The ^{1}H and ^{13}C NMR Data of 19 Methoxyflavonol Derivatives

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Received September 17, 2007

In the present study, we report ^{1}H and ^{13}C NMR data of 19 methoxyflavonol derivatives with different substitution patterns on A- and B-ring. In addition, the influence of the methoxy substituents in A- and B-ring on the ^{1}H and ^{13}C NMR chemical shifts is discussed: the ^{1}H and ^{13}C chemical shifts of and the number of methoxyl groups provided information allowing elimination of many structural isomers from consideration and in certain instances greatly simplified structural elucidation.

Key Words : NMR, Methoxyflavonol derivatives, Structure

Introduction

Flavonoids are a large group of polyphenolic compounds possessing a basic flavan moiety with two aromatic rings (A- and B-ring) interconnected by a three-carbon-atom heterocyclic ring (C-ring). The most widespread flavonoids contain a double bond between C-2 and C-3 and a keto function at C-4 of C-ring. Here, the double bond is attached to B ring at C-2 (flavone) or at C-3 (isoflavone). As a result of a number of further modifications on all three rings, particularly on C ring, flavonoids represent one of the largest and the most diverse class of plant secondary metabolites.[1] Variations on the basic structure of flavonoids yield different classes of flavonoids.[2] These structural variations may explain the observed differences in the bioactivity of these related compounds. Flavonoids have been known for a long time to exert diverse biological effects and in particular to act as antioxidants, antitumour, antiangiogenetic, anti-inflammatory, antiallergic, and antiviral properties.[3] The structural diversity of flavonoids is the result of a number of substitution reaction, such as hydroxylation, methoxylation, glycosylation, methylation, and acylation.[2] The methylation of flavonols not only reduces the chemical activity of their phenolic hydroxyl groups but also increases their lipophilicity. Many methoxyflavonol derivatives have been found from natural sources, and whenever they were isolated, they have been identified by instrumental analysis. Especially, NMR spectroscopy has been successively employed to establish the environment and nature of the carbon and hydrogen atoms and the information gained by this technique is fundamental for flavonoid structure determination. Therefore, the complete NMR data of methoxyflavonol derivatives can help us identify derivatives isolated from natural products. In the present study, we report ^{1}H and ^{13}C NMR data of flavonol (**1**) and 18 methoxyflavonol derivatives (**2-19**) with different substitution patterns on A- and B-ring. In addition, we discussed the influence of the methoxy substituents in A- and B-ring on the ^{1}H and ^{13}C NMR chemical shifts.

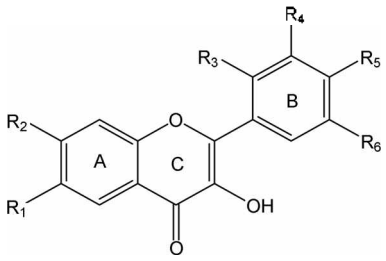
Materials and Methods

Materials. Nineteen methoxyflavonol derivatives, flavonol (**1**), 6-methoxyflavonol (**2**), 7-methoxyflavonol (**3**), 2'-methoxyflavonol (**4**), 3'-methoxyflavonol (**5**), 4'-methoxyflavonol (**6**), 6,3'-dimethoxyflavonol (**7**), 6,4'-dimethoxyflavonol (**8**), 7,3'-dimethoxyflavonol (**9**), 7,4'-dimethoxyflavonol (**10**), 2',3'-dimethoxyflavonol (**11**), 2',4'-dimethoxyflavonol (**12**), 6,2',3'-trimethoxyflavonol (13), 6,2',4'-trimethoxyflavonol (**14**), 6,3',4'-trimethoxyflavonol (**15**), 7,2',3'-trimethoxyflavonol (**16**), 7,2',4'-trimethoxyflavonol (**17**), 3',4',5'-trimethoxyflavonol (**18**), and 7,3',4',5'-tetramethoxyflavonol (**19**), were purchased from INDOFINE chemical company, Inc. (Hillsborough, NJ). Their structures and nomenclatures are shown in Figure 1. The chemicals were used for the experiments without further purification, which were supplied from the company at the purity of 98%.

NMR spectra. All NMR measurements were carried out on a Bruker Avance 400 spectrometer system (9.4 T, Karlsruhe, Germany) at 298 K. The concentrations of the samples for ^{1}H NMR, ^{13}C NMR, DEPT, COSY, HMQC, HMBC, and NOESY experiments were approximately 50 mM in DMSO-d_6. The number scans for the ^{1}H NMR were 16 and the 32 K data points were acquired with a 1 sec relaxation delay. Its 90° pulse was 10.2 μsec with a spectral width of 12 ppm. The ^{13}C NMR and DEPT spectra were obtained with a spectral width of 210 ppm using 64 K data points. Their 90° pulses were 10.3 μsec. All two-dimensional spectra except NOESY were acquired with 2,048 data points for t_2 and 256 for t_1 increments using magnitude mode. The long-ranged coupling time for HMBC was 70 msec. Prior to fourier transformation, zero filling of 2 K and sine squared bell window function were applied using XWIN-NMR (Bruker) and all NMR data were analyzed in Sparky.[4,5]

Results and Discussion

The structures and nomenclatures of methoxyflavonol



Derivative	Nomenclature	R_1	R_2	R_3	R_4	R_5	R_6
1	flavonol	H	H	H	H	H	H
2	6-methoxyflavonol	OMe	H	H	H	H	H
3	7-methoxyflavonol	H	OMe	H	H	H	H
4	2'-methoxyflavonol	H	H	OMe	H	H	H
5	3'-methoxyflavonol	H	H	H	OMe	H	H
6	4'-methoxyflavonol	H	H	H	H	OMe	H
7	6,3'-dimethoxyflavonol	OMe	H	H	OMe	H	H
8	6,4'-dimethoxyflavonol	OMe	H	H	H	OMe	H
9	7,3'-dimethoxyflavonol	H	OMe	H	OMe	H	H
10	7,4'-dimethoxyflavonol	H	OMe	H	H	OMe	H
11	2',3'-dimethoxyflavonol	H	H	OMe	OMe	H	H
12	2',4'-dimethoxyflavonol	H	H	OMe	H	OMe	H
13	6,2',3'-trimethoxyflavonol	OMe	H	OMe	OMe	H	H
14	6,2',4'-trimethoxyflavonol	OMe	H	OMe	H	OMe	H
15	6,3',4'-trimethoxyflavonol	OMe	H	H	OMe	OMe	H
16	7,2',3'-trimethoxyflavonol	H	OMe	OMe	OMe	H	H
17	7,2',4'-trimethoxyflavonol	H	OMe	H	OMe	OMe	H
18	3',4',5'-trimethoxyflavonol	H	H	H	OMe	OMe	OMe
19	7,3',4',5'-tetramethoxyflavonol	H	OMe	H	OMe	OMe	OMe

Figure 1. Structures and nomenclatures of 19 methoxyflavonol derivatives

derivatives **1-19** are shown in Figure 1. Of nineteen methoxyflavonol derivatives, the NMR data of eleven methoxyflavonol derivatives (**1**, **3-10**, **16**, **19**) have been previously reported.[6-10] Since the ^{1}H and ^{13}C chemical shifts of the remaining eight derivatives (**2**, **11-15**, **17**, **18**) were not reported yet, we carried out their complete assignments and considered the effect of methoxyl substituents of flavonol. Derivative **2** is 6-methoxyflavonol which is rarely found in natural sources. Fourteen peaks were observed in the ^{13}C NMR spectrum and the peak at 55.8 ppm suggested the presence of one methoxy carbon. In addition, the most downfield shifted peak at 172.6 ppm was assigned to C-4 which was long-range coupled to two ^{1}H peaks at 7.44 and 9.50 ppm in the HMBC spectrum. The protons closest to C-4 are H-5 and 3-OH so that they are assigned correspondingly. According to the HMQC data, C-5 resonates 103.9 ppm because C-5 is long-range coupled to proton with 7.40 ppm, which was assigned to H-7. Here, the ^{1}H peak at 7.40 ppm shows two coupling constants of 3.0 and 9.0 Hz. Since H-5 shows the coupling constant of 3.0 Hz, the ^{1}H peak with the coupling constant of 9.0 Hz should be H-8 which was found at 7.72 ppm. This correlation was confirmed on the based of the COSY interpretation. In HMBC, the H-5 signal showed a long-range coupling with three ^{13}C peaks at 123.6, 149.6, and 172.6 ppm. Because two signals at 123.6 and 172.6 ppm were already assigned to C-7 and C-4, respectively, the signal at 149.6 ppm corresponds to C-9. The H-8 signal showed a long-range coupling with two ^{13}C peaks at 121.9 and 156.0 ppm in the HMBC spectrum. Those peaks should be assigned to C-6 and/or C-10, respectively. However, the chemical shift of C-6, an oxygen-bearing carbon, was more downfield shifted than that of C-10, so 156.0 ppm was assigned to C-6 and 121.9 ppm was assigned to C-10. In the HMBC spectrum, C-6 was long-range coupled to the ^{1}H peak at 3.80 ppm, which was attached directly to the ^{13}C peak at 55.8 ppm. Therefore, 3.80 ppm should be assigned to a 6-methoxy proton, and 55.8 ppm was a 6-methoxy carbon. Since two ^{1}H peaks at 7.56 and 8.20 ppm showed double intensities, they were H-2'/H-6' and/or H-3'/H-5'. In the COSY spectrum, two cross-peaks between 8.20 and 7.56 ppm, and 7.56 and 7.48 ppm were observed. The ^{1}H peak at 7.56 ppm was correlated with both 8.20 ppm and 7.48 ppm, so it should be H-3'/5'. The ^{1}H peak at 8.20 ppm which was double intensities should be H-2'/6'. The ^{1}H peak at 7.48 ppm was assigned to H-4'. Three singlet carbons of C-2, C-3 and C-1' remained. From HMBC, the singlet carbon observed at 131.4 ppm showed a long-ranged coupling with H-3'/H-5', so it was considered to be C-1'. Another singlet carbon observed at 145.1 ppm showed a long-ranged coupling with H-2'/H-6', so it was considered as C-2. The remaining singlet carbon, 138.7 ppm, was assigned to C-3. The complete assignments of the ^{1}H and ^{13}C chemical shifts of 6-methoxyflavonol are listed in Tables 1 and 2, respectively. The NMR data of the remained seven methoxyflavonol derivatives (**11-**

Table 1. The ^{1}H chemical shifts of 19 methoxyflavonol derivatives **1-19**

	$\delta$of ^{1}H (J, Hz)								
position	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**
5	8.10 (dd, 1.5, 8.0)	7.44 (d, 3.0)	7.98 (d, 8.9)	8.14 (dd, 1.5, 8.0)	8.10 (dd, 1.4, 8.0)	8.11 (dd, 1.5, 8.0)	7.42 (d, 3.0)	7.42 (d, 3.0)	7.99 (d, 8.9)
6	7.42 (m)	–	7.03 (dd, 2.4, 8.9)	7.46 (m)	7.44 (m)	7.46 (ddd, 1.3, 6.6, 8.0)	–	–	7.05 (dd, 2.4, 8.9)
7	7.76 (ddd, 1.5, 7.1, 8.5)	7.40 (dd, 3.0, 9.1)	–	7.76 (ddd, 1.5, 7.1, 8.5)	7.78 (m)	7.78 (m)	7.39 (dd, 3.0, 9.1)	7.37 (dd, 3.0, 9.1)	–
8	7.71 (d, 8.5)	7.72 (d, 9.1)	7.25 (d, 2.4)	7.61 (d, 8.5)	7.75 (m)	7.76 (m)	7.73 (d, 9.1)	7.68 (d, 9.1)	7.30 (d, 2.4)
2'	8.20 (dd, 1.4, 7.2)	8.20 (dd, 1.5, 7.5)	8.20 (d, 7.3)	–	7.77 (s)	8.21 (d, 9.0)	7.77 (dd, 2.6, 1.6)	8.17 (d, 9.1)	7.77 (dd, 2.6, 1.6)
3'	7.54 (m)	7.56 (dd, 7.3 7.5)	7.55 (dd, 7.3, 7.3)	7.19 (d, 8.3)	–	7.13 (d, 9.0)	–	7.11 (d, 9.1)	–
4'	7.42 (dd, 7.2, 7.2)	7.48 (dd, 7.3, 7.3)	7.49 (dd, 7.3, 7.3)	7.51 (m)	7.07 (dd, 2.4, 8.2)	–	7.07 (ddd, 1.6, 2.6, 8.1)	–	7.08 (ddd, 1.6, 2.6, 8.2)
5'	7.54 (m)	7.56 (dd, 7.3, 7.5)	7.55 (dd, 7.3, 7.3)	7.08 (dd, 7.4, 7.4)	7.46 (m)	7.13 (d, 9.0)	7.48 (dd, 8.1, 8.1)	7.11 (d, 9.1)	7.47 (dd, 7.9, 8.2)
6'	8.20 (dd, 1.4, 7.2)	8.20 (d, 1.5, 7.5,)	8.20 (d, 7.3)	7.49 (m)	7.80 (m)	8.21 (d, 9.0)	7.81 (ddd, 1.6, 1.6, 8.1)	8.17 (d, 9.1)	7.82 (ddd, 1.6, 1.6, 7.9)
3-OH	9.60 (s)	9.50 (s)	9.47 (s)	8.94 (s)	9.63 (s)	9.45 (s)	9.52 (s)	9.34 (s)	9.28 (s)
6-OMe		3.80 (s)					3.88 (s)	3.86 (s)	
7-OMe			3.90 (s)						3.92 (s)
2'-OMe				3.79 (s)					
3'-OMe					3.82 (s)		3.83 (s)		3.86 (s)
4'-OMe						3.85 (s)		3.83 (s)	
5'-OMe									

	$\delta$of ^{1}H (J, Hz)									
position	**10**	**11**	**12**	**13**	**14**	**15**	**16**	**17**	**18**	**19**
5	7.98 (d, 8.9)	8.15 (dd, 1.6, 8.0)	8.12 (dd, 1.5, 8.0)	7.47 (d, 3.1)	7.46 (d, 3.1)	7.44 (d, 3.1)	8.03 (d, 8.9)	8.01 (d, 8.9)	8.12 (d, 8.0)	7.99 (d, 8.9)
6	7.03 (dd, 2.4, 8.9)	7.48 (m)	7.45 (m)	–	–	–	7.05 (dd, 2.4, 8.9)	7.03 (dd, 8.9, 2.4)	7.46 (dd, 8.0, 8.0)	7.05 (dd, 8.9, 2.2)
7	–	7.77 (m)	7.75 (m)	7.38 (dd, 3.1, 9.2)	7.35 (dd, 3.1, 9.2)	7.39 (dd, 3.1, 9.1)	–	–	7.81 (m)	–
8	7.26 (d, 2.4)	7.63 (d, 7.5)	7.59 (d, 8.3)	7.60 (d, 9.2)	7.55 (d, 9.2)	7.74 (d, 9.1)	7.08 (m)	7.08 (d, 2.4)	7.80 (m)	7.32 (d, 2.2)
2'	8.19 (d, 9.2)	–	–	–	–	7.80 (d, 2.0)	–	–	7.56 (s)	7.55 (s)
3'	7.12 (d, 9.2)	–	6.71 (d, 2.3)	–	6.71 (d, 2.3)	–	–	6.71 (d,2.3)	–	–
4'	–	7.22 (dd, 1.8, 8.2)	–	7.23 (dd, 1.9, 8.2)	–	–	7.22 (d, 8.3, 2.0)	–	–	–
5'	7.12 (d, 9.2)	7.17 (m)	6.65 (dd, 2.3, 8.4)	7.18 (dd, 7.4, 8.2)	6.65 (dd, 2.3, 8.5)	7.14 (d, 8.6)	7.12 (m)	6.65 (dd, 8.4, 2.3)	–	–
6'	8.19 (d, 9.2)	7.10 (dd, 1.8, 7.3)	7.41 (d, 8.4)	7.10 (dd, 1.9, 7.4)	7.40 (d, 8.5)	7.86 (dd, 2.0, 8.6)	7.80 (m)	7.39 (d, 8.4)	7.56 (s)	7.55 (s)
3-OH	9.28 (s)	9.02 (s)	8.78 (s)	9.00 (s)	8.69 (s)	9.38 (s)	8.88 (s)	8.60 (s)	9.63 (s)	9.45 (s)
6-OMe				3.87 (s)	3.88 (s)	3.88 (s)				
7-OMe	3.90 (s)						3.87 (s)	3.87 (s)		3.93 (s)
2'OMe		3.79 (s)	3.79 (s)	3.78 (s)	3.79 (s)		3.80 (s)	3.79 (s)		
3'-OMe		3.87 (s)		3.83 (s)		3.85 (s)	3.87 (s)		3.86 (s)	3.87 (s)
4'-OMe	3.85 (s)		3.84 (s)		3.84 (s)	3.85 (s)		3.84 (s)	3.75 (s)	3.76 (s)
5'-OMe									3.86 (s)	3.87 (s)

15. **17**. **18**) were completely assigned based on the interpretation of 1D and 2D experiments as the same manner as derivative **2**. Their complete assignments of the ^{1}H and ^{13}C chemical shifts are listed in Tables 1 and 2, respectively.

We investigated the influence of the methoxy substituents in A- and B-ring on the ^{1}H and ^{13}C NMR chemical shifts. In

Table 2. The ^{13}C chemical shifts of 19 methoxyflavonol derivatives **1-19**

	δ of ^{13}C																		
position	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**	**15**	**16**	**17**	**18**	**19**
2	145.1	145.1	144.4	147.2	144.9	145.7	144.7	145.4	144.1	144.8	146.7	147.3	146.6	147.1	145.3	146.0	146.4	145.0	144.3
3	139.1	138.7	138.7	139.2	139.2	138.2	138.7	137.7	138.8	137.7	139.0	139.0	138.6	138.5	137.9	138.8	138.6	138.8	138.5
4	173.0	172.6	172.3	172.8	173.0	172.7	172.5	172.2	172.3	172.0	172.7	172.6	172.3	172.1	172.2	172.2	172.0	172.8	172.2
5	124.8	103.9	126.2	124.9	124.8	124.8	103.8	103.8	126.1	126.0	124.8	124.8	103.9	104.0	103.9	126.3	126.1	124.7	126.1
6	124.5	156.0	114.7	124.5	124.6	124.5	155.9	155.8	114.9	114.4	124.5	124.3	155.9	155.8	155.9	114.5	114.3	124.5	114.7
7	133.6	123.6	163.6	133.4	135.0	133.6	123.5	123.1	163.7	163.5	133.5	133.3	123.3	123.0	123.2	163.5	163.3	133.6	163.7
8	118.3	120.1	100.2	118.4	118.5	118.4	120.1	119.9	100.3	100.2	118.3	118.3	119.9	119.9	120.1	100.2	100.1	118.5	100.4
9	154.5	149.6	156.5	155.1	154.6	154.5	149.5	149.6	156.5	156.3	154.8	154.9	149.8	149.9	149.3	156.8	156.8	154.4	156.4
10	121.3	121.9	115.1	122.0	121.3	121.4	121.7	121.8	115.0	115.1	121.9	121.9	122.5	122.5	121.8	115.8	115.7	121.2	115.1
1'	131.3	131.4	131.3	119.9	132.5	123.6	132.5	123.6	132.6	123.6	125.2	112.4	125.3	122.5	123.7	125.4	112.5	126.5	126.7
2'	127.6	127.7	127.4	157.2	113.5	129.5	113.3	129.3	113.1	129.1	146.9	158.5	146.9	158.5	111.0	147.0	158.4	105.8	105.5
3'	128.5	128.5	128.5	112.1	159.2	114.1	159.1	114.0	159.1	113.9	152.7	98.8	152.7	98.8	148.4	152.7	98.7	152.7	152.7
4'	129.8	129.9	129.6	131.9	115.2	160.5	115.1	160.3	115	160.2	114.8	162.3	122.3	162.2	150.3	114.8	162.1	139.3	139.0
5'	128.5	128.5	128.5	120.2	129.7	114.1	129.6	114.0	129.5	113.9	123.8	105.2	123.8	105.1	111.5	123.8	105.1	152.7	152.7
6'	127.6	127.7	127.4	131.1	120.1	129.5	120.0	129.3	119.8	129.1	122.3	131.8	114.8	131.9	121.5	122.4	131.8	105.8	105.5
6-OMe		55.8					55.7	55.68					55.7	55.7	55.68				
7-OMe			56.06						56.08	56.0						56.0	55.9		56.2
2'-OMe				55.8							60.5	55.5	60.6	55.8		60.6	55.8		
3'-OMe					55.3		55.2		55.2		55.9		55.9		55.58	55.9		56.1	56.2
4'-OMe						55.4		55.33		55.3		55.8		55.4	55.66		55.4	60.2	60.2
5'-OMe																		56.1	56.2

general. A-ring and/or B-ring methoxylation does not result in long-range chemical shift perturbations: A-ring methoxylation has no effect on B-ring chemical shifts and B-ring methoxylation has no effect on A-ring chemical shifts. In addition, the substitution of methoxyl groups affects the chemical shifts of *ortho*-position of flavonol to move the upfield in A- and B-ring of monomethoxyflavonols (**2-10**). Especially, in monomethoxylated B-ring (**6**, **8**, **10**), the B-ring signals were readily assigned by consideration of symmetry and standard chemical shift effects of a single oxygen on an aromatic ring. The ^{13}C chemical shifts of mono-substituted methoxyl carbons usually appear about 55 ppm. However, the resonances of aromatic methoxyl groups attached to di-*ortho* substituted carbons (**11**, **13**, **16**) occur considerably downfield at about 60 ppm. This provides a useful diagnostic tool for structural analysis of natural products. The presence of symmetry was useful in assigning structures to the flavonols with trimethoxylated B-rings (**18**, **19**). A signal corresponding to a degenerate pair of methine carbons appeared in those spectra indicating that these carbons were symmetrically disposed about the C-1' - C-4' axis. In conclusion, the ^{1}H and ^{13}C chemical shifts and the number of methoxyl groups based on the elucidation of Tables 1 and 2 provided information allowing elimination of many structural isomers from consideration and in certain instances greatly simplified structural elucidation.

Acknowledgments. This work was supported by grant KRF-2006-005-J03402 (KRF). Biogreen 21 (Korea Ministry of Agriculture and Forestry), and grant from the second BK21 (MOE). Y. Park and B-H. Moon contributed equally to this work.

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