Complete Assignments of the ^1H and ^{13}C NMR Data of Flavone Derivatives

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The ^1H and ^{13}C chemical shifts of flavone and its five derivatives were determined completely using the basic 1D and 2D NMR experiments and molecular modeling. Of the six compounds used for our experiments, the NMR data of three compounds were published previously, but we found that the data of two compounds included wrong assignments. Therefore, we report the corrected data and the complete assignments of NMR data of the other three compounds.

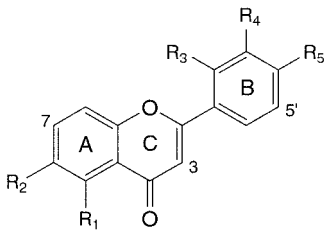
Key Words : NMR, Flavone derivatives, Structure

Introduction

Flavonoids are one of secondary metabolites produced by plants. They have C6-C3-C6 skeleton where two aromatic rings are linked through pyran ring.[1] Based on the oxidation state of pyran ring, flavonoids can be classified by anthocyanins, flavans, proanthocyanidins, C-glycosylflavonoids, biflavonoids, triflavonoids, isoflavonoids, neoflavonoids, flavones, flavonols, flavone glycosides, and flavonol glycosides.[2] They play many important roles such as pigmentation, pathogen resistance, UV light protection, growth, and development in plants.[3] From a human point of view, flavonoids can provide protection against many diseases because of their antioxidant activities.[4]

Until now, over 4,000 flavonoids were found. Many flavonoid derivatives are being discovered still. In order to identify them, a comparison of their retention time obtained from HPLC is being used.[5] Since most of them were isolated as natural products, it is difficult to secure their authentic samples. Whenever a flavonoid derivative is isolated, its authentic sample should be required for a comparison using HPLC. However, if its NMR data is known, its authentic sample is not needed anymore.

Of flavonoid classes, flavones include ketone group in pyran ring. Many flavone derivatives such as luteolin and apigenin are known.[4] According to the different hydroxylated substitution and the number of hydroxyl groups, the ^1H and ^{13}C chemical shifts are changed. We carried out the basic 1D and 2D NMR experiments for flavone, 2'-hydroxylated flavone, 3'-hydroxylated flavone, 4'-hydroxylated flavone, 5-hydroxylated flavone, and 6-hydroxylated flavone (Fig. 1). The ^1H and ^{13}C chemical shifts of flavone and its five derivatives were assigned. Of 6, the NMR data of flavone, 2-hydroxylated flavone, and 6-hydroxylated flavone were reported previously,[6,7] and the other three were not. We found the NMR data of 2'-hydroxylated flavone and 6-hydroxylated flavone included wrong data. Therefore, we report here the corrected NMR data and the complete assignments of the ^1H and ^{13}C NMR data of the other three flavone derivatives.



flavone (**1**)	R_1=R_2=R_3=R_4=R_5=H
derivative **2**	R_1=R_2=R_4=R_5=H, R_3=OH
derivative **3**	R_1=R_2=R_3=R_5=H, R_4=OH
derivative **4**	R_1=R_2=R_3=R_4=H, R_5=OH
derivative **5**	R_2=R_3=R_4=R_5=H, R_1=OH
derivative **6**	R_1=R_3=R_4=R_5=H, R_2=OH

Figure 1. Structures and nomenclatures of flavone (**1**) and its derivatives **2-6**.

Materials and Methods

Materials. Flavone (**1**) and its derivatives, 2'-hydroxylated flavone, 3'-hydroxylated flavone, 4'-hydroxylated flavone, 5-hydroxylated flavone, and 6-hydroxylated flavone (**2-6**) were purchased from INDOFINE chemical company, Inc. (Hillsborough, NJ). The chemicals were used for the NMR experiments without further purification, which were supplied from the company at the purity of 98%.

NMR spectra. All NMR measurements were performed on a Bruker Avance 400 spectrometer system (9.4 T, Karlsruhe, Germany) at a temperature of 298 K. The NMR spectra of ^1H NMR, ^{13}C NMR, DEPT, COSY, HMQC, HMBC, and NOESY were collected in DMSO-d_6. The concentrations of the samples were approximately 50 mM. For the ^1H NMR analysis, 16 transients were acquired with a 1 sec relaxation delay using 32 K data points. Its 90° pulse was 10.2 μsec with a spectral width of 5,000 Hz. The ^{13}C NMR and DEPT spectra were obtained with a spectral width of 23,809 Hz 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 NOESY spectra were collected with 2,048 data points for t_2 and 256 for t_1

using time proportional phase increments at the mixing time of 1 sec. 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).[8] All NMR data were analyzed in Sparky.[9]

Calculations. Molecular modeling was carried out using InsightII software (Accelrys, San Diego, CA) on a Silicon Graphics workstation O2 R12,000. The force field was consistent valence force field (cvff). The molecules were subjected to energy minimization by InsightII/Discover module. Steepest descents were carried out until maximum derivative of 1.0 kcal/molÅ, and conjugate gradients were followed until maximum derivative of 0.1 kcal/molÅ. After energy minimization, molecular dynamics was performed at 300 K, 1 atm for 500 psec with 1 fsec each step. The output conformers were collected at every 2.5 psec. As a result, 200 conformers were saved in the history file. The energy profile was analyzed using InsightII/Analysis module. Of 200 conformers, the conformer with the lowest energy was chosen.[10]

Results and Discussion

The structures and nomenclatures of flavone (**1**) and its derivatives **2-6** are shown in Figure 1. Thirteen peaks were observed in the ^{13}C NMR spectrum of flavone. The DEPT experiments of flavone gave five singlets and eight doublets. The most downfield shifted peak was 177.0 ppm which was assigned ketone group (C-4). The next downfield shifted peak, 162.4 ppm was C-2. In the HMBC spectrum, C-2 was long-range coupled to the ^{1}H peak at 6.96 ppm which was attached directly to the ^{13}C peak at 106.8 ppm in HMQC. Its possible assignments included C-3, C-1', and C-2'/C-6'. C-1' did not have an attached proton and C-2'/C-6' should show a double intensity of its ^{13}C peak, but the ^{13}C peak at 106.8 ppm did not belong to both of them, so that it was assigned C-3. Since two peaks at 126.2 and 128.9 ppm showed double intensities, they were C-3'/C-5' and/or C-2'/C-6'. In the HMBC spectrum, C-2 was long-range coupled to the ^{1}H peak at 8.01 ppm which was attached directly to the ^{13}C peak at 126.2 ppm in HMQC. Therefore, 126.2 ppm should be assigned C-2'/C-6'. The ^{1}H peaks of H-2' and H-3' could be determined from the HMQC spectrum, which were 8.01 and 7.51 ppm, respectively. According to the interpretation of the COSY spectrum, H-4' was 7.53 ppm. The singlet carbon observed at 131.0 ppm showed a long-ranged coupling with H-3'/H-5', so that it was considered C-1'. Now, only two singlet carbons, 123.2 and 155.5 ppm, are remained. The former should be C-10, and the latter is C-9. From COSY, four ^{1}H peaks at 7.43, 7.67, 7.76, and 8.02 ppm were correlated with each other. In HMBC, while C-10 was long-range coupled to two ^{1}H peaks at 7.43 and 7.67 ppm, C-9 showed long ranged coupling with two ^{1}H peaks at 7.76 and 8.02 ppm. As a result, the former peaks should be assigned H-6 and/or H-8, and the letter peaks were assigned H-5 and/or H-7. In the HMBC spectrum, C-4 was long-range coupled

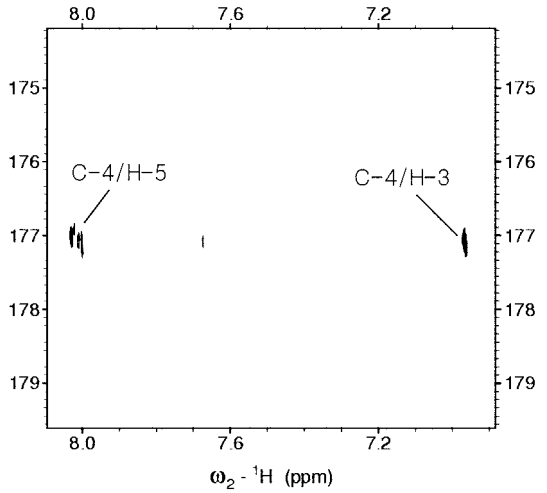


Figure 2. The partial HMBC spectrum of flavone (**1**). The long-ranged coupling time was 70 msec.

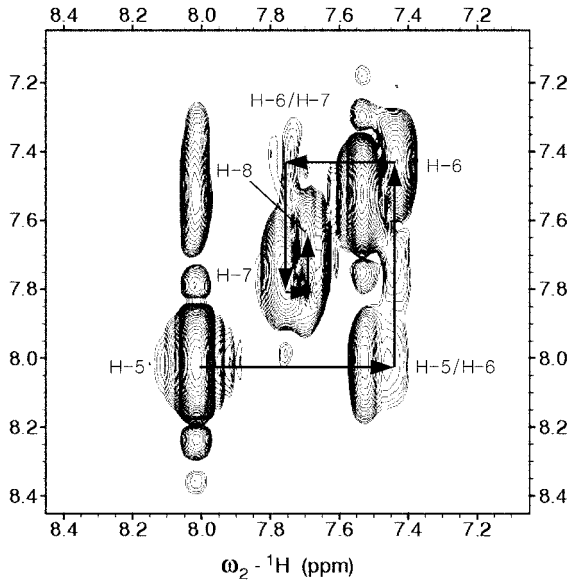


Figure 3. The partial COSY spectrum of flavone (**1**).

to the ^{1}H peak at 8.02 ppm, so that the ^{1}H peak was assigned H-5 (Fig. 2). As a result, the ^{1}H peak at 7.76 ppm should be H-7. Based on the interpretation of three cross-peaks observed in COSY (8.02 ppm → 7.43 ppm → 7.76 ppm → 7.67 ppm), they were assigned H-5, H-6, H-7, and H-8, respectively (Fig. 3). The complete assignments of the ^{1}H and ^{13}C chemical shifts of flavone are listed in Table 1.

The derivative **2** is 2'-hydroxylated flavone (Fig. 1). Its ^{13}C NMR spectrum showed fifteen peaks. Comparing them with the ^{13}C peaks of flavone, seven chemical shifts of C-4~C-10 could be assigned easily, because they showed only small differences within 0.3 ppm. The chemical shift of C-10 was 123.1 ppm which was long-range coupled to the ^{1}H peak at 7.14 ppm in the HMBC spectrum. C-10 could show four possible long-ranged couplings with the neighboring protons, H-3, H-5, H-6, and H-8. Because the ^{1}H peak at 7.14 ppm was a singlet, it was assigned H-3. In HMBC, H-3

Table 1. The complete assignments of the ^1H and ^{13}C chemical shifts of flavone

Assignment	$\delta^{13}C$	CH_n	δ^1H (*J*, Hz)	HMBC	COSY
2	162.4	s	–	C-2/H-2',6', C-2/H-3	–
3	106.8	d	6.96 (s)	–	–
4	177.0	s	–	C-4/H-3, C-4/H-5	–
5	124.7	d	8.02 (m)	C-5/H-7	H-5/H-6
6	125.3	d	7.43 (ddd, 1.1, 7.0, 7.9)	C-6/H-8	H-6/H-5, H-6/H-7
7	134.1	d	7.76 (ddd, 1.6, 7.0, 8.3)	C-7/H-5	H-7/H-6, H-7/H-8
8	118.3	d	7.67 (dd, 1.1, 8.3)	C-8/H-6	H-8/H-7
9	155.5	s	–	C-9/H-5, C-9/H-7, C-9/H-8	–
10	123.2	s	–	C-10/H-3, C-10/H-6, C-10/H-8	–
1'	131.0	s	–	C-1/H-2',6', C-1'/H-3	–
2'	126.2	d	8.01 (m)	C-2'/H-4', C-2'/H-6'	H-2'/H-3'
3'	128.9	d	7.51 (m)	C-3'/H-4', C-3'/H-5'	H-3'/H-2', H-3'/H-4'
4'	131.6	d	7.53 (m)	C-4'/H-2',6', C-4'/H-3',5'	H-4'/H-3',5'
5'	128.9	d	7.51 (m)	C-5'/H-3', C-5'/H-4'	H-5'/H-4', H-5'/H-6'
6'	126.2	d	8.01 (m)	C-6'/H-2', C-6'/H-4'	H-6'/H-5'

was long-range coupled to the ^{13}C peak at 160.7 ppm, so that the ^{13}C peak was assigned C-2. Then, the most downfield shifted ^{13}C peak at 156.6 ppm except C-2 and C-4 should be C-2'. A singlet peak determined from the DEPT experiments, 117.7 ppm was C-1'. In the HMBC spectrum, C-2 was long-range coupled to the ^1H peak at 7.92 ppm. Therefore, it should be H-6'. Based on the interpretation of the coupling constants of H-3', H-4', and H-5', they were assigned. In order to clarify the assignments of H-3', H-4', and H-5', the long-ranged couplings observed in the HMBC spectrum were analyzed. The expected couplings were found among C-1'/H-5', C-1'/H-3', C-6'/H-4', and C-5'/H-3'. The ^{13}C chemical shifts of the derivative **2** were reported by Kingsbury and Looker.[6] Except C-5 and C-6, their chemical shifts agree with ours. In our assignments, C-5 and C-6 are 124.7 and 125.2 ppm, respectively, but in their assignments, C-5 and C-6 are 125.2 and 124.8 ppm, respectively. In order to prove whether our assignments are correct, the interpretation of the HMBC data was carried out carefully. C-4 showed long-ranged coupling with the ^1H peak at 8.04 ppm and C-9 did with the same ^1H peak too. Therefore, this ^1H peak must be H-5. The partial HMBC spectrum showing these couplings is shown in Figure 4. Likewise, C-10 was long-range coupled to the ^1H peak at 7.47 ppm and C-8, the same ^1H peak too (Fig. 4). The ^1H peak at 7.47 ppm must be H-6. As a result, C-5 and C-6 are assigned 124.7 and 125.2 ppm, respectively. The complete assignments of ^1H and ^{13}C chemical shifts of 2'-hydroxylated flavone are listed in Tables 2 and 3, respectively.

The derivative **3** is 3'-hydroxylated flavone (Fig. 1). Like the derivative **2**, nine chemical shifts of C-2~C-10 could be determined easily based on the comparison of its ^{13}C chemical shifts with those of flavone. The ^{13}C peak at 157.9 ppm should be C-3' and the singlet ^{13}C peak at 132.4 ppm was assigned C-1'. Four ^1H peaks at 7.00, 7.36, 7.43, and 7.49 ppm were not assigned. As shown in Figure 5, two cross-peaks between 7.00 and 7.36 ppm, and 7.36 and 7.49 ppm were observed in the COSY spectrum. However, the ^1H peak at 7.43 ppm did not show any correlated cross-peak, so that it was assigned H-2'. Because the ^1H peak at 7.36 ppm was correlated with both 7.00 ppm and 7.49 ppm, it should be H-5'. In the HMBC spectrum, C-2 (162.7 ppm) was long-range coupled to the ^1H peak at 7.49 ppm, so that it was assigned H-6' (Fig. 6). The complete assignments of ^1H and ^{13}C chemical shifts of 3'-hydroxylated flavone are listed in Tables 2 and 3, respectively.

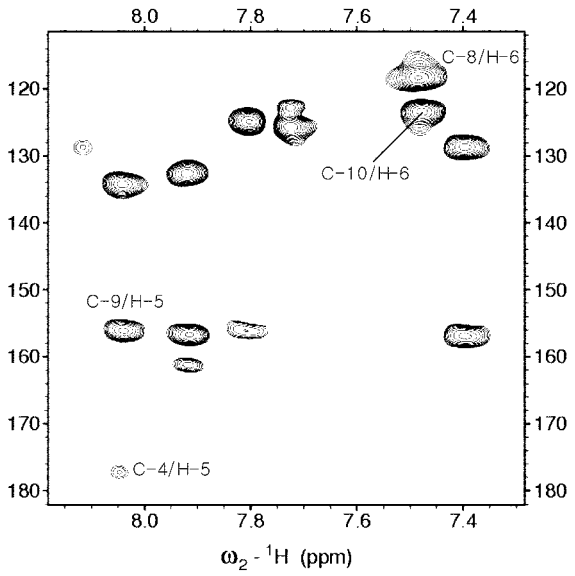


Figure 4. The partial HMBC spectrum of derivative **2**. The long-ranged coupling time was 70 msec.

The derivative **4** is 4'-hydroxylated flavone (Fig. 1). Like flavone, the ^{13}C NMR spectrum of the derivative **4** showed thirteen peaks. By comparing the ^{13}C NMR data with those of flavone, nine ^{13}C chemical shifts of C-2~C-10 were determined. Two ^{13}C peaks showing double intensities at 115.9 and 128.3 ppm were observed in the ^{13}C NMR spectrum, which were assigned C-3' and/or C-2'. In the HMBC spectrum, C-2 was long-range coupled to the ^1H

Table 2. The ^{1}H chemical shifts, δ^1H (*J*, Hz), of flavone derivatives **2**, **3**, **4**, **5**, and **6**

position	**2**	**3**	**4**	**5**	**6**
3	7.14 (s)	6.90 (s)	6.87 (s)	7.10 (s)	6.96 (s)
5	8.04 (dd, 1.7, 7.9)	8.03 (dd, 1.5, 7.9)	8.03 (dd, 1.3, 7.9)	–	7.34 (d, 3.0)
6	7.47 (ddd, 1.0, 7.0, 7.9)	7.46 (m)	7.47 (ddd, 1.1, 7.0, 7.9)	6.81 (d, 8.3)	–
7	7.80 (ddd, 1.7, 7.0, 8.5)	7.80 (ddd, 1.5, 7.1, 8.3)	7.80 (ddd, 1.3, 7.0, 8.5)	7.68 (dd, 8.3, 8.3)	7.24 (dd, 3.0, 9.0)
8	7.72 (dd, 1.0, 8.5)	7.72 (d, 8.3)	7.72 (dd, 1.1, 8.5)	7.19 (d, 8.3)	7.59 (d, 9.0)
2'	–	7.43 (dd, 2.0, 2.0)	7.96 (d, 8.8)	8.11 (dd, 1.6, 6.9)	8.01 (dd, 2.0, 7.7)
3'	7.06 (dd, 1.0, 8.3)	–	6.93 (d, 8.8)	7.59 (m)	7.51 (m)
4'	7.39 (ddd, 1.7, 7.5, 8.3)	7.00 (dd, 2.0, 7.9)	–	7.61 (m)	7.53 (m)
5'	7.00 (ddd, 1.0, 7.5, 7.9)	7.36 (dd, 7.9, 7.9)	6.93 (d, 8.8)	7.59 (m)	7.51 (m)
6'	7.92 (dd, 1.7, 7.9)	7.49 (m)	7.96 (d, 8.8)	8.11 (dd, 1.6, 6.9)	8.01 (dd, 2.0, 7.7)
OH-2'	10.74 (s)	–	–	–	–
OH-3'	–	9.90 (s)	–	–	–
OH-4'	–	–	10.33 (s)	–	–
OH-5	–	–	–	12.65 (s)	–
OH-6	–	–	–	–	10.04 (s)

Table 3. The ^{13}C chemical shifts (ppm) of flavone derivatives **2**, **3**, **4**, **5**, and **6**

Position	**2**	**3**	**4**	**5**	**6**
2	160.7	162.7	163.0	164.1	162.1
3	111.0	106.9	104.8	105.7	105.9
4	177.2	177.0	176.8	183.2	177.0
5	124.7	124.8	124.7	159.8	107.5
6	125.2	125.5	125.3	111.0	149.3
7	134.1	134.3	134.0	135.9	123.0
8	118.4	118.4	118.3	107.5	119.7
9	155.8	155.6	155.6	155.9	154.9
10	123.1	123.3	123.3	110.1	124.2
1'	117.7	132.4	121.5	130.5	131.3
2'	156.6	112.8	128.3	126.6	126.2
3'	117.0	157.9	115.9	129.2	129.0
4'	132.5	118.8	160.9	132.3	131.5
5'	119.4	130.2	115.9	129.2	129.0
6'	128.5	117.1	128.3	126.6	126.2

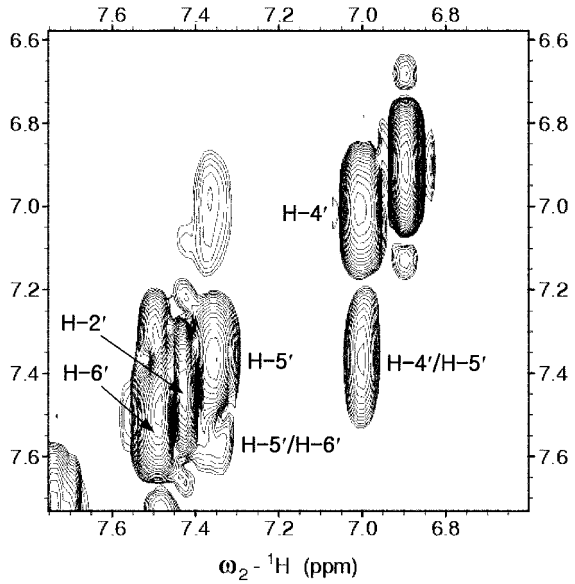


Figure 5. The partial COSY spectrum of derivative **3**.

peak at 7.96 ppm which was attached directly to the ^{13}C peak at 128.3 ppm (Fig. 7). Therefore, 7.96 ppm should be assigned H-2', and 128.3 ppm was C-2'. The ^{13}C peak at 160.9 ppm was C-4' and the remained peak, 121.5 ppm, should be C-1'. The complete assignments of ^{1}H and ^{13}C chemical shifts of 4'-hydroxylated flavone are listed in Tables 2 and 3, respectively.

The derivative **5** is 5-hydroxylated flavone (Fig. 1). The ^{13}C chemical shifts of the B-ring should be the same as those of flavone. Comparing the ^{13}C chemical shifts of the derivative **5** with those of flavone, C-2, C-3, C-7, and C-9 could be determined. The most downfield shifted peak at 183.2 ppm was assigned C-4. Of four remained peaks, the peak at 159.8 ppm should be C-5. The singlet peak at 110.1 ppm was assigned C-10, because C-6 and C-8 could not be a singlet. Two ^{13}C peaks at 111.0 and 107.5 ppm were attached to the ^{1}H peaks at 6.81 and 7.19 ppm, respectively. In order to distinguish them, the NOESY experiment was carried out. As shown in Figure 8, a cross-peak between 6.81 and 12.65 ppm was observed. Because 12.65 ppm was caused by the hydroxyl proton, the ^{1}H peaks at 6.81 and 7.19 ppm were H-6 and/or H-8. Of two protons, H-6 can show an nOe cross-peak with the hydroxyl proton, so that 6.81 ppm should be H-6. As a result, H-8 was 7.19 ppm. In order to prove this result, molecular modeling was performed. The distance r_{ij} between two protons i and j was calculated from the nOe η_{ij} based on the equation, $\eta_{ij}/\eta_{kl} = (r_{kl}/r_{ij})^6$ where k and l denote two neighboring protons of benzene ring and r_{kl} is 2.45 Å.[11] As shown in Figure 9, while the calculated distance between H-6 and OH-5 was 3.54 Å, the distance between H-8 and OH-5 was 5.65 Å. Therefore, it was expected that the nOe cross-peak of H-6/OH-5 was observed in the NOESY spectrum but that of H-8/OH-5 was not. The molecular modeling simulation agreed to the result obtained from the NOESY experiment. In addition, a hydrogen bond between

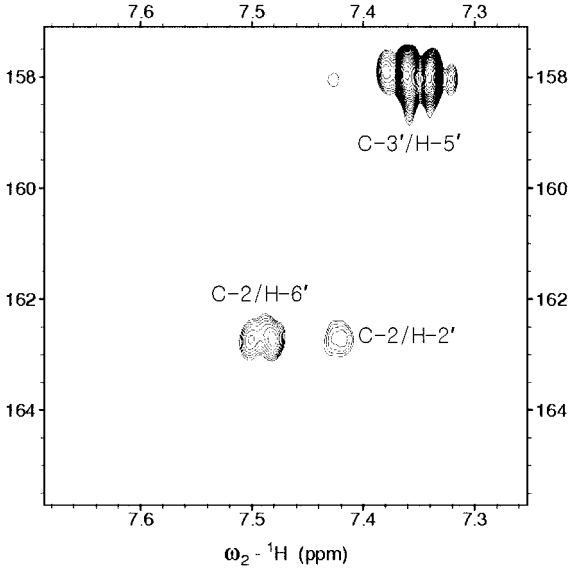


Figure 6. The partial HMBC spectrum of derivative **3**. The long-ranged coupling time was 70 msec.

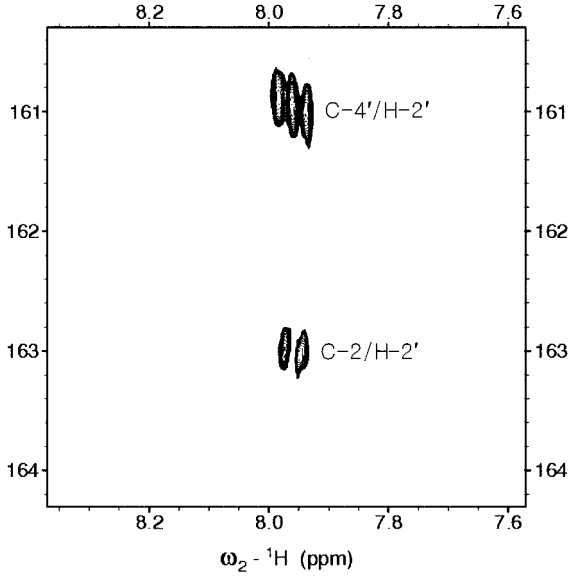


Figure 7. The partial HMBC spectrum of derivative **4**. The long-ranged coupling time was 70 msec.

OH-5 and C=O-4 was observed and its distance was 2.12 Å. The ^{13}C chemical shifts of the derivative **5** were reported by Ternai and Marham.[7] In their data, C-6 and C-8 were 107.2 and 110.8 ppm, respectively, but in our data, 111.0 and 107.5, respectively. As mentioned before, our assignments of C-6 and C-8 were carried out based on the interpretation of the NOESY data and they were proved by the molecular modeling calculations. The complete assignments of ^{1}H and ^{13}C chemical shifts of 5-hydroxylated flavone are listed in Tables 2 and 3, respectively.

The derivative **6** is 6-hydroxylated flavone (Fig. 1). Like the derivative **5**, the ^{13}C chemical shifts of the B-ring could be determined easily. C-2, C-3, C-4, C-8, C-9, and C-10 were assigned based on the comparison of the chemical

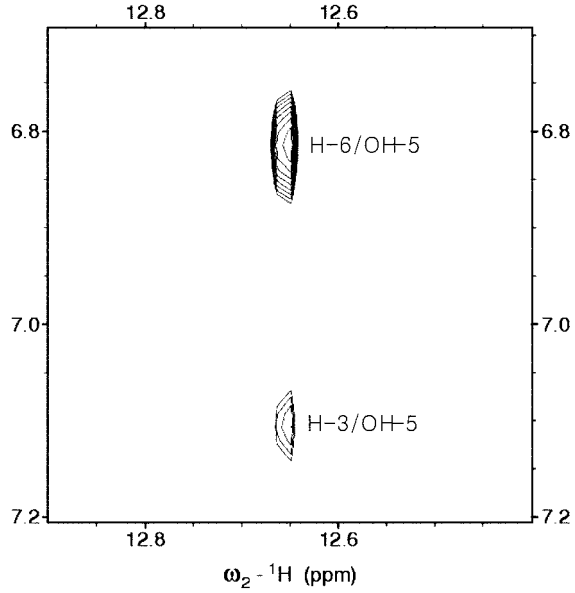


Figure 8. The partial NOESY spectrum of derivative **5**. The mixing time was 1 sec.

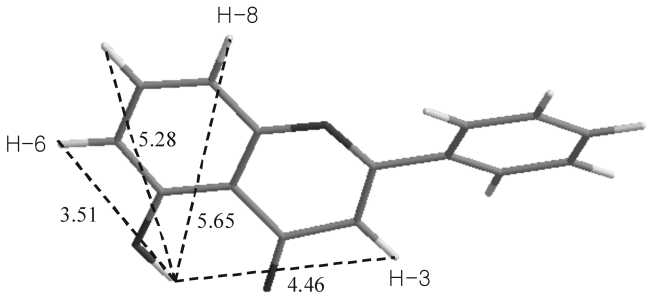


Figure 9. The distances of H-6/OH-5 and H-8/OH-5 calculated by molecular modeling for derivative **5**.

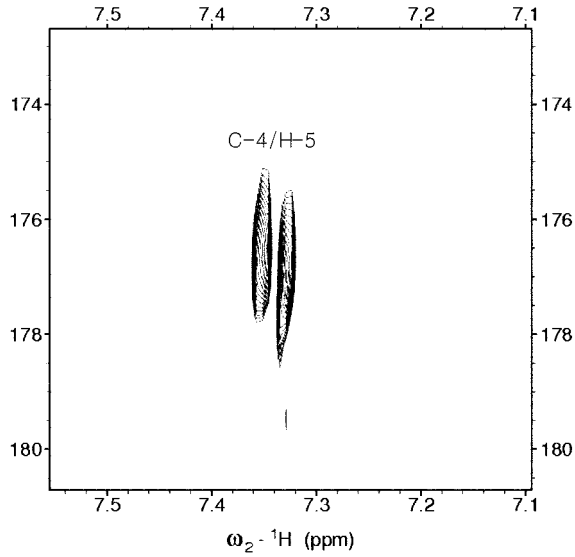


Figure 10. The partial HMBC spectrum of derivative **6**.

shifts with flavone. The ^{13}C peak at 149.3 ppm should be C-6. In the HMBC spectrum, C-4 was long-range coupled to

the ^{1}H peak at 7.34 ppm which was attached directly to the ^{13}C peak at 107.5 ppm (Fig. 10). Therefore, they were assigned H-5 and C-5, respectively. The complete assignments of ^{1}H and ^{13}C chemical shifts of 6-hydroxylated flavone are listed in Tables 2 and 3, respectively.

As a result, we assigned the ^{1}H and ^{13}C NMR data of flavone and its five derivatives. Of them, the ^{13}C NMR data of the derivatives **2** and **5** were reported previously.[6,7] However, we found their data contained partially wrong assignments. Here we report the corrected data. In addition, as mentioned in the introduction section, flavone derivatives are found in the metabolites of many plants. The complete assignments of ^{1}H and ^{13}C chemical shifts of the derivatives reported here can help us identify the natural products such as flavone derivatives based on the simple comparison of the NMR data.

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