

^1H and ^{13}C NMR Data on Hydroxy/methoxy Flavonoids and the Effects of Substituents on Chemical Shifts

Hyuk Yoon, Sunglock Eom, Jiye Hyun, Geunhyeong Jo, Doseok Hwang, Sunhee Lee, Yeonjoong Yong, Jun Cheol Park,[†] Young Han Lee,[‡] and Yoongho Lim*

Division of Bioscience and Biotechnology, BMIC, Konkuk University, Seoul 143-701, Korea. *E-mail: yoongho@konkuk.ac.kr

[†]National Institute of Animal Science, Rural Development Administration, Suwon 441-706, Korea

[‡]Department of Biomedical Science and Technology, Research Center for Transcription Control, Konkuk University, Seoul 143-701, Korea

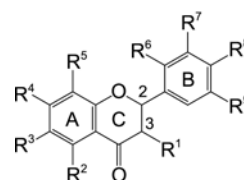
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Polyphenols are secondary metabolites that naturally occur in plants. Unlike alkaloids and terpenoids, they are not used widely as a therapeutic drug because of their mild biological activities. However, if these compounds have low toxicities, they may be used as adjuvants for drugs.¹ Polyphenols have recently been examined for such applications, and they are classified based on their carbon skeletons: phenolic acids with C6-C1 skeleton, hydrocinammates with C6-C3 skeleton, stilbenes with C6-C2-C6 skeleton, and flavonoids with C6-C3-C6 skeleton.² Of these compounds, flavonoids are ubiquitously found in most plants. Since flavonoids belong to polyphenols, they have many hydroxy groups. From a bioavailability point of view, hydroxy groups prevent cell membrane transport, and hydroxyflavonoids can be metabolized by *O*-methyltransferases.^{3,4} However, methoxylated flavonoids may not have these problems. Hydroxylated or methoxylated flavonoids are found from natural sources. Nuclear magnetic resonance (NMR) spectroscopy is widely used to identify different compounds including hydroxylated or methoxylated flavonoids. Because the position and the number of substituted hydroxy or/and methoxy groups will change the ^1H and ^{13}C chemical shifts, it is important to understand these changes so that the structures of newly isolated hydroxy/methoxy-flavonoids can be easily identified.

In order to study the effects of substituents on ^1H and ^{13}C chemical shifts in flavonoids, 16 derivatives were chosen. Their structures and nomenclature are shown in Figure 1. Compounds **1-15** belong to flavone and compound **16** is a class of flavanone. The former contains a double bond between C2 and C3, and the latter contains a single bond. The ^1H and ^{13}C NMR data of compounds **1** and **12**, the ^1H NMR data of compounds **5** and **14**, and the ^{13}C NMR data of compounds **8** and **15** have been reported, but NMR data on the other ten compounds have not yet been reported.⁵⁻¹⁰ The ^1H and ^{13}C NMR data of 6 compounds were assigned and compared with the published data and complete NMR data was obtained for the ten compounds that had not been previously examined by NMR. The procedure used for the complete assignment of **10**, 2',3,6-trihydroxyflavone was as

follows: Fifteen peaks were observed in the ^{13}C NMR spectrum. According to distortionless enhancement by polarization transfer (DEPT) experiments, seven methines and eight quaternary carbons were identified. Four ^1H peaks at 6.91, 6.97, 7.33, and 7.41 ppm were correlated in the two dimensional correlated spectroscopy (COSY) spectrum, and four ^{13}C peaks at 118.7, 116.4, 131.3, and 130.9 ppm were attached to the ^1H peaks directly in the heteronuclear multiple quantum coherence (HMQC), respectively. Therefore, these peaks were assigned as protons and carbons of the B-ring. Based on the interpretation of the nuclear Overhauser and enhanced spectroscopy (NOESY) and heteronuclear multiple bonded connectivities (HMBC) spectra, they were determined to be H-5', H-3', H-4', and H-6', respectively. From the long-ranged couplings of C-1' and H-5', and C-2'



Derivatives	C2-C3	R ¹	R ²	R ³	R ⁴	R ⁵	R ⁶	R ⁷	R ⁸	R ⁹
1	Double	H	H	OH	OH	H	H	H	H	H
2	Double	H	H	OCH ₃	OCH ₃	H	H	H	H	H
3	Double	H	H	H	OH	OH	H	H	H	H
4	Double	H	H	H	OCH ₃	OCH ₃	H	H	H	H
5	Double	H	H	OH	H	H	H	H	OH	H
6	Double	H	H	OCH ₃	H	H	H	H	OCH ₃	H
7	Double	H	H	H	H	H	H	OH	H	OH
8	Double	H	H	H	H	H	OCH ₃	H	H	OCH ₃
9	Double	H	H	OH	H	H	OH	H	H	H
10	Double	OH	H	OH	H	H	OH	H	H	H
11	Double	H	H	H	OH	OH	H	H	H	OH
12	Double	H	H	H	OCH ₃	OH	H	H	H	H
13	Double	H	OH	OH	OCH ₃	H	H	H	H	H
14	Double	OH	H	CH ₃	H	H	H	H	OCH ₃	OCH ₃
15	Double	H	OH	H	OH	H	H	OH	OH	OH
16	Single	H	H	OCH ₃	H	H	OCH ₃	OCH ₃	H	H

Figure 1. Structures and nomenclature of hydroxy/methoxy flavonoids **1-16**.

Table 2. The ^{13}C chemical shift of hydroxy/methoxy flavonoids **1-16**

		δ of ^{13}C							
Position	1	2	3	4	5	6	7	8	
2	161.5	161.7	162.0	162.1	162.8	162.4	163.0	160.4	
3	106.0	106.2	106.2	106.4	104.0	104.7	106.7	111.7	
4	176.3	176.1	177.1	176.6	176.9	176.7	177.0	177.2	
5	107.6	103.6	115.3	120.1	107.7	104.8	124.8	125.4	
6	144.7	147.4	114.2	110.8	154.8	156.5	125.5	124.7	
7	152.4	154.3	150.7	156.4	122.8	123.0	134.3	134.3	
8	103.2	100.7	133.3	136.4	119.6	120.0	118.4	118.6	
9	150.9	151.7	146.8	149.8	149.3	150.3	155.6	155.9	
10	116.2	116.5	117.1	118.0	124.3	124.0	123.3	123.1	
1'	131.6	131.3	131.6	131.3	121.9	123.3	132.9	120.4	
2'	126.0	126.0	126.5	126.1	128.2	128.1	104.4	151.8	
3'	129.1	129.0	129.1	129.1	116.0	114.6	159.0	114.3	
4'	131.3	131.4	131.5	131.7	160.8	162.1	105.9	118.0	
5'	129.1	129.0	129.1	129.1	116.0	114.6	159.0	153.1	
6'	126.0	126.0	126.5	126.1	128.2	128.1	104.4	113.9	
6-CH ₃	-	-	-	-	-	-	-	-	
6-OCH ₃	-	55.7	-	-	-	55.7	-	-	
7-OCH ₃	-	56.3	-	56.4	-	-	-	-	
8-OCH ₃	-	-	-	61.1	-	-	-	-	
2'-OCH ₃	-	-	-	-	-	-	-	56.4	
3'-OCH ₃	-	-	-	-	-	-	-	-	
4'-OCH ₃	-	-	-	-	-	55.5	-	-	
5'-OCH ₃	-	-	-	-	-	-	-	55.7	

		δ of ^{13}C							
Position	9	10	11	12	13	14	15	16	
2	160.4	147.3	162.1	162.1	163.2	145.3	164.1	74.2	
3	110.1	138.1	106.1	106.0	104.7	138.2	102.9	42.8	
4	177.2	172.4	177.1	177.0	182.4	172.5	181.5	191.6	
5	107.4	106.8	115.2	114.7	146.1	123.8	161.5	107.4	
6	154.8	149.1	114.1	110.1	130.1	134.0	98.8	153.7	
7	123.0	122.9	150.6	151.6	154.7	134.7	164.1	124.6	
8	119.8	119.7	133.3	134.7	91.4	118.2	93.7	119.4	
9	149.6	153.9	146.8	145.7	149.9	152.8	157.3	155.8	
10	124.0	122.8	117.1	118.0	105.3	121.0	103.7	120.5	
1'	118.0	118.5	132.9	131.4	130.8	121.5	120.4	132.2	
2'	156.4	155.4	113.0	126.3	126.4	111.0	105.7	146.0	
3'	117.0	116.4	157.9	129.0	129.2	148.4	146.3	152.4	
4'	132.4	131.3	118.7	131.5	132.0	150.3	137.8	113.2	
5'	119.5	118.7	130.3	129.0	129.2	111.6	146.3	124.2	
6'	128.6	130.9	117.3	126.3	126.4	123.7	105.7	118.6	
6-CH ₃	-	-	-	-	-	20.4	-	-	
6-OCH ₃	-	-	-	-	-	-	-	55.6	
7-OCH ₃	-	-	-	56.4	56.4	-	-	-	
8-OCH ₃	-	-	-	-	-	-	-	-	
2'-OCH ₃	-	-	-	-	-	-	-	60.7	
3'-OCH ₃	-	-	-	-	-	55.7	-	55.8	
4'-OCH ₃	-	-	-	-	-	55.6	-	-	
5'-OCH ₃	-	-	-	-	-	-	-	-	

and H-4', two ^{13}C peaks at 118.5 and 155.4 ppm were assigned C-1' and C-2', respectively. C-2' was determined based on the long-ranged coupling with H-6' in HMBC. The

peak corresponding to the Ketone group at C-4 was at 172.4 ppm. C-5, C-6, C-7, C-8, C-9, and C-10 were determined by interpreting the HMBC spectrum. Only the ^{13}C peak at 138.1 ppm was not assigned, which should be C-3. Three hydroxy groups were observed at 9.59 and 9.91 ppm. According to the long ranged coupling of the HMBC spectrum, the ^1H peak at 9.91 ppm was 6-OH. 3-OH and 2'-OH were not distinguished. The NMR spectra used for the assignments of compound **10** are provided as supplementary materials. The ^1H and ^{13}C NMR data are listed in Tables 1 and 2, respectively. In a similar manner, the ^1H and ^{13}C NMR data of other 15 flavonoids were acquired and listed in Tables 1 and 2, respectively. As mentioned above, the ^1H and ^{13}C NMR data of compounds **1** and **12**, the ^1H NMR data of compounds **5** and **14**, and the ^{13}C NMR data of compounds **8** and **15** had been previously reported, and their spectra matched well with the NMR data in the present study.

Compound **1** contains dihydroxy groups at C-6 and C-7, and compound **2** contains dimethoxy groups at the same positions. Likewise, compounds **3** and **4** have dihydroxy and dimethoxy groups at C-7 and C-8, respectively. In addition, compounds **5** and **6** have dihydroxy and dimethoxy groups at C-6 and C-4', respectively. When the NMR data was compared, the ^{13}C chemical shifts of the carbons with the hydroxy groups were found to be more upfield shifted than those with methoxy groups. The ^1H chemical shifts of the neighboring protons of the carbons with hydroxy or methoxy groups show the same patterns; the chemical shifts of H-5 and H-8 of compound **1** with 6-OH and 7-OH were more upfield shifted than those of compound **2** with 6-OCH₃ and 7-OCH₃. Likewise, H-5 and H-6 of compound **3** (7-OH and 8-OH) were more upfield shifted than those of compound **4** (7-OCH₃ and 8-OCH₃) and H-5 and H-7 of compound **5** (6-OH) was more upfield shifted than those of compound **6** (6-OCH₃). In addition, the H-3'/H-5' of compound **5**, which contains a 4'-OH, were upfield shifted than those of compound **6**, which contains a 4'-OCH₃. The substitution of hydroxy/methoxy groups at C-2' makes the chemical shift of C-2' upfield shifted, and the 5-OH substitute causes a downfield shift of C-4 and an upfield shift of C-10. While compounds **1-15** belong to flavone, compound **16** does flavanone. The double bond between C-2 and C-3 causes downfield shifts of their ^{13}C chemical shifts by approximately 90 and 60 ppm, respectively because of their sp^2 hybrid orbitals. In addition, it has an effect on the ^{13}C chemical shift of C-4 by at least 10 ppm. The substitution of hydroxy/methoxy group at C-2' causes the downfield shift at C-3 and the upfield shift at C-2. Mono-hydroxy/methoxy group in A-ring occurs to the upfield shift at C-3 by about 2 ppm. In compounds with unsubstituted B-ring, **1**, **2**, **3**, **4**, **12**, and **13**, the ^{13}C chemical shifts of C-1', C-2'/C-6', C-3'/C-5', and C-4' in B-ring have the values of 131.2 ± 0.4 , 126.3 ± 0.2 , 129.1 ± 0.1 , and 131.7 ± 0.3 ppm, respectively. Likewise, in compounds with unsubstituted A-ring, **7** and **8**, the change of the ^{13}C chemical shifts of C-5, C-6, C-7, C-8, C-9, and C-10 in A-ring is ranged within ± 0.2 ppm. The substitution of hydroxy/methoxy group at C-7 and/or C-8 occurs to the

upfield shift at C-9 and C-10.

Comparing the ^{13}C chemical shifts of compound **10** with those of compound **9**, only one more hydroxy group at C-3 of the former changes chemical shifts of most carbons consisting of the compound. In addition, 3-OH of compound **10** causes the ^{13}C chemical shift by 5.7 ppm at the other hydroxylated carbon, C-6, but does by 1.0 ppm at C-2'. While the bond distance between C-3 and C-6 is four, that between C-3 and C-2' is three, but C-6 showed more chemical shift change. Because flavone can have polysubstituted positions, various cases such as mentioned above would be found in the NMR data of flavone. As a result, the NMR data as obtained in this experiment can help us identify structures of newly isolated hydroxy/methoxy-flavones.

Experimental Section

Hydroxy/methoxy flavonoids **1-16** were purchased from INDOFINE chemical company, Inc. (Hillsborough, NJ, USA). The chemicals were used in the NMR experiments without further purification, which were supplied from the company at a purity of 98%.¹¹

All NMR experiments were carried out on a Bruker Avance 400 spectrometer (9.4 T, Karlsruhe, Germany). The samples were dissolved in $\text{DMSO-}d_6$ at 298 K and transferred into 5 mm NMR tube. The concentrations of the samples were approximately 50 mM. For the ^1H and ^{13}C NMR experiments, 1 sec and 3 sec relaxation delays were used, respectively, and data was collected at 32 K and 64 K, respectively. In addition, the 90° pulses were 10.2 and 10.3 μsec , respectively, and their spectral widths were 6,000 Hz and 24,000 Hz, respectively. All two-dimensional spectra were collected with $2,048 \times 256$ data points ($t_2 \times t_1$). The long-ranged coupling time for HMBC was 70 msec. The NMR data were processed using XWIN-NMR (Bruker) and

Sparky.^{12,13}

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Supplementary Materials

 ^1H and ^{13}C NMR Data on Hydroxy/methoxy Flavonoids and the Effects of Substituents on Chemical ShiftsHyuk Yoon, Sunglock Eom, Jiye Hyun, Geunhyeong Jo, Doseok Hwang, Sunhee Lee, Yeonjoong Yong, Jun Cheol Park,[†] Young Han Lee,[‡] and Yoongho Lim^{*}

Division of Bioscience and Biotechnology, BMIC, Konkuk University, Seoul 143-701, Korea. *E-mail: yoongho@konkuk.ac.kr

[†]National Institute of Animal Science, Rural Development Administration, Suwon 441-706, Korea[‡]Department of Biomedical Science and Technology, Research Center for Transcription Control, Konkuk University, Seoul 143-701, Korea

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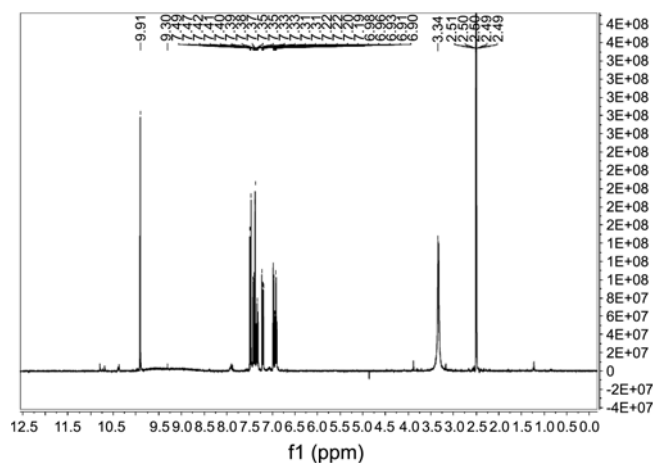
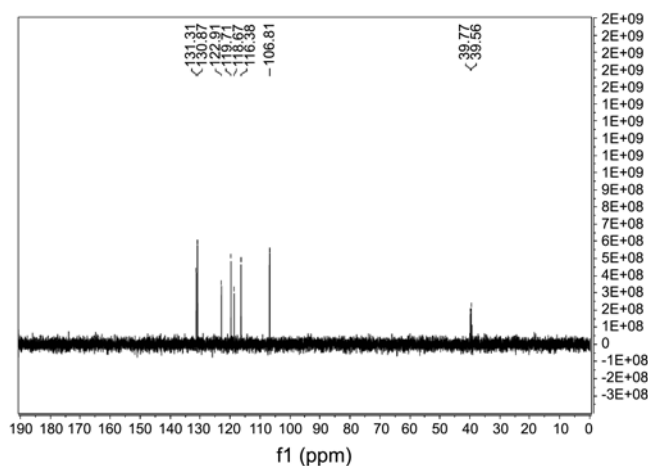
Fig. 1. The ^1H NMR spectrum of compound 10.

Fig. 3. The DEPT spectrum of compound 10.

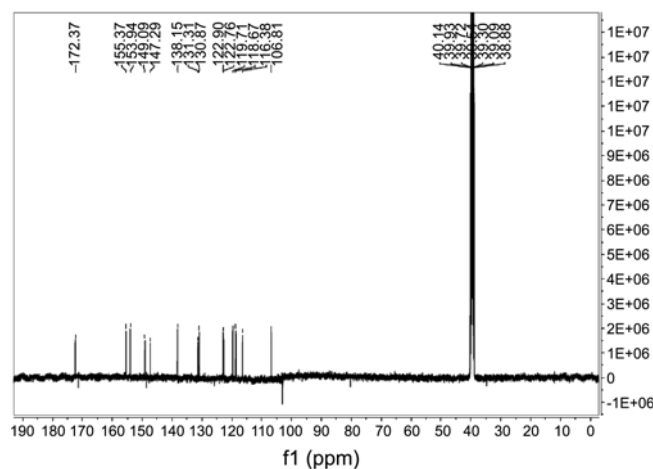
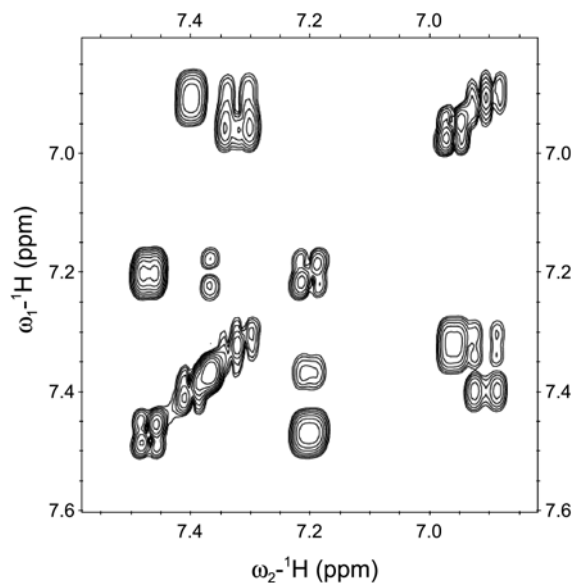
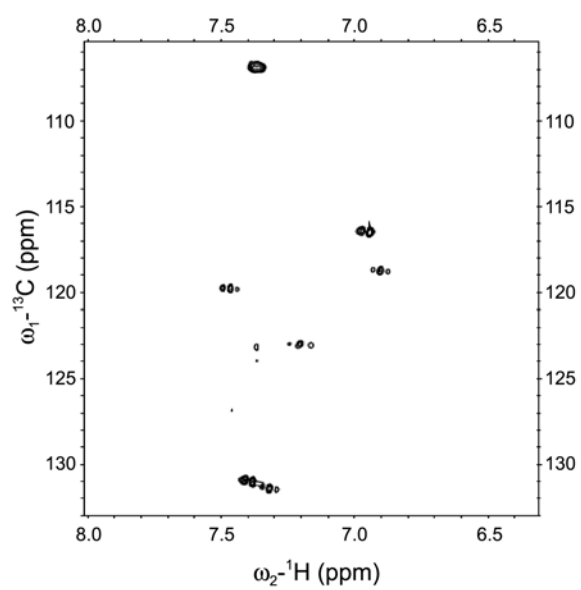
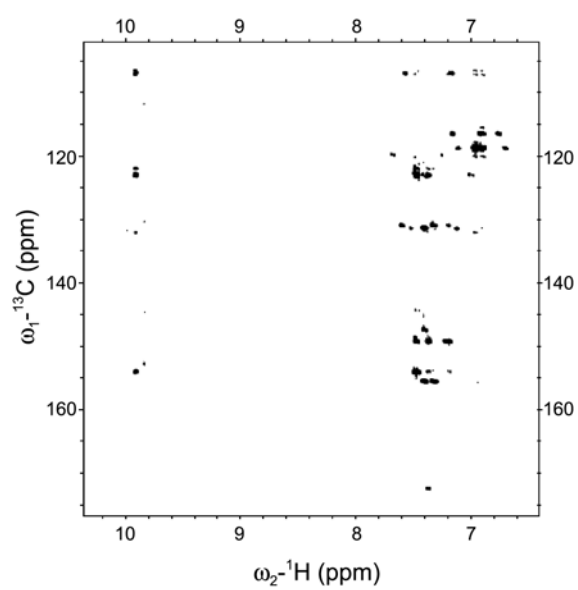
Fig. 2. The ^{13}C NMR spectrum of compound 10.

Fig. 4. The COSY spectrum of compound 10.

**Fig. 5.** The HMQC spectrum of compound **10**.**Fig. 6.** The HMBC spectrum of compound **10**.