New iodine derivatives of flavonol and isoflavone

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ABSTRACT

The reaction of the flavonol 3,7,3′, 4′-tetra-O-methylquercetin (**1**) and of the isoflavone 7,4′-di-O-methylgenistein (**2**) with alkaline iodine in methanol afforded four new iodine derivatives: 8-iodo-5-hydroxy-3,7,3′, 4′-tetramethoxy-flavone (**1a**) and 6-iodo-5-hydroxy-3,7,3′, 4′-tetramethoxyflavone (**1b**) from **1**; **2** afforded a mixture of two compounds, identified as a racemic mixture of (±)-*trans*-5-hydroxy-2,3,7,4′-tetramethoxy-8-iodo-isoflavanone (**2a**) and (±)-*trans*-5-hydroxy-2,3,7,4′-tetramethoxy-6,8-diiodo-isoflavanone (**2b**). The formation of these different products reveals a significant difference involving the chemical interaction between the reactive site of α, β-unsaturated ketones of flavonol and isoflavone under the tested reaction conditions (using I_2/KOH/MeOH). Furthermore, the *trans* stereoselectivity is noteworthy in the nucleophylic addition of methanol at the isoflavone α, β-unsaturated system. The structures were identified on the basis of spectral data, mainly 1D and 2D NMR and mass spectra.

Key words: flavonol, isoflavone, iodoflavonoid, iodoisoflavonoid, iodine derivatives.

INTRODUCTION

In previous reports we have described the isolation and identification of natural flavonoids in Solanaceae (Silva 2002, Silva et al. 2004) and Leguminosae (Silva et al. 2006, 2007), preparation of some derivatives and made the unambiguous proton and carbon-13 chemical shifts assignments (Carvalho et al. 2006). Citations concerning the synthesis (Guo-Qiang and Zhong 1997, Zembower and Zhang 1998, Quintin and Lewing, 2004, Bekker et al. 1998) and biological importance of flavonoids and biflavonoids besides incorporation of any

groups to improve those activities have been frequently observed (Dejjermm 1997, Paulo and Mota-Filipe 2006). The iodination of natural flavones is a procedure that has been used to obtain some useful intermediates in the synthesis of biflavonoids (Zheng et al. 2004, Ali and Ilyas 1986).

Halogenated derivatives of natural flavonoids are relatively rare in the literature, particularly with the iodine derivate, whose synthetic utility is largely due to the increase in reactivity of this particular halogen, as in nucleophilic aromatic substitutions (Yaipakdee and Robertson 2001). Iodine derivatives are described in the synthesis of biflavonoids, by using expensive reagents difficult to obtain (Rho et al. 2001, Bovicelli et al. 2001).

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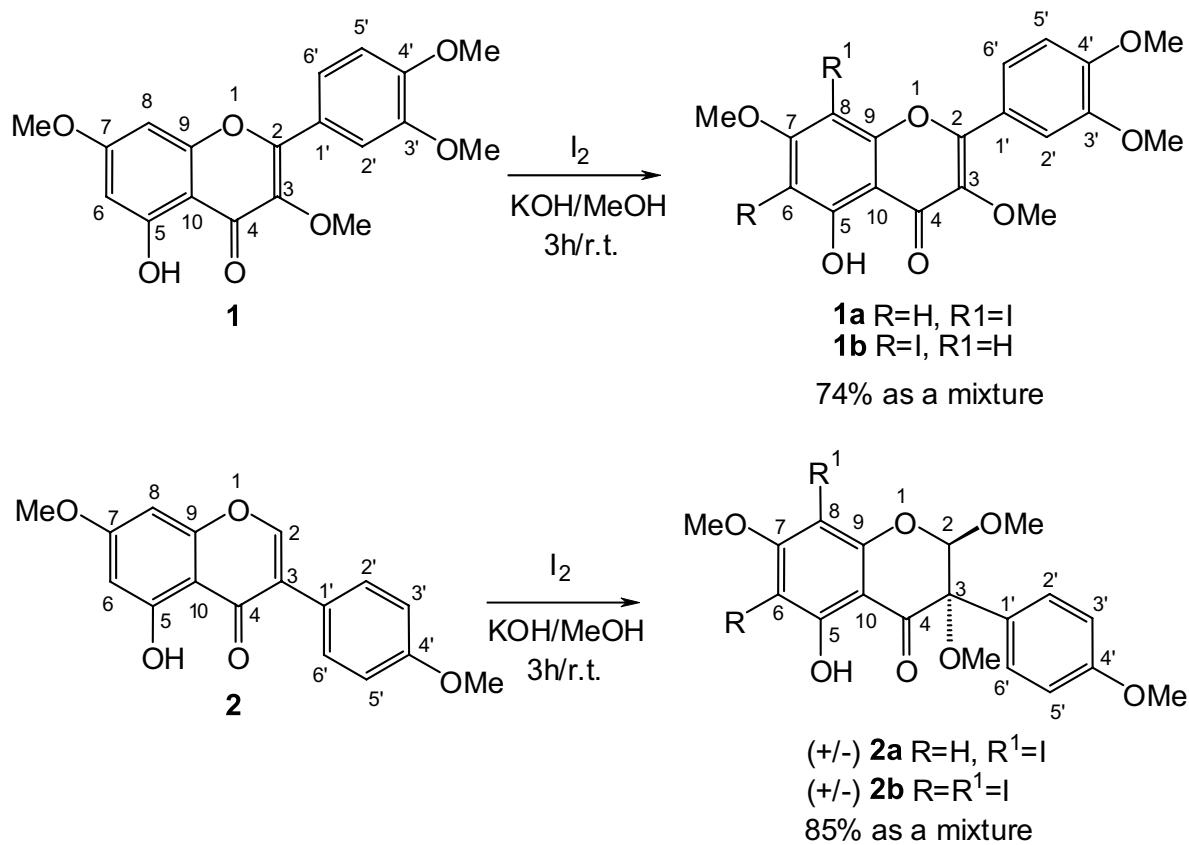E-mail: mgeraldo@ufrrj.br

Fig. 1 – Structure and reactions of flavonoids **1** and **2**.

In this paper we describe the first iodination reaction of two flavonoids (Fig. 1), the flavonol quercetin 3,7,3′, 4′-tetramethyl ether (**1**, 3,7,3′, 4′-tetra-*O*-methyl-quercetin, retusin) isolated from *Solanum* species (Silva 2002, Silva et al. 2004) and the isoflavone 7,4′-di-*O*-methylgenistein (**2**, 5-hydroxy-4′, 7-dimethoxyisoflavo-ne) obtained by selective methylation of bichanin A (5,7-dihydroxy-4′-methoxyisoflavone) isolated from *Andira* species (Silva et al. 2006, 2007), using iodine as an inexpensive and easily available reagent. These flavonoids have C-6 or C-8 positions as two sites for electrophylic substitutions. The functionality of similar nucleus in synthesis of bichalcones and other biflavonoids have been reported (Ali and Ilyas 1986). This work led us to synthesize four new iodine derivatives: 5-hydroxy-8-iodo-3,7,3′, 4′-tetramethoxyflavone (**1a**) and the C-6 corresponding regioisomer (**1b**) from **1** in 74% yield and **2** afforded a mixture constituted by (±)-*trans*-5-hydroxy-2,3,7,4′-tetramethoxy-8-iodo-isoflavanone (**2a**)

and (±)-*trans*-5-hydroxy-2,3,7,4′-tetramethoxy-6,8-di-iodo-isoflavanone (**2b**) in 85% yield. The products structures were identified on the basis of spectral data, mainly 1D and 2D NMR and mass spectra. The 1D and 2D NMR spectra were also used to the complete ^{1}H and ^{13}C chemical shift assignments of the four new products. These new derivatives of natural flavonoids, **1a**, **1b**, **2a** and **2b**, can be used as intermediate in synthetic procedure and to include useful group to study the mechanism of flavonoids biological activity.

MATERIALS AND METHODS

GENERAL EXPERIMENTAL PROCEDURE

NMR: Bruker DRX-500 (500 MHz for ^{1}H and 125 MHz for ^{13}C) and Bruker AMX-300 (300 MHz for ^{1}H and 75 MHz for ^{13}C) were used to obtain the 1D and 2D ^{1}H and ^{13}C NMR spectra and Bruker AC-200 was used to make the NOEDIFF experiments, in CDCl$_3$ as sol-
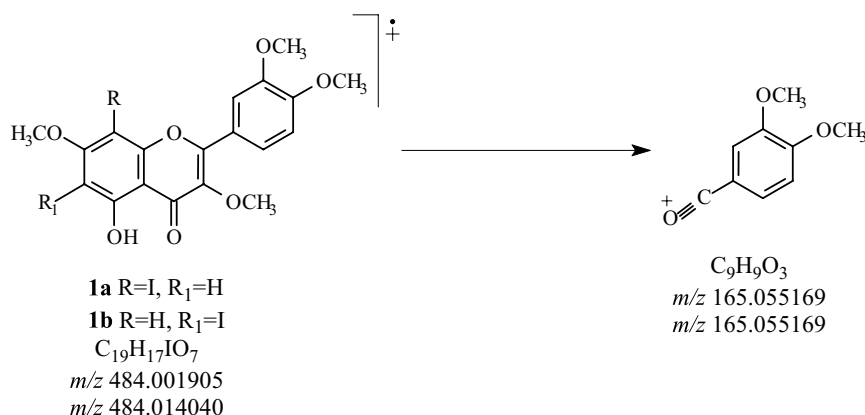
Fig. 2 – Proposed fragmentation patterns for **1a/1b** derivatives based on the HREIMS.

vent and the signals at δ_H 7.24 (CHCl$_3$) and δ_C 77.00 (CDCl$_3$) as internal standards. The LRMS were obtained on a GC-MS Varian Saturn 2000 with ion trap and IE ionization, at 70 eV. The HRESI-MS were recorded on a VG 7070E-HF spectrometer using methanol:H$_2$O, Ar as CAD, DE 20 eV for MS and 45 eV for MS-MS using methanol:H$_2$O/formic acid in positive mode. The reactions were monitored by aluminum-backed silica gel TLC plates and visualized under UV (λ_{max} 254 nm) or using 1% AlCl$_3$ in ethanol as revealing spray solution.

SYNTHESIS OF THE COMPOUNDS

Derivative **2** (Fig. 1) was prepared by treating a methanol solution of biochanin A (4′-O-methylgenistein, 50.0 mg) with ethereal diazomethane solution followed by solvent evaporation (Silva et al. 2006, 2007). The natural flavonoid **1** (Fig. 1) (Silva 2002) (20.0 mg, 0.056 mMol) and derivative **2** (28.0 mg, 0.093 mMol), were dissolved in 10 mL of methanol, mixed with potassium hydroxide (25.0 mg in each reaction), under continuous stirring, and to this mixture iodine was added in small portions to slight excess. The solutions were kept at room temperature under stirring for 3h. Aliquots of the reaction mixture were periodically analyzed by TLC until complete reaction. The organic solvents were removed under reduced pressure, and the remaining aqueous solution was extracted with dichloromethane (3 × 15 mL). The combined organic solutions were dried with anhydrous sodium sulfate and the solvent was evaporated under reduced pressure. Reaction of **1** (Fig. 1) yielded a brown-yellowish gum (**1a** + **1b**, 20.0 mg, 74%) and the reaction

of **2** (Figs. 1 and 3) yielded a yellow gum as a mixture of **2a** and **2b** (23.8 mg, 85%).

RESULTS AND DISCUSSION

The flavonoid 3,7,3′, 4′-tetramethylquercetin (**1**) afforded a mixture of 8-iodo-3,7,3′, 4′-tetramethylquercetin (**1a**) and 6-iodo-3,7,3′, 4′-tetramethylquercetin (**1b**) in a 3(**1a**):1(**1b**) ration, as determined by inspection of ^{1}H NMR data. The reaction afforded a mixture of regioisomers **1a** and **1b** involving the C-8 and C-6 positions, respectively, reflecting the higher reactivity of the C-8 position under the experimental conditions (Fig. 1). The HREIMS of **1a** and **1b** mixture gave [M]$^+$ at m/z 484.01404 corresponding to the molecular formula C$_{19}$H$_{17}$IO$_7$ (Calcd. for C$_{19}$H$_{17}$IO$_7$ m/z 484.00190). From the molecular ion m/z 484.014040 was obtained a fragment of m/z 165.055169 as base peak corresponding to the fragment involving the B-ring (Fig. 2).

The ^{1}H NMR spectrum of **1a/1b** mixture showed signals of **1a** (component present in major proportion) in the aromatic region corresponding to four hydrogen atoms and the hydrogen-bonded absorption at δ_H 12.92 (s) of hydroxyl group at C-5. The singlet at δ_H 6.42 was attributed to H-6 at A-ring. The chemical shifts, the multiplicity and the coupling constants values (J) of the signals in δ_H 8.06 (dd, J=1.8 and 8.7 Hz, H-6′), δ_H 7.98 (d, J=1.8 Hz, H-2′), and 7.03 (d, J=8.7 Hz, H-5′) allowed to recognize the ABC system of B-ring with substitutions in the positions 3′ and 4′ (Table I). The 2D ^{1}H-^{1}H-COSY confirmed the interactions spin-spin for these
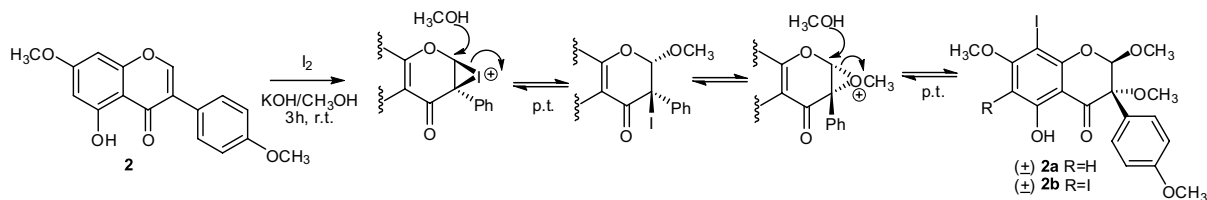
Fig. 3 – Proposed pathway to the observed *trans* stereochemistry in isoflavone iodination.
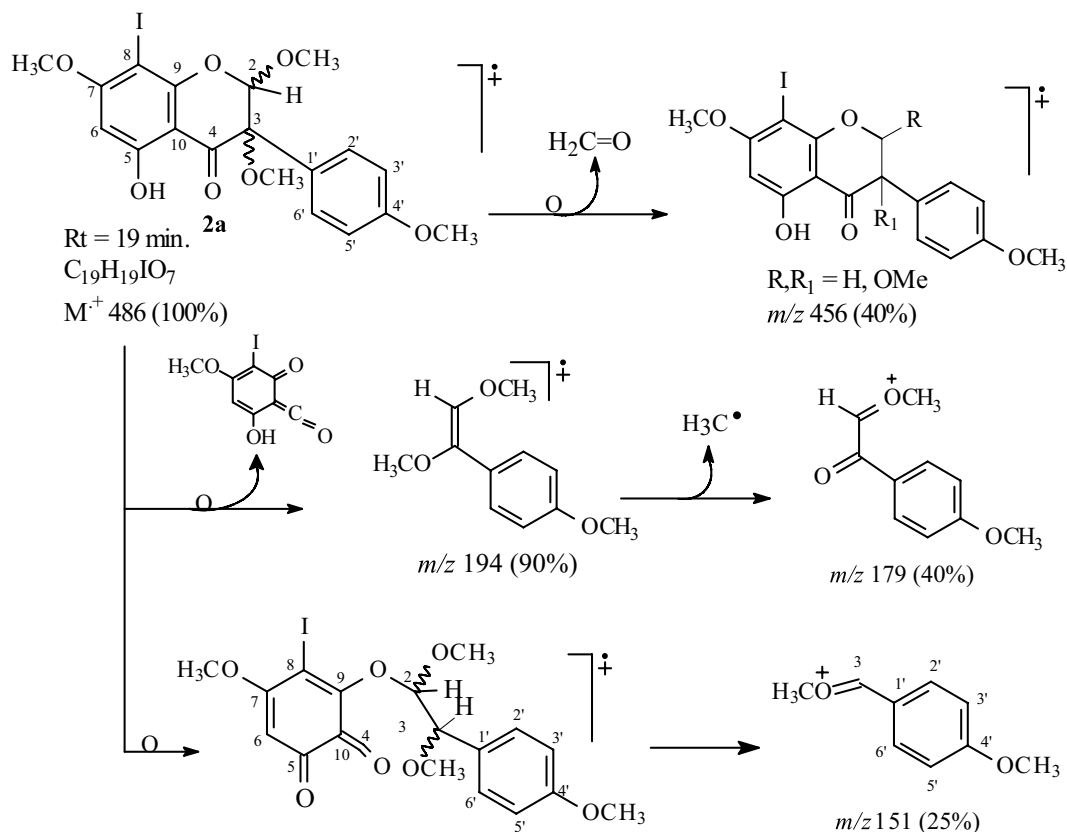

Fig. 4 – Proposed fragmentation patterns for **2a** (only peaks classified as principals).

hydrogen atoms. These data and the singlet between 3.91-4.0 ppm corresponding to four methoxyl groups allowed us to characterize the product as a 5-hidroxy-tetramethoxy-flavonol. The ^{13}C NMR-PENDANT spectrum revealed signals for nineteen carbons, being four methynes, four methoxyl groups, and eleven quarternary carbon atoms. The 2D ^{1}H-^{13}C-COSY-$^{1}J_{CH}$ (HMQC) spectrum was used to recognize the direct correlations ($^{1}J_{CH}$) of the hydrogen and carbon atoms corresponding to methynes and methoxyl groups (Table I). The correct position of methyne carbons was confirmed by heteronuclear interactions at long range ($^{2}J_{CH}$ and $^{3}J_{CH}$) of the

C-5 (δ_C 163.34), C-7 (δ_C 163.99), C-8 (δ_C 60.98), and C-10 (δ_C 106.15) with H-6 (δ_H 6.42). The heteronuclear interactions of OH-5 (δ_H 12.92) with both C-5 (δ_C 163.34, $^{2}J_{CH}$) and CH-6 (δ_C 95.45, $^{3}J_{CH}$) were used to characterize definitively the **1a** structure. Other correlations revealed by HMBC are shown in Table I. The iodine derivative **1b** was also characterized by same procedure, which revealed the data described in Table I.

However, the same reaction using the isoflavone (**2**) yielded different products when compared with those obtained of flavonol **1**, by the presence of two additional methoxyl groups located at CH-2 and C-3 of the enone
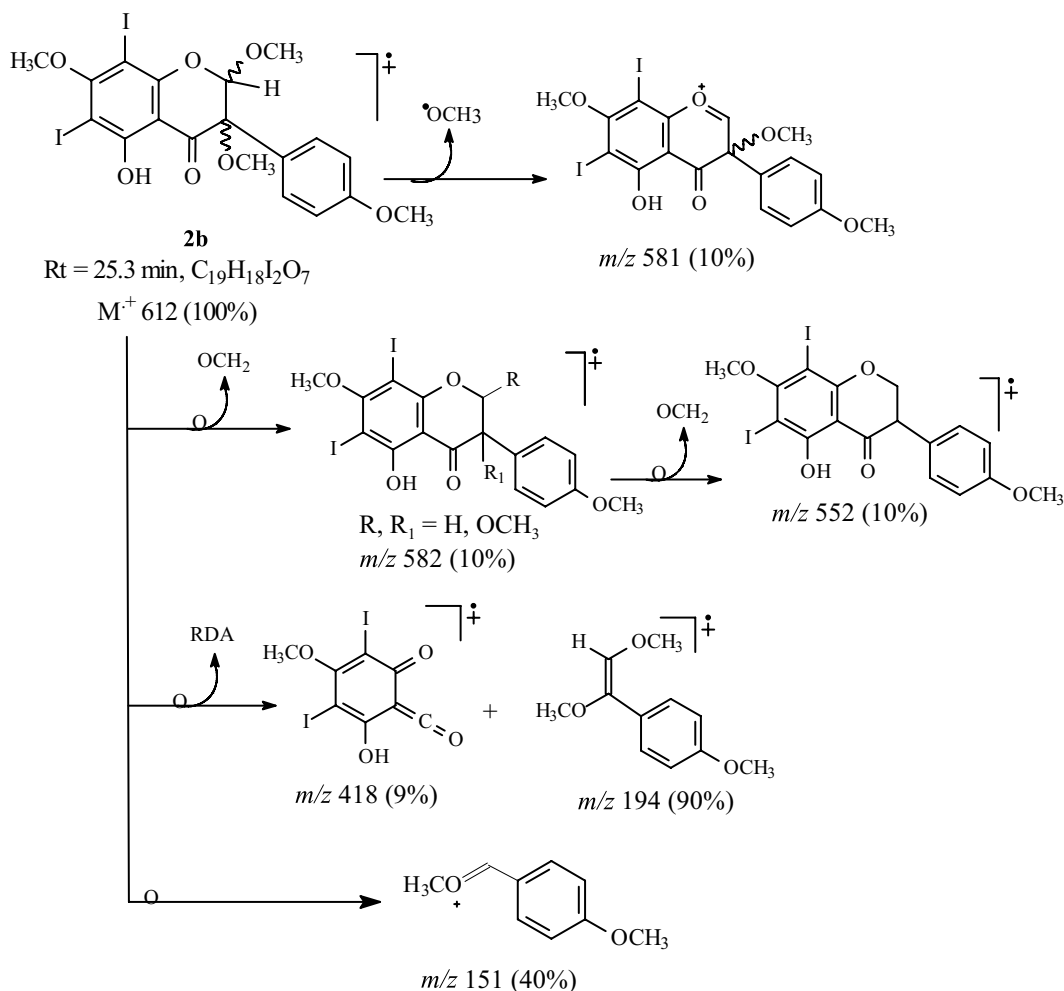
Fig. 5 – Proposed fragmentation patterns for **2b** (only peaks classified as principals).

system (Figs. 1 and 3). The CG-LREIMS of the mixture containing the compounds **2a** and **2b** showed two peaks [T_R 19.0 min (58.14%) and T_R 25.3 (41.86%)] and the corresponding molecular ions peaks identified at m/z 486 (100%, **2a**) and 612 (100%, **2b**), as summarized in Figures 4 and 5. Besides these data, the analysis of ^{1}H and ^{13}C NMR let us to identify the chemical shifts represented by two close values (Table I), suggesting similar structures compatible with the presence of these two enantiomeric pairs (**2a** and **2b**). The detailed mass spectral data analysis and the NOEDIFF experiments justified both similar structures with two additional methoxyl groups with one and two incorporated iodine in **2a** and **2b**, respectively. These structures and locations of those groups were recognized by analysis of

additional peaks in the mass spectra m/z (%): **2a**: 486 (100, $C_{19}H_{19}O_7I$); 456 (40, M-30), 194 (90), 179 (40), 151 (25) Figure 4; **2b**: m/z 612 (100, $C_{19}H_{18}O_7I_2$), 582 (10), 552 (10), 418 (9), 194 (90), 151 (40); been 194 = $H_3COCH=COCH_3(C_6H_4$-$OCH_3)$ by RDA, Figure 5. The data of ^{1}H and ^{13}C NMR ({^{1}H} and DEPT) 1D- and 2D (^{1}Hx^{1}H-COSY, HMQC and HMBC) spectra analysis are described in Table I. The NOEDIFF experiments were also used to confirm these structures, contributing to complete ^{1}H and ^{13}C NMR assignments (Table I) and to identify the relative configuration of C-2 and C-3. The irradiations at δ_H 3.63 (H$_3$CO-2, **2a** and **2b**) yield NOE at H-2 [5.22 (**2a**) and δ_H 5.18 (**2b**)], irradiation at 3.19 (H$_3$CO-3, **2b**) yield NOE at H-2 (δ_H 5.18, **2b**), HO-5 (δ_H 12.17) and at H-2′, 6′(δ_H 7.42); irradi-

TABLE I

^{1}H and ^{13}C NMR spectral data for *1a, 1b* (^{1}H: 300 MHz and ^{13}C: 75 MHz), (±)*2a* and (±)*2b* (^{1}H: 500 MHz and ^{13}C: 125 MHz), in CDCl$_3$ as solvent. Chemical shifts are described in δ (ppm) and coupling constants (J in parenthesis) in Hz.*

	1a*			1b*			2a			2b		
	HMQC		HMBC	HMQC		HMBC	HMQC		HMBC	HMQC		HMBC
C	$δ_C$	$δ_H$	$(^{2,3}J)$	$δ_C$	$δ_H$	$(^{2,3}J)$	$δ_C$	$δ_H$	$(^{2,3}J)$	$δ_C$	(^1J)	$(^{2,3}J)$
2	156.15		H-2'	156.96			106.30	5.22 (s)	CH$_3$O-2	106.36	5.18 (s)	CH$_3$O-2
3	139.01		CH$_3$O-3	139.02		CH$_3$O-3	80.16		CH$_3$O-3	80.47		CH$_3$O-3
4	178.64			177.99			192.92		H-2	193.61		H-2
5	163.34		H-6	162.13		HO-5	166.03		HO-5; H-6	163.61		HO-5
6	95.45	6.42 (s)		75.19		HO-5	93.96	6.23	HO-5	73.39		HO-5
7	163.99		H-6, CH$_3$O-7	165.39		H-8	165.76		H-6	167.02		C$_3$CO-7
8	60.98		H-6	94.74	6.42 (s)		70.62		H-6	64.86		H-2
9	156.15			155.10			157.47		H-2	156.86		HO-5
10	106.56		H-6	108.56			102.89		H-6; HO-5	104.74		
1'	122.97		H-5'	122.48			123.73		H-3';5'	124.50		H-3';5'
2'	111.61	7.98 (d, 1.8)	H-6'	111.61	7.96 (d, 1.9)		130.19	7.42 (d, 8.5)	H-3';5'	130.22	7.39 (d, 8.5)	H-3';5'
3'	149.08		H-2'; CH$_3$O-3'; H-5'	149.17			113.50	6.98 (d, 8.5)	H-2';6'	113.56	6.96 (d, 8.5)	H-2';6'
4'	151.79		H-5'; CH$_3$O-4'; H-2'; H-6'	152.18			159.67		CH$_3$O-4'; H-2';6'	159.84		CH$_3$O-4'; H-2';6'
5'	111.17	7.03 (d, 8.7)		111.25	7.04 (d, 1.9)		113.50	6.98 (d, 8.5)	H-2';6'	113.56	6.96 (d, 8.5)	H-2';6'
6'	123.21	8.06 (dd, 1.8, 8.7)	H-2'	123.47	8.06 (dd)		130.19	7.42 (d, 8.5)	H-3';5'	130.22	7.39 (d, 8.5)	H-3';5'
MeO-2							57.30	3.36 (s)		57.40	3.36 (s)	
MeO-3	60.23	3.91 (s)		61.41	3.97 (s)		52.90	3.19 (s)		53.00	3.21 (s)	
MeO-7	57.18	3.98 (s)					56.90	3.95 (s)		60.90	3.97 (s)	
MeO-3'	56.34	4.01 (s)					55.30	3.86 (s)		55.30	3.87 (s)	
MeO-4'	56.20	3.99 (s)										
HO-5		12.92 (s)			13.86 (s)			12.17 (s)			12.81 (s)	

*Number of hydrogens bound to carbon atoms deduced by comparative analysis of {^{1}H}- and APT-^{13}C NMR spectra. Coupling constants (J) in Hz for hydrogen atoms were obtained of the 1D ^{1}H NMR spectra.

ation at δ_H 3.86 (H$_3$CO-4$''$ of **2a**) yielded NOE at δ_H 6.98 (H-3$'$, 5$'$) and at δ_H 3.95 (H$_3$CO-7 of **2a**) yielded NOE at 6.23 (H-6); irradiation at δ_H 3.21 (H$_3$CO-3, **2a**) yield NOE at δ_H 5.22 (H-2), δ_H 7.39 (H-2$'$, 6$'$) and HO-5 (δ_H 12.81); no signal was observed in the spectrum obtained from irradiation at δ_H 3.97 (H$_3$CO-7 of **2b**); on the other hand a doublet at δ_H 6.96 (H-3$'$, 5$'$) was observed in the spectrum obtained by irradiation at δ_H 3.87 (H$_3$CO-4$'$ of **2b**). Thus the NOE observed at H-2 and H-3 with irradiation at the H$_3$CO-2 was used to justify the *trans* correlation relationship between methoxyl groups at 2 and 3 positions, according to the proposed mechanism summarized in Figure 3. The detailed analysis of ^{1}H and ^{13}C NMR (1D and 2D) spectra let us to make the complete data assignments of these new derivatives (Table I).

In this work we rationalize *trans* stereoselectivity observed at the α, β-enone system of **2** as a well established addition of methanol to an intermediate halonium species (Bateman et al. 1983), followed by alkoxide substitution of the resulting β-alkoxy-(or β-hydroxy-)-α-halo derivative intermediate (Bird et al. 1983). Presumably, solvolytic conditions in methanol drives the incipient benzylic carbenium formed to be anchimeric assisted by a methoxy group (Smith et al. 1965), thus explaining the *trans* selectivity observed in the products. The reactions performed in the present work are of exploratory nature, carried out to ascertain experimental conditions and to ensure the observed products.

As far as we know, this is the first work that describes the incorporation of nuclear iodine atoms in flavonoids **1** and **2** along with the stereoselective *trans* incorporation of two methoxyl groups at the 2 and 3 positions of compound **2**.

RESUMO

A reação do flavonol 3,7,3$'$, 4$'$-tetra-*O*-metilquercetina (**1**) e da isoflavona 7,4$'$-di-*O*-metilgenisteina (**2**) com iodo/KOH em metanol forneceu como produto quatro derivados iodados: 8-iodo-5-hidroxi-3,7,3$'$, 4$'$-tetrametoxiflavona (**1a**) e 6-iodo-5-hidroxi-3,7,3$'$, 4$'$-tetrametoxiflavona (**1b**) a partir da iodação de **1**; a partir de **2** foi obtida uma mistura racêmica composta de ($\pm$)-*trans*-5-hidroxi-2,3,7,4$'$-tetrametoxi-8-iodo-isoflavanona (**2a**) e ($\pm$)-*trans*-5-hidroxi-2,3,7,4$'$-tetrametoxi-6,8-diiodo-isoflavanona (**2b**). A formação destes diferentes produtos revela a significante diferença envolvendo a interação química entre o sitio reativo de cetonas α, β-insaturadas de flavonol e de isoflavonas nas condições experimentais testadas (usando I$_2$/KOH/MeOH). Além disso, ressalta-se a estereosseletividade *trans* na adição de metanol ao sistema α, β-insaturado da isoflavona. As estruturas foram identificadas com análise nos dados espectrométricos de RMN 1D e 2D e massas.

Palavras-chave: flavonol, isoflavona, iodoflavonóide, iodo-isoflavonóide, derivados de iodo.

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