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Unambiguous ^{1}H- and ^{13}C-NMR Assignments of Isoflavones from *Virola caducifolia*

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A inequívoca atribuição dos deslocamentos químicos dos átomos de carbono e hidrogênio dos isoflavonóides 5,7-diidroxi-4'-metoxiisoflavona (biochanina A, **1**), 2',5,7-triidroxi-4'-metoxiisoflavona (**2**) e 5,7-dihidroxi-2',4'-dimetoxiisoflavona (**3**) envolveu a análise de espectros bidimensionais de correlação homonuclear de hidrogênio (^{1}Hx^{1}H-COSY) e heteronuclear de carbono-13 e hidrogênio através de uma ligação (^{1}Hx^{13}C-COSY-$^1J_{CH}$) e a longa distância (^{1}Hx^{13}C-COSY-$^nJ_{CH}$, n = 2 e 3), além de experiências de NOE homonuclear por subtração de espectros de hidrogênio (^{1}H {^{1}H}-NOE). Essas isoflavonas foram isoladas de *Virola caducifolia.*

Unambiguous ^{1}H- and ^{13}C-NMR assignments of the isoflavonoids 5,7-dihydroxy-4'-methoxyisoflavone (biochanin A, **1**), 2',5,7-trihydroxy-4'-methoxyisoflavone (**2**), 5,7-dihydroxy-2',4'-dimethoxyisoflavone (**3**) involved the homonuclear 2D ^{1}Hx^{1}H-COSY and heteronuclear 2D direct CH (^{1}Hx^{13}C-COSY-$^1J_{CH}$), via long-range CH coupling [^{1}Hx^{13}C-COSY-$^nJ_{CH}$ (n = 2 and 3), COLOC] experiments, along with NOE difference spectra (^{1}H {^{1}H}-NOE). These isoflavones were isolated from *Virola caducifolia.*

Keywords: *isoflavones; ^{1}H- and ^{13}C-NMR spectral data*

Introduction

In a previous paper the structural determination of the isoflavonoids 5,7-dihydroxy-4'-methoxyisoflavone (biochanin A, **1**), 2',5,7-trihydroxy-4'- methoxyisoflavone (**2**), and 5,7-dihydroxy-2',4'-dimethoxyisoflavone (**3**), isolated from *Virola caducifolia* (family Myristicaceae), was reported on the basis of ^{1}H-NMR (100 MHz), EIMS, IR and UV spectral data[1].

In this paper we describe the analysis of the data obtained by NOE difference (^{1}H {^{1}H}-NOE), homonuclear 2D ^{1}Hx^{1}H-COSY and 2D ^{1}Hx^{13}C-COSY-$^nJ_{CH}$ (n = 1, direct CH coupling; n = 2 and 3, long-range coupling) spectra[2] of **1-3**, following additional purification by TLC. The ^{1}H- and ^{13}C-NMR spectral data allowed the confirmation of the structures of these isoflavonoids and the unambiguous attribution of the chemical shifts of the hydrogen and carbon-13 atoms.

Results and Discussion

The multiplicity of the signals corresponding to carbon-13 atoms of isoflavonoids **1-3** was deduced by comparative analysis involving PND (Proton Noise Decoupling) and DEPT (Distortionless Enhancement by Polarization Transfer) ^{13}C-NMR spectra.

The heteronuclear 2D ^{1}Hx^{13}C-COSY-$^nJ_{CH}$ (n = 1, direct CH coupling; n = 2 and 3, long-range CH coupling-COLOC) NMR spectra **1** were used to confirm ^{13}C chemical shifts assignments described in the literature for biochanin A (**1**) [3]. Only the chemical shift of the carbon atoms C-1' and C-3 need to be interchanged. The couplings via three bonds ($^3J_{CH}$) between carbon C-3 (δ_C 123.55) and hydrogens H-2',6' (δ_H 7.53), and between C-1' (δ_C 124.10) and H-3', 5' (δ_H 6.98) were used to confirm these correlations (Table 1). The presence of a methoxy group at C-4' was also confirmed by the ^{1}H {^{1}H}-NOE difference spectra performed by irradiation at δ_H 3.82 (s, MeO-4') resulting in enhancement (3%) at δ_H 6.98 (d, J = 8.9 Hz, H-3',5').

Irradiation at MeO-4' [δ_H 3.77 (s)] of **2** resulted in NOE at δ_H 6.52 and 6.50 (H-3' and H-5', respectively, 7%), which indicated the presence of a methoxyl group at C-4'. These results together with the heteronuclear 2D ^{1}Hx^{13}C-COSY-$^1J_{CH}$ and ^{1}Hx^{13}C-COSY-$^nJ_{CH}$ (n = 2 and 3, COLOC) NMR spectra, were used to unambiguously assign the chemical shifts of the carbon atoms of this isoflavonoid, and consequently to confirm the 2'-hydroxy-4'-methoxy substitution pattern for the aromatic ring, definitely ruling out the 2'-methoxy-4'-hydroxy alternative (Table 2). On the basis of these data, the assignments described in the literature[3] for compound **4** were also confirmed (Table 2).

The presence of methoxy groups at C-2' and C-4' of **3** was definitively confirmed by irradiations at δ_H 3.75 (s, MeO-2') and δ_H 3.80 (s, MeO-4'), enhancement at δ_H 6.62 (H-3', NOE = 4%) being observed in the first case and enhancements at δ_H 6.62 (H-3', NOE = 3%) and 6.52 (H-5', NOE = 5%) being observed in the second (Table 3). The chemical shifts of the carbon atoms were also assigned by heteronuclear 2D 1Hx^{13}C-COSY-$^1J_{CH}$ and ^{1}Hx$_{13}$C-COSY-$^nJ_{CH}$ (n = 2 and 3) spectra, as shown in Table 3. The upfield shift of the signal corresponding to carbon C-3' (δ_C 99.25) of **3**, when compared with that of the same carbon of **2** (δ_H 103.12), can be attributed to the γ-effect [$\Delta\delta_C$ = 99.25 (**3**)-103.12 (**2**) = -3.87 ppm) of the methyl group of the methoxy function at C-2'.

As anticipated, the presence of a hydroxy or methoxy group at C-2' promotes an upfield shift (γ-effect) of the signal corresponding to C-3 [$\Delta\delta_C$ = ~121.67 (**2** + **3**/2) - 123.55 (**1**) = -1.87 ppm)], along with shielding at C-1' [$\Delta\delta_C$ = ~112.51 (**2** + **3**/2) - 124.10 (**1**) = -11.58 ppm)] and C-3' [$\Delta\delta_C$ = ~ 101.18 (**2** + **3**/2) - 114.43 (**1**) = -13.24 ppm)] by the *ortho*-electronic mesomeric effect, and at C-5' [$\Delta\delta_C$ = ~105.83 (**2** + **3**/2) - 114.43 = -8.6 ppm] by a *para*-electronic effect (Tables 1-3) [4].

Table 1. ^{1}H- (200 MHz) and ^{13}C- (50.3) NMR spectral data for **1** compared with values described in the literature[3] for biochanin A (**1**), in acetone-d$_6$.*

	1					
	^{1}Hx^{13}C-COSY-$^1J_{CH}$		^{1}Hx^{13}C-COSY-$^nJ_{CH}$ (n = 2, 3)			
C	δ_C	δ_H	$^2J_{CH}$	$^3J_{CH}$	Biochanin A (**1**)^a	
2	154.30	8.16(s)			154.50	153.60
3	123.55	-	H-2	H-2', 6'	124.10^b	123.90^c
4	181.35	-		H-2	181.60	181.10
5	163.70	-	H-6		163.90	163.30
6	99.91	6.27(d, J = 1.8)		H-8	99.90	99.60
7	165.36	-	H-6,H-8		165.00	164.50
8	94.49	6.40(d, J = 1.8)		H-6	94.50	94.20
9	158.91	-	H-8	H-2	159.00	158.60
10	105.81	-		H-6, H-8	106.20	105.70
1'	124.10	-		H-3', 5'	123.80^b	123.50^c
2',6'	131.06	7.53(d, J = 8.9)			131.10	130.60
3',5'	114.43	6.98(d, J = 8.9)			114.50	114.20
4'	160.49	-		H-2',6'; MeO-4'	161.70	160.30
MeO-4'	55.52	3.82(s)			55.60	58.40
HO-5	-	12.98(brs)			-	-

* The chemical shifts are reported in δ (ppm) and the coupling constants (J) in hertz (Hz). The multiplicity of the signals of the carbon-13 atoms was deduced by comparative analysis of PND- and DEPT- ^{13}C-NMR spectra. The ^{1}H-NMR spectral values were obtained from a one-dimensional (1D) spectrum; the assignments were also based on the homonuclear 2D ^{1}Hx^{1}H-COSY spectrum.

a) Reference 3 reports the values described in two publications. b,c) Assignments with the same letter are interchanged in the column.

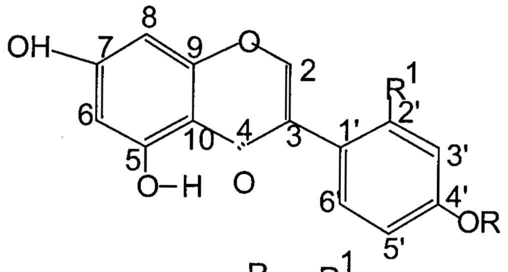
Table 2. ^{1}H (200 MHz) and ^{13}C (50.3 MHz) NMR spectral data for the isoflavonoid **2** (acetone-d_6) compared with values described in the literature[3] for **4** (DMSO-d_6).*

	2						4
	^{1}Hx^{13}C-COSY-$^{1}J_{CH}$		^{1}Hx^{13}C-COSY-$^{n}J_{CH}$		^{1}H{^{1}H}-NOE		
C	δ_C	δ_H	$^{2}J_{CH}$	$^{3}J_{CH}$	H	%	δ_C
2	156.30	8.14(s)					155.40
3	121.85	-	H-2	H-6'			120.60
4	181.95	-		H-2			180.60
5	163.42	-	H-6				162.00
6	100.15	6.29(d, J = 2.0)		H-8			99.00
7	165.76	-	H-6, H-8				164.30
8	94.62	6.43(d, J = 2.0)					93.80
9	158.94	-	H-8	H-2			157.80
10	105.66	-		H-6, H-8			104.60
1'	111.91	-		H-2, H-3', H-5'			108.80
2'	157.60	-	H-3'	H-6'			156.60
3'	103.12	6.52(d, J = 2.5)		H-5'			102.80
4'	162.15	-	H-3'	H-6', MeO-4'			159.80
5'	106.38	6.50(dd, J = 8.6,2.5)		H-3'			106.40
6'	132.71	7.19(d, J = 8.6)					132.30
MeO-4'	55.42	3.77(s)			3',5'	7	-

* The chemical shifts are described in d (ppm) and the coupling constants (J) in hertz (Hz). The multiplicity of the signals corresponding to carbon-13 atoms was deduced by comparative analysis of PND- and DEPT- ^{13}C-NMR spectra. The ^{1}H-NMR spectral values were obtained from a one-dimensional (1D) spectrum; the assignments were also based on the homonuclear 2D ^{1}Hx^{1}H-COSY spectrum.

The results published in this paper can be used to confirm or modify assignments reported in the literature for other analogous isoflavonoids.

The unambigous attribution of hydrogen and carbon-13 atom chemical shifts, including ^{1}Hx^{1}H coupling information, is essential for incorporation into computer programs for structural elucidation from spectral data. These data are important not only for new compounds but also for any known compounds for which complete ^{1}H and ^{13}C chemical shift assignments have not been previously made[5].



	R	R^{1}
1	Me	H
2	Me	OH
3	Me	OMe
4	H	OH

Figure 1.

Experimental

The NMR spectra were recorded in the Fourier transform mode on a Bruker AC-200 (^{1}H: 200 MHz; ^{13}C: 50.3 MHz) spectrometer. The solvent used was acetone-d_6 and the chemical shifts are given in δ (ppm) with TMS as the internal reference. The coupling constant (J) values are given in hertz (Hz). The pulse sequences used in the homonuclear (^{1}Hx^{1}H-COSY) and heteronuclear (^{1}Hx^{13}C-COSY-$^{1}J_{CH}$ and ^{1}Hx^{13}C-COSY$^{n}J_{CH}$, $n = 2$ and 3) 2D shift-correlated NMR spectra were the Bruker programs COSY-AU and XH CORR-AU [modulated with $^{1}J_{CH} = 140$ Hz ($D3 = 0.5 / J_{CH}$ and $D4 = 0.5 / 2J_{CH}$, coupling via one bond) and $^{n}J_{CH} = 7$ Hz ($n = 2$ and 3, COLOC – correlation via long-range coupling; $D3 = 0.5 / J_{CH}$ and $D4 = 0.5 / 2J_{CH}$)], respectively. The NOE (Nuclear Overhauser Effect) difference spectra experiments were obtained by the Bruker programs NOEDIFF-AU, and the DEPT (Distortionless Enhancement by Polarization Transfer) spectra by DEPTVAR-AU ($\Theta = 90°$ and $\Theta = 135°$).

Table 3. ^{1}H- (200 MHz) and ^{13}C- (50.3 MHz) NMR spectral data for the isoflavonoid **3** (acetone-d_6) compared with **2** (Table 2).*

	3						**2**
	^{1}Hx^{13}C-COSY-$^1J_{CH}$		^{1}Hx^{13}C-COSY-$^nJ_{CH}$		^{1}H{^{1}H}-NOE		
C	δ_C	δ_H	$^2J_{CH}$	$^3J_{CH}$	H	%	δ_C
2	156.65	7.94(s)					156.30
3	121.50	-	H-2	H-6'			121.85
4	181.38	-		H-2			181.95
5	163.66	-	H-6				163.42
6	99.73	6.26(d, J = 1.9)		H-8			100.15
7	164.96	-	H-6, H-8				165.76
8	94.42	6.39(d, J = 1.9)		H-6			94.62
9	158.93	-	H-8	H-2			158.94
10	105.97	-		H-6, H-8			105.66
1'	113.12	-		H-3', H-5'			111.91
2'	159.63	-		H-6, MeO-2'			157.60
3'	99.25	6.62(d, J = 2.3)					103.12
4'	162.24	-		H-6', MeO-4'			162.15
5'	105.28	6.52(dd, J = 8.2,2.3)					106.38
6'	133.03	7.19(d, J = 8.2)					132.71
MeO-2'	55.92	3.75(s)			3'	4	-
MeO-4'	55.63	3.80(s)			3'	3	55.42
					5'	5	

* The chemical shifts are reported in δ (ppm) and the coupling constants (J) in hertz (Hz). The multiplicity of the signals corresponding to carbon-13 atoms was deduced by comparative analysis involving PND- and DEPT- ^{13}C-NMR spectra. The ^{1}H-NMR spectral values were obtained from a one-dimensional (1D) spectrum; the assignments were also based on the homonuclear 2D ^{1}Hx^{1}H-COSY spectrum.

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