **1**.

The structure of **2** (Figure 1) was established by HRESIMS, 1D and ^{1}H NMR spectral analyses, as well as by comparison with literature data.[7] This finding is the second report of 4,2',4'-trihydroxychalcone-(3→*O*→4'')-2''',4''',-dihydroxychalcone (**2**) as a natural product. This compound was isolated for first time from *Luxemburgia octandra* St. Hil (Ochnaceae), and it was previously named luxenchalcone.[7] The soluble-dichloromethane fraction of the MeOH extract from the leaves of *S. brasiliensis* yielded compounds **3**-**7**, whose structures were also established from comparison of their spectroscopic data and optical rotation, with those reported in literature.[8-10]

Both extracts showed acetylcholinesterase-inhibiting activities employing Marston's TLC colorimetric method based on the hydrolysis of naftil acetate.[5] However, when the isolates were submitted to the Elmann's test employing acetyl and butyrylcholinesterase, only compounds **1** and **2** showed weak inhibition of these enzymes when compared with serine (Table 2). Despite the isolated biflavonoids present weak inhibitory activities, there are few examples in literature of this class of compounds showing AChE or BuChE activities.[11]

Table 1. NMR data [500 (^{1}H) and 75 MHz (^{13}C), $(CD_3)_2CO$, δ (ppm), *J* (Hz)] for compound **1**

Position	δ_H		δ_C	HMBC
2	5.98	(d; 12.2)	84.8	C-3, C-4, C-9, C-1'
3	2.76	(dd; 1.4, 12.2)	51.6	C-2, C-4, C-1', C-3'' C-4''
4	–		191.6	
5	7.71	(d; 8.7)	129.8	C-4, C-6, C-9
6	6.58	(dd; 2.3, 8.7)	111.4	C-7, C-8, C-10
7	–		165.7	
8	6.32	(d; 2.3)	103.4	C-6, C-7, C-9, C-10
9	–		164.1	
10	–		114.9	
1'	–		130.1	
2'/6'	7.07	(d; 7.6)	130.0	C-2, C-1', C-3'/C-5', C-4'
3'/5'	6.94	(d; 7.6)	114.8	C-1', C-2'/C-6', C-4'
4'	–		161.2	
2''	5.92	(d; 12.2)	85.0	C-3'', C-4'', C-9'', C-1''', C-6'''
3''	2.63	(dd; 1.4, 12.2)	51.8	C-4, C-3', C-2'', C-4'', C-1'''
4''	–		191.5	
5''	7.71	(d; 8.7)	129.8	C-4'', C-6'', C-9''
6''	6.58	(dd; 2.3, 8.7)	111.4	C-7'', C-8'', C-10''
7''	–		165.7	
8''	6.32	(d; 2.3)	103.4	C-6'', C-7'', C-9'', C-10''
9''	–		164.1	
10''	–		114.9	
1'''	–		131.0	
2'''	6.62	(d; 2.0)	115.1	C-2'', C-3''', C-4''', C-6'''
3'''	–		147.7	
4'''	–		149.2	
5'''	6.95	(d; 8.0)	112.2	C-1''', C-3''', C-4''', C-6'''
6'''	6.63	(dd; 2.0, 8.0)	120.2	
4'-OCH_3	3.84	(s)	55.6	C-4'
4'''-OCH_3	3.89	(s)	56.3	C-4'''

Carbon multiplicities obtained by DEPT experiments.

Table 2. IC_{50} values of acetyl and butyrilcholinesterase inhibition by compounds **1** and **2**

Compound	IC_{50} / (μmol L^{-1})	
	Inhibition of acetylcholinesterase	Inhibition of butyrylcholinesterase
1	2932.3	5375.2
2	4729.89	3878.3
Eserine	271.2	138.6

Conclusions

The present study of leaves and stems of *Schinopsis brasiliensis* to the isolation of biflavonoids (2*R**,3*R**,2''*R**,3''*R**)-7-hydroxy-4'-methoxy-flavanone-(3→3'')-3''',7''-dihydroxy-4'''-methoxy-flavanone (**1**) and 4,2',4'-trihydroxychalcone-(3→*O*→4")-2"',4"',-dihydroxychalcone (**2**) which showed weak AchE and BuChE activities. The compounds isolated from leaves (**3**-**7**) are not active. This is the first occurence of these flavonoids in Anacardiaceae family and, besides, compound **1** presents an unusual linkage of the two flavonoid moieties.

Supplementary Information

Supplementary information is available free of charge at http://jbcs.sbq.org.br as PDF file.

Acknowledgements

The authors are grateful to the CNPq, CAPES and FAPESB/PRONEM for fellowships and grants. We are also indebted to Prof Edilberto R. Silveira and PhD Daniel E. Uchoa (CENAUREM - UFC) for the 2D-NMR spectra acquisition.

References

1. Paes, J. B.; Morais, V. M.; Lima, C. R.; *Rev. Árvore* **2004**, *28*, 275; Rizini, C. T. In *Árvores e Madeiras Úteis do Brasil. Manual de Dendrologia Brasileira*, 2ª ed.; Ed. Blucher: São Paulo, 1978, p. 296; Williams, R. S.; Miller, R.; Gangstad, J.; *Wood Fiber Sci*. **2001**, *33*, 618.
2. Albuquerque, U. P.; Medeiros, P. M.; Almeida, A. L. S.; Monteiro, J. M.; Lins Neto, E. M. F.; Melo, J. G.; Santos, J. P.; *J. Ethnopharmacol*. **2007**, *114*, 325.
3. Correia, S. J.; David, J. M.; David, J. P.; *Quim. Nova* **2006**, *29*, 1287.
4. Cardoso, M. P.; David, J. M.; David, J. P.; *Nat. Prod. Res.* **2005**, *19*, 431.
5. Marston, A.; Kissling, J.; Hostettmann, K.; *Phytochem. Anal.* **2002**, *13*, 51.
6. Ellman, G. L.; Courtney, K. D.; Andres, V.; Featherstone, R. M.; *Biochem. Pharmacol*. **1961**, *7*, 88.
7. Carvalho, M. G.; Alves, C. C. F.; Silva, K. G. S.; Eberlin, M. N.; Werle, A. A.; *J. Braz. Chem. Soc.* **2004**, *15*, 146.
8. Baranowska, M. K.; *Acta Pol. Pharm*. **2004**, *61*, 199.
9. Yan, X.; Murphy, B. T.; Hammond, G. B.; Vinson, J. A.; Neto, C. C.; *J. Agric. Food Chem*. **2002**, *50*, 5844.
10. Takeda, Y.; Zhang, H.; Masuda, T.; Honda, G.; Otsuka, H.; Sezik, E.; Yesilada, E.; Sun, H.; *Phytochemistry* **1997**, *44*, 1335.
11. Rajachan, O.; Kanokmedhakul, S.; Nasomjai, P.; Kanokmedhakul, K.; *Nat. Prod. Res*. **2014**, *28*, 268.

Submitted: January 28, 2015
Published online: April 28, 2015