Substitution Effect of Hydroxyl Groups on the ^{1}H and ^{13}C Chemical Shifts in Hydroxyflavonols

Sunhee Lee, Younghee Park, Byoung-Ho Moon, Eunjung Lee, Sungwon Hong, and Yoongho Lim*

Division of Bioscience and Biotechnology, BMIC, Research Center for Drugs, RCTC, Konkuk University, Seoul 143-701, Korea
**E-mail: yoongho@konkuk.ac.kr*
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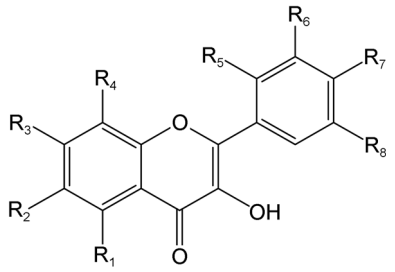
Flavonoids belong to the secondary metabolites naturally occurring in plants. They have C6-C3-C6 framework and can be classified into several subclasses based on the oxidation level of the central pyran ring.[1] Their diverse structures are prepared by oxidation, alkylation, glycosylation, and so on. The variety of their biological activities such as anti-cancer, anti-viral, anti-inflammatory, and anti-oxidative activities is related with their structures.[2,3] Most flavonoids are known to show anti-oxidative effects. Of them, scavenging effects of flavonol derivatives were reported previously.[4] While the scavenging effect of 2'-hydroxyflavonol was 84%, that of 3'-hydroxyflavonol was 39%. Vitamin C showed the effect of 89% in the same experimental condition. That is, the anti-oxidative effects of flavonol derivatives are dependent upon the number of hydroxyl groups and their positions.

Many flavonol derivatives are being isolated from natural sources. Nuclear magnetic resonance (NMR) spectroscopy is widely used for their identification. Because the position and the number of substituted hydroxyl groups cause the changes of the ^{1}H and ^{13}C chemical shifts, it is important to elucidate the substitution effect of hydroxyl groups. The substitution effect on flavonol derivatives can be used to identify the structures of the newly isolated hydroxyflavonol derivatives. In order to elucidate the substitution effects of hydroxyl groups on ^{1}H and ^{13}C chemical shifts in hydroxyflavonols, 18 derivatives were chosen. Their structures and nomenclatures are shown in Figure 1. Of eighteen hydroxyflavonol derivatives, the NMR data of eleven hydroxyflavonol derivatives (**1-6**, **8-11**, **17**) have been previously reported.[5-7] Because the ^{1}H and ^{13}C chemical shifts of the remaining seven derivatives (**7**, **12-16**, **18**) were not reported yet, we carried out their complete assignments. The ^{1}H and



derivative	nomenclature	R1	R2	R3	R4	R5	R6	R7	R8
1	flavonol	H	H	H	H	H	H	H	H
2	6-hydroxyflavonol	H	OH	H	H	H	H	H	H
3	7-hydroxyflavonol	H	H	OH	H	H	H	H	H
4	2-hydroxyflavonol	H	H	H	H	OH	H	H	H
5	3-hydroxyflavonol	H	H	H	H	H	OH	H	H
6	4-hydroxyflavonol	H	H	H	H	H	H	OH	H
7	5,7-dihydroxyflavonol (galangin)	OH	H	OH	H	H	H	H	H
8	6,3'-dihydoxyflavonol	H	OH	H	H	H	OH	H	H
9	6,4'-dihydoxyflavonol	H	OH	H	H	H	H	OH	H
10	7,3'-dihydoxyflavonol	H	H	OH	H	H	OH	H	H
11	7,4'-dihydoxyflavonol	H	H	OH	H	H	H	OH	H
12	5,7,2'-trihydroxyflavonol (datiscetin)	OH	H	OH	H	OH	H	H	H
13	5,7,4'-trihydroxyflavonol (kaempferol)	OH	H	OH	H	H	H	OH	H
14	6,2',3'-trihydroxyflavonol	H	OH	H	H	OH	OH	H	H
15	7,3',4'-trihydroxyflavonol (fisetin)	H	H	OH	H	H	OH	OH	H
16	7,3',4',5'-tetrahydroxyflavonol	H	H	OH	H	H	OH	OH	OH
17	5,7,3',4'-tetrahydroxyflavonol (quercetin)	OH	H	OH	H	H	OH	OH	H
18	5,7,8,3',4'-pentahydroxyflavonol (gossypetin)	OH	H	OH	OH	H	OH	OH	H

Figure 1. Structures and nomenclatures of hydroxyflavonol derivatives **1-18**.

Table 1. The ^{1}H chemical shifts of 18 hydroxyflavonol derivatives **1-18**

	δ of ^{1}H (J, Hz)								
position	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**
5	8.10 (dd, 1.5, 8.0)	7.37 (d, 3.0)	7.96 (d, 8.4)	8.14 (dd, 1.5, 8.0)	8.10 (dd, 1.3, 7.6)	8.09 (m)	–	7.37 (d, 3.0)	7.35 (d,3.0)
6	7.42 (m)	–	6.91 (dd, 2.2 8.4)	7.46 (m)	7.43 (dd, 7.6, 7.6)	7.43 (dd, 7.8, 7.8)	6.12 (d, 2.0)	–	–
7	7.76 (ddd, 1.5, 7.1, 8.5)	7.25 (dd, 9.0, 3.0)	–	7.76 (ddd, 1.5, 7.0, 8.5)	7.75 (m)	7.73 (m)	–	7.26 (dd, 3.0, 9.0)	7.22 (dd, 3.0, 9.1)
8	7.71 (d, 8.5)	7.62 (d, 9.0)	6.94 (d, 2.2)	7.61 (d, 8.5)	7.69 (m)	7.70 (d, 8.3)	6.46 (d, 2.0)	7.60 (d, 9.0)	7.58 (d, 9.1)
2'	8.20 (dd, 1.4, 7.2)	8.20 (m)	8.16 (d, 7.3)	–	7.72 (s)	8.11 (d, 9.0)	8.13 (d, 7.3)	7.67 (dd, 1.6, 2.4)	8.07 (d, 9.0)
3'	7.54 (m)	7.55 (m)	7.53 (dd, 7.3, 7.3)	6.99 (dd, 0.8, 8.1)	–	6.95 (d, 9.0)	7.5 (m)	–	6.93 (d, 9.0)
4'	7.42 (dd, 7.2, 7.2)	7.48 (m)	7.46 (dd, 7.3, 7.3)	7.35 (ddd, 1.7, 7.4, 8.1)	6.92 (dd, 2.0, 8.0)	–	7.5 (m)	6.90 (ddd, 2.4, 2.4, 8.0)	–
5'	7.54 (m)	7.55 (m)	7.53 (dd, 7.3, 7.3)	6.93 (ddd, 0.8, 7.4, 7.4)	7.35 (dd, 8.0, 8.0)	6.95 (d, 9.0)	7.5 (m)	7.34 (dd, 8.0, 8.0)	6.93 (d, 9.0)
6'	8.20 (dd, 1.4, 7.2)	8.20 (m)	8.16 (d, 7.3)	7.44 (m)	7.65 (d, 8.0)	8.11 (d, 9.0)	8.13 (d, 7.3)	7.63 (ddd, 1.6, 2.4, 8.0)	8.07 (d, 9.0)
3-OH	9.60 (s)	9.40 (s)	9.32 (s)	9.40 (bs)	9.55 (s)	9.32 (s)	9.62 (s)	9.32 (s)	9.10 (s)
5-OH	–	–	–	–	–	–	12.44 (s)	–	–
6-OH	–	9.98 (s)	–	–	–	–	–	9.95 (s)	9.89 (s)
7-OH	–	–	10.83 (s)	–	–	–	10.87 (s)	–	–
8-OH	–	–	–	–	–	–	–	–	–
2-OH	–	–	–	9.40 (bs)	–	–	–	–	–
3-OH	–	–	–	–	9.74 (s)	–	–	9.69 (s)	–
4-OH	–	–	–	–	–	10.10 (s)	–	–	10.04 (s)
5-OH	–	–	–	–	–	–	–	–	–

position	**10**	**11**	**12**	**13**	**14**	**15**	**16**	**17**	**18**
5	7.94 (d, 9.3)	7.92 (d, 8.6)	–	–	7.38 (d, 2.9)	7.92 (d, 9.3)	7.92 (d, 8.7)	–	–
6	6.93 (dd, 2.2, 9.3)	6.90 (dd, 2.3, 8.6)	6.20 (d, 1.9)	6.18 (d, 2.0)	–	6.92 (m)	6.89 (dd, 2.2, 8.7)	6.18 (d, 2.0)	6.25 (s)
7	–	–	–	–	7.22 (dd, 2.9, 9.0)	–	–	–	–
8	6.91 (d, 2.1)	6.92 (d, 2.3)	6.31 (d, 1.9)	6.43 (d, 2.0)	7.47 (d, 9.0)	6.92 (m)	6.85 (d, 2.2)	6.40 (d, 2.0)	
2'	7.63 (dd, 1.8, 2.4)	8.05 (d, 9.0)	–	8.03 (dd, 2.0, 6.9)	–	7.69 (d, 2.0)	7.24 (s)	7.67 (d, 2.2)	7.77 (d, 2.2)
3'	–	6.91/6.93 (d, 9.0)	6.97 (d, 8.2)	6.92 (dd, 2.0, 6.9)	–	–	–	–	–
4'	6.88 (dd, 2.4, 8.0)	–	7.34 (ddd, 1.6, 7.5, 8.2)	–	6.91 (dd, 1.2, 7.8)	–	–	–	–
5'	7.33 (dd, 8.0, 8.0)	6.91/6.93 (d, 9.0)	6.91 (dd, 7.5, 7.5)	6.92 (dd, 2.0, 6.9)	6.75 (dd, 7.8, 7.8)	6.89 (m)	–	6.88 (d, 8.5)	6.88 (d, 8.5)
6'	7.59 (m)	8.05 (d, 9.0)	7.40 (dd, 1.6, 7.5)	8.03 (dd, 2.0, 6.9)	6.88 (dd, 1.2, 7.8)	7.56 (dd, 2.0, 8.5)	7.24 (s)	7.54 (dd, 2.2, 8.5)	7.64 (dd, 2.2, 8.5)
3-OH	9.26 (s)	9.26 (s)	9.43 (bs)	9.38 (s)	8.89 (bs)	9.00 (s)	8.98 (s)	9.34 (s)	9.26 (s)
5-OH	–	–	12.54 (s)	12.48 (s)	–	–	–	12.48 (s)	11.91 (s)
6-OH	–	–	–	–	9.89 (s)	–	–	–	–
7-OH	10.78 (s)	9.66 (s)	10.79 (s)	10.79 (s)	–	10.72 (s)	10.73 (s)	10.76 (s)	10.37 (s)
8-OH	–	–	–	–	–	–	–	–	8.56 (s)
2-OH	–	–	9.43 (bs)	–	9.41 (s)	–	–	–	–
3-OH	9.66 (s)	–	–	–	–	9.25 (s)	9.16 (s)	9.34 (s)	9.23 (s)
4-OH	–	10.78 (s)	–	10.11 (s)	–	9.49 (s)	8.70 (s)	9.57 (s)	9.53 (s)
5-OH	–	–	–	–	–	–	9.16 (s)	–	–

Table 2. The ^{13}C chemical shift of 18 hydroxyflavonol derivatives **1**-**18**

	δ of ^{13}C																	
position	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**	**15**	**16**	**17**	**18**
2	145.1	145.0	144.2	147.6	145.3	146.1	145.8	144.9	145.8	144.1	145.0	148.2	146.8	147.2	145.0	145.1	146.7	146.5
3	139.1	138.5	138.5	138.9	139.1	137.8	137.2	138.3	137.2	138.3	137.1	136.9	135.7	138.0	137.1	137.3	135.6	135.3
4	173.0	172.7	172.4	172.8	173.0	172.5	176.3	172.5	172.1	172.2	172.0	176.4	175.9	172.3	171.9	171.9	175.7	175.9
5	124.8	106.9	126.6	124.9	124.8	124.7	160.9	106.7	106.8	126.5	126.4	161.4	160.7	106.8	126.4	126.5	160.6	152.1
6	124.5	154.2	115.0	124.4	124.6	124.5	98.4	154.0	154.0	114.8	114.6	98.2	98.2	153.9	114.6	114.6	98.1	97.8
7	133.6	123.4	162.6	133.4	133.7	133.4	164.3	123.3	122.8	162.5	162.2	163.9	163.9	122.9	162.2	162.2	163.8	152.5
8	118.3	119.9	102.1	118.4	118.2	118.3	93.7	119.6	119.5	101.8	101.9	93.5	93.5	119.7	101.8	101.7	93.3	124.6
9	154.5	148.7	156.6	155.1	154.6	154.4	156.5	148.5	148.4	156.4	156.3	157.0	156.2	149.1	156.3	156.2	156.0	144.76
10	121.3	122.2	114.3	122.0	121.3	121.4	103.3	121.9	122.1	114.1	114.2	103.7	103.1	122.7	114.2	114.1	102.9	102.6
1'	131.3	131.6	131.5	118.3	132.5	122.0	131.3	132.5	122.2	132.5	122.2	118.0	121.7	119.1	122.5	121.3	121.9	122.2
2'	127.6	127.7	127.4	155.4	114.7	129.6	127.6	114.4	129.5	114.2	129.2	155.4	129.5	143.8	114.9	107.0	115.0	115.2
3'	128.5	128.6	128.5	116.4	157.4	115.5	128.6	157.2	115.4	157.4	115.3	116.4	115.4	145.9	145.0	135.3	145.0	144.85
4'	129.8	129.8	129.6	131.5	117.1	159.2	130.0	116.8	159.0	116.6	158.7	131.6	159.2	116.7	147.2	145.7	147.6	147.5
5'	128.5	128.6	128.5	118.7	129.6	115.5	128.6	129.4	115.4	129.4	115.3	118.8	115.4	118.8	115.5	135.3	115.5	115.3
6'	127.6	127.7	127.4	131.0	118.3	129.6	127.6	118.3	129.5	118.1	129.2	130.9	129.5	120.6	119.6	107.0	119.9	120.1

^{13}C chemical shifts of 18 derivatives are listed in Tables 1 and 2, respectively.

The substitution effect on the changes of the ^{1}H and ^{13}C chemical shifts in hydroxyflavonol derivatives was investigated based on the elucidation of the data listed in Tables 1 and 2. Generally, the substitution of hydroxyl groups affects *ortho* and *para*-position of flavonol. This effect causes the ^{1}H and ^{13}C chemical shifts of those positions to move upfield. The result has been ascribed to the increasing electron density supplied by resonance-based lone pair electron distribution into the A- or B-rings. In addition, the hydroxylation at A-ring has no effect on the ^{1}H and ^{13}C chemical shift changes at B-ring, and *vice versa*.[8] In particular, the authors found two distinctive features of the substitution effect on the ^{1}H and ^{13}C chemical shifts changes of hydroxyflavonol derivatives.

First of all, the ^{1}H NMR data show the different effects of the introduction of a hydroxyl group in position 5. 5-OH protons (12.51 ± 0.07 ppm) showed more downfield shifted value than 3-OH protons (9.31 ± 0.31 ppm). The reason is the effect of the intramolecular hydrogen bonding between C-4 keto and C-5 hydroxyl groups, forming a six-membered ring. The hydrogen bonding causes the ^{1}H chemical shift of 5-OH position to move 3.2 ppm more downfield shift than the ^{1}H chemical shift of 3-OH position. In the ^{13}C NMR data, the presence of a C-5 hydroxyl group causes a downfield shift of the C-4 resonance of about 3.1 ± 0.3 ppm too because of the intramolecular hydrogen bonding. In the other aspect, the effect of hydroxylation on the ^{13}C NMR spectra can be assessed by comparing the ^{13}C chemical shifts of flavonol to those of *ortho*-position of the substituted flavonol ($\Delta\delta_{\text{hydroxylation}} = \delta_{\text{flavonol}} - \delta_{\textit{ortho}\text{-position}}$). For example, the hydroxylation effect of derivative **4**, 2-hydroxyflavonol, shows that the ^{13}C chemical shift of the *ortho*-position moves upfield about 12.2 ppm; $\delta_{\text{C-3 of flavonol}}$ (128.6 ppm) – $\delta_{\text{C-3 of 2-hydroxyflavonol}}$ (116.4 ppm) = $\Delta\delta_{\text{hydroxylation}}$ (12.2 ppm). The substitution effect in the A-ring may be considered to be a complicated result of the ^{13}C chemical shifts of hydroxyflavonol because of the effect of oxygen of C-9 position and carbonyl group of C-10 position. However, the effect of substitution in the B-ring obviously shows that the *ortho* effect of the hydroxyl substituent on the ^{13}C chemical shifts of the benzene ring is upfield of 12.7 ± 0.6 ppm. Their *para* effect appears upfield of 9.5 ± 0.4 ppm. For illustration, a polyhydroxylated flavonol, derivative **18** known as gossypetin, shows that the ^{13}C chemical shift of C-4 position (175.9 ppm) moves 2.9 ppm more downfield than that of C-4 position (173.0 ppm) of flavonol because of hydrogen bonding between 5-OH and C-4 carbonyl oxygen. The ^{13}C chemical shifts of C-2 and C-5 position (115.2 and 115.3 ppm) move 12.8 ± 0.4 ppm more upfield than those of C-2 and C-5 position (127.6 and 128.5 ppm) of flavonol due to the *ortho* effect of the OH substituent in the B-ring.

Experimental Section

Hydroxyflavonol derivatives **1**-**18** were purchased from INDOFINE chemical company, Inc. (Hillsborough, NJ, USA). The chemicals were used for the NMR experiments without further purification, which were supplied from the company at the purity of 98%.[5]

All NMR experiments were performed on a Bruker Avance 400 spectrometer (9.4 T, Karlsruhe, Germany). We prepared the samples in DMSO-d_6 at 298 K, and their concentrations as approximately 50 mM. For the ^{1}H and ^{13}C NMR experiments, 1 sec and 3 sec relaxation delays were used, respectively. The data points for ^{1}H NMR and ^{13}C NMR were 32 K and 64 K, respectively, and their 90° pulses were 10.2 and 10.3 μsec, respectively. The spectral widths for ^{1}H NMR and ^{13}C NMR were 6,000 Hz and 23,809 Hz, respectively. All two-dimensional spectra were acquired with 2,048 × 256 data points ($t_2 \times t_1$) with magnitude mode.

The long-ranged coupling time for HMBC was 70 msec. The zero filling of 2 K and sine squared bell window function were applied using XWIN-NMR (Bruker).[6]

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