^{1}H NMR CHEMICAL SHIFT OF THE ISOFLAVANONE 5-HYDROXYL PROTON AS A CHARACTERIZATION OF 6- OR 8-PRENYL GROUP[1]

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Abstract ——— ^{1}H Nmr examination of 6- or 8-prenylated isoflavanone derivative has shown that the location of prenyl side chain on A ring can be deduced from the chemical shift of the 5-hydroxyl proton. The effects of substituents of B ring for the chemical shift are also discussed.

In the mass spectrometry, flavonoids typically give prominent *retro*-Diels-Alder fragments corresponding to A and B rings. The fragment ions often allow ring substituents to be partially identified. Simple 6- and 8-prenylated flavonoids (except flavanones) give characteristic fragment ions, respectively, and the location of the 3-methyl-2-butenyl (prenyl) group can be identified by this mass spectrometry.[2] However, this method is not able to use for complex prenylflavonoids (*e.g.* prenyl groups located at both A and B rings) because the compounds give many fragment ions.[2] In the ^{13}C nmr spectra of 5,7-dihydroxyflavonoids (flavones, isoflavones, and flavonols), the chemical shifts of A ring carbons indicate the location of the prenyl group at the A ring: the C-8 signal of the 6,8-unsubstituted flavonoids appears at more upfield than the C-6 signal (*ca.* 5 ppm), and a substitution of C-6 or C-8 in the 5,7-dihydroxyflavonoids does not result in a marked shift (less than 1 ppm) of the signal of the other carbon atom.[3] However, this ^{13}C nmr method is not able to use for isoflavanone derivatives, as well as flavanone derivatives, because the chemical shifts of A ring carbons of 6-prenylated and 8-prenylated isoflavanones are almost the same. The signals of prenylated carbons (near δ 109) and unsubstituted carbons (near δ 96) of 6-prenylated and 8-prenylated isoflavanones appear at similar regions (within *ca.* 1 ppm, see Table 3). Most certain spectroscopic method of the structure determination of these 6- and 8-prenylated isoflavanone is the observation of long-range coupling between hydrogen-bonded hydroxyl proton (OH-5) and 6-carbon signals (long-range selective proton decoupling technique, COLOC, HMBC spectra *etc.*). Although, this method needs relatively large amount of sample (more than 2 mg in a routine measurement).

Recently, we reported a new ^{1}H nmr method for the structure determination of prenylated flavonoids by use of chemical shift of OH-5 signal.[4-8] This method is useful for the structure determination of minor prenylated flavonoids, since the measurement needs only a minute amount of sample (less than 200 micrograms).

In this paper, we discuss the application of this method for isoflavanone derivatives.

In our previous ^{1}H nmr study of isoprenoid substituted flavonoids (isoflavone, flavanone, flavone, and flavonol),[4-8] we reported that the signal of OH-5 of 6-prenylated flavonoids appears at lower field (Δ 0.25 - 0.30 ppm) than that of 6-unsubstituted flavonoids having the same B and C rings when measured in acetone-d_6. In contrast, the OH-5 signal of 8-prenylated flavonoids shows upfield shift (Δ 0.04 - 0.10 ppm) compared with that of flavonoids having same B and C rings and no side chain.

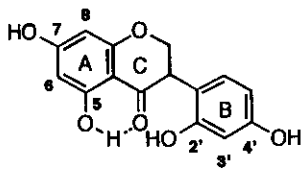
In the case of isoflavanone derivatives, the prenylation of C-6 resulted in a marked downfield shift (Δ 0.30 ppm) of the OH-5 signal [comparison between darbergioidin (**1**, δ 12.37) and diphysolone (**2**, δ 12.67)[9]]. In contrast, the prenylation of C-8 resulted in a small upfield shift (Δ 0.04 ppm): the OH-5 signal of glyasperin J (**3**, δ 12.36)[10] appeared at upper field than that of glyasperin F (**4**, δ 12.40).[10] These prenylation effects on OH-5 signal were similar to those of other flavonoids.[4-8]

The prenylation at C-3' position of 2',4'-dihydroxylated isoflavanone caused a upfield shift (Δ 0.29 ppm): the OH-5 signal of kanzonol G (**5**, δ 12.22)[11] appeared at upper field than that of glyasperin B (**6**, δ 12.51).[12] Similar upfield shift has been observed in the case of isoflavone derivative: the OH-5 signal of 2'-hydroxylupalbigenin (**7**, δ 12.80)[13] appears at upper field (Δ 0.25 ppm) than that of luteone (**8**, δ 13.05).[14]

In the previous paper,[5] we reported that the hydroxylation at C-2' of 3'-unsubstituted isoflavone derivative results in a marked upfield shift (Δ 0.26 ppm). Although, in the case of 3'-unsubstituted isoflavanone derivative, the OH-5 signal shifted to lower field (Δ 0.12 ppm) by the hydroxylation at C-2': the OH-5 signal of ferreirin (**9**, δ 12.34) appeared at lower field than that of dihydrobiochanin A (**10**, δ 12.22). Similar downfield shifts have been observed in the case of flavonol (Δ 0.10 ppm)[5] and flavone derivatives [comparison between **11** (δ 13.15) and **12** (δ 13.02)].

The effects of methylation of OH-2' and OH-4' on the OH-5 signal were less than 0.05 ppm.[15] The methylation of OH-4' of **1** resulted in a small upfield shift [0.03 ppm; comparison between **1** (δ 12.37) and **9** (δ 12.34)], and the chemical shift of cajanol (**13**, δ 12.35) is the same as that of dihydrocajanin (**14**).

The methylation of OH-7 of 6,8-unsubstituted isoflavanone caused a small upfield shift [comparison between **1** (δ 12.37) and **14** (δ 12.35)], but the methylation of OH-7 of 6-prenylated isoflavanone resulted in a marked upfield shift: the OH-5 signal of glyasperin K (**15**, δ 12.46)[16] was observed at upper field (Δ 0.16 ppm) than that of diphysolidone (**16**, δ 12.62).[9] The same upfield shift was observed in comparison between **2** and



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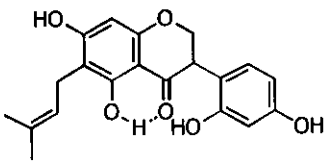

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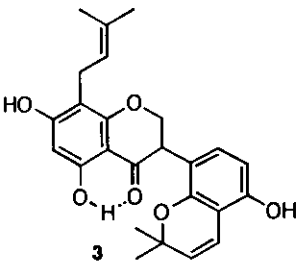

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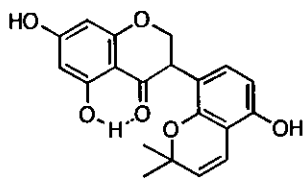

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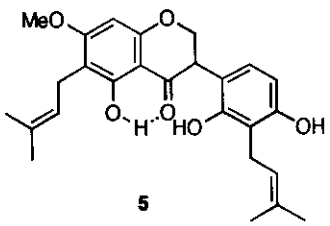

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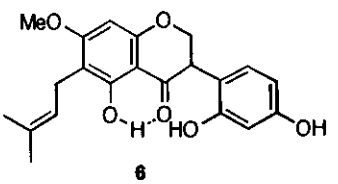

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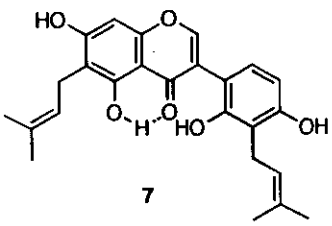

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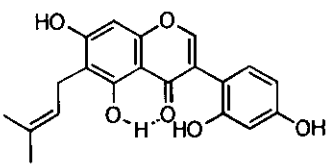

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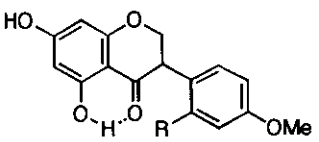

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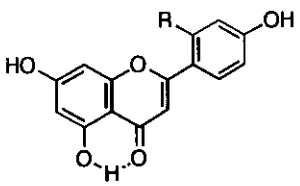
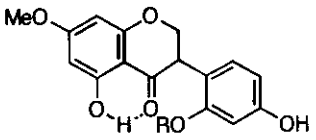
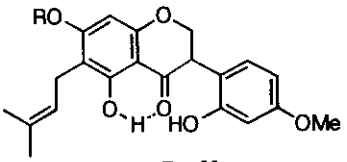


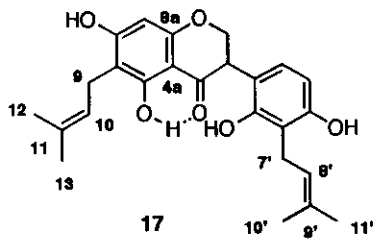
11: R = OH
12: R = H



13: R = Me
14: R = H



15: R = Me
16: R = H



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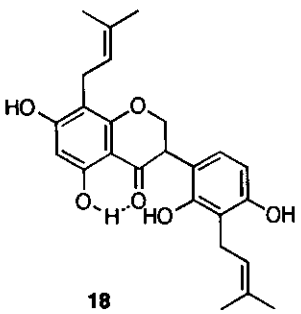

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Table 1. Substituent parameters (ppm) on OH-5 of isoflavanone (in acetone-d_6)

	substituent parameter
1. prenylation at C-6	+ 0.30
2. prenylation at C-8	− 0.04
3. prenylation at C-3' (2',4'-dihydroxylated derivative)	− 0.29
4. hydroxylation at C-2' (3'-unsubstituted)	+ 0.12
5. methylation of OH-2' or OH-4'	0 ~ − 0.05
6. methylation of OH-7	
(6,8-unsubstituted)	− 0.02
(6-prenylated)	− 0.16
7. cyclization between OH-2' and 3'-prenyl groups*	+ 0.35

*: Comparison between **3** and **18**.

Table 2. ^{1}H Nmr data of **17** and **18**

	17	**18**
H-2	4.58 (dd, J = 5 and 11 Hz)	4.66 (dd, J = 5 and 11 Hz)
	4.67 (dd, J = 7 and 11 Hz)	4.76 (dd, J = 7 and 11 Hz)
H-3	4.14 (dd, J = 5 and 7 Hz)	4.14 (dd, J = 5 and 7 Hz)
H-6	.....	6.03 (s)
H-8	6.03 (s)	.....
H-5'	6.41 (d, J = 8 Hz)	6.43 (d, J = 8 Hz)
H-6'	6.93 (d, J = 8 Hz)	6.98 (d, J = 8 Hz)
H_2-9	3.22 (br d, J = 7 Hz)	3.23 (br d, J = 7 Hz)
H-10	5.20 (br t, J = 7 Hz)	5.19 (br t, J = 7 Hz)
H_2-7'	3.40 (br d, J = 7 Hz)	3.39 (br d, J = 7 Hz)
H-8'	5.23 (br t, J = 7 Hz)	5.23 (br t, J = 7 Hz)
Me	1.62 (br d, J = 1 Hz)	1.63 (6H, br s)
	1.63 (br d, J = 1 Hz)	1.74 (br s)
	1.73 (br d, J = 0.7 Hz)	1.75 (br s)
	1.75 (br d, J = 0.7 Hz)	
OH	7.67 (br s)	7.73 (br s)
	8.24 (br s)	8.25 (br s)
	9.62 (br)	9.65 (br)
OH-5	12.36 (s)	12.01 (s)

6 (Δ 0.16 ppm).

The above effects on OH-5 signal were summarized as substituent parameters in Table 1. These parameters are useful for the structure determination of new prenylated isoflavanones. The suitable model compound provides a chemical shift of newly discovered isoflavanone with the calculation by use of these substituent parameters.

To confirm the certainty of the new structure determination method, we applied the method for two isoflavanone derivatives isolated from licorice.

In earlier papers, we reported the structure determinations of isoprenoid-substituted flavonoids from Chinese licorice.[17] In our continuous research of Chinese licorice, we isolated two known diprenylated isoflavanone derivatives (**17** and **18**)[18,19] form *Glycyrrhiza glabra*.

The ^{1}H nmr, ^{13}C nmr, and mass spectra of **17** and **18** indicated that these compounds were 3'-prenyl-5,7,2',4'-tetrahydroxyisoflavanons having a prenyl group at C-6 or C-8 position. The ^{1}H nmr and ^{13}C nmr spectra were resemble each other except chemical shifts of H-2 × 2 and OH-5 (Tables 2 and 3). To identify the structures of these compounds, we used the new method described above.

The calculated value for the structure (**17**) was δ 12.38 obtained from the chemical shift of OH-5 of **2** (δ 12.67) and substitution parameter 3 (- 0.29, Table 1). The value was also obtained from the chemical shift of **1** (δ 12.37) and substitution parameters 1 (+ 0.30) and 3 (- 0.29). The calculated chemical shift of OH-5 for the structure (**18**) was δ 12.04 obtained from the chemical shift of **1** and the substituent parameters 2 (- 0.04) and 3 (- 0.29).

The calculated values for these structures were similar to the chemical shifts of OH-5 of the diprenylated isoflavanones isolated from *G. glabra* (Table 2). The similarity between the calculated and found values indicated that the compounds (**17**) and (**18**) were 6-prenylated and 8-prenylated isoflavanones, respectively.

The identification was further confirmed with HMBC spectrum of **17**. The proton signal of the OH-5 showed long-range correlation to C-4a (δ 102.88), C-6 (δ 109.37), and C-5 (δ 162.89) in the spectrum.

Thus, this new method is useful for structure determination of minor isoflavanone derivatives.

EXPERIMENTAL

^{1}H and ^{13}C Nmr spectra were measured in acetone-d_6 with JEOL JNM-EX-400 NMR Spectrometer. For tlc (silica gel) and preparative tlc (silica gel), Wakogel B-5FM and B-5F were used. The prenylated isoflavanones used in the present study were isolated in the course of our study of *Glycyrrhiza* species[17,20] unless noted otherwise.

Table 3. ^{13}C Nmr data of **17** and **18**

C	**17**	**18**†
2	71.00 (br T, J = *ca.* 148 Hz)*	70.94 (Td, J = 4 and 150 Hz)
3	46.80 (br D, J = *ca.* 128 Hz)	46.46 (br D, J = 128 Hz)
4	198.80 (Sm)	198.87 (St, J = 5 Hz)
4a	102.88 (br S)	102.77 (Sdd, J = 4 and 5 Hz)
5	162.89(br S)	163.53 (br S)
6	109.37 (br S)	96.54 (Dd, J = 7 and 161 Hz)
7	165.33 (br S)	165.36 (br S)
8	95.33 (D, J = 161 Hz)	108.30 (br S)
8a	162.15 (br S)	161.05 (Sm)
1'	115.68 (br S)	115.45 (Sm)
2'	154.95 (br S)	154.89 (Sm)
3'	117.15 (br s)	117.03 (br S)
4'	156.57 (br S)	156.43 (Sm)
5'	108.58 (D, J = 158 Hz)	108.44 (D, J = 158 Hz)
6'	127.09 (Dd, J = 5 and 157 Hz)	126.91 (Dd, J = 5 and 157 Hz)
9	21.76 (Td, J = 4 and 125 Hz)	22.05 (Td, J = 4 and 123 Hz)
10	123.59 (br D, J = *ca.* 145 Hz)	123.69 (br D, J = *ca.* 145 Hz)
11	131.44 (br S)	131.31 (br S)
12	18.06 (br Q, J = *ca.* 125 Hz)	17.83 (br Q, J = *ca.* 125 H)
13	25.96 (br Q, J = *ca.* 125 Hz)	25.88 (br Q, J = *ca.* 125 Hz)
8'	23.43 (Td, J = 4 and 125 Hz)	23.27 (Td, J = 5 and 123 Hz)
9'	123.92 (br D, J = *ca.* 145 Hz)	123.81 (br D, J = *ca.* 145 Hz)
10'	131.80 (br S)	131.61 (br S)
11'	17.96 (br Q, J = *ca.* 125 Hz)	17.95 (br Q, J = *ca.* 125 Hz)
12'	25.96 (br Q, J = *ca.* 125 Hz)	25.88 (br Q, J = *ca.* 125 Hz)

*: Capital letters refer to the coupling pattern resulting from directly bonded proton(s) and lowercase letters to long-range ^{13}C-^{1}H coupling.

†: The sample measured here was obtained from *Glycyrrhiza aspera* (ref. 10).

Isolation of diprenylisoflavanones (17 and 18) from Glycyrrhiza glabra

The licorice was obtained from a market in Hojin Province, Xinjiang Uegur Autonomous, China and identified as *Glycyrrhiza glabra* L. by Dr. L. Zeng, Purdue University. The licorice (400 g) was extracted at room temperature with hexane (1 l, three times) and then with benzene (1 l, x 3) (each 3 days). Evaporation of the hexane and benzene solutions to dryness yielded 1.1 g and 4.4 g of the residues, respectively. The benzene extract (4.4 g) was chromatographed on silica gel (100 g) successively with benzene (Fr. 1 -6), benzene-ether = 99:1 (Fr. 7 - 10), benzene-ether = 49:1 (Fr. 11 and 12) as an eluent (column A), each fraction (eluent volume 500 ml) being monitored by tlc. The fraction 9 (0.4 g) was rechromatographed on silica gel (10 g) with hexane-

chloroform as an eluent (each 100 ml, column B). The fraction 10 of column B (eluted with hexane-chloroform=2:3, 60 mg) was purified by preparative tlc (benzene-acetone=4:1) to give **17** (6 mg). The fractions 10 -12 of column A (0.46 g) was rechromatographed on silica gel (10 g) with hexane-ethyl acetate as an eluent (each volume 100 ml, column C). The fraction 2 of column C (eluted with hexane-ethyl acetate=3:1, 0.32 g) was rechromatographed on silica gel (10 g) with benzene-acetone as an eluent (each 100 ml, column D). The fraction 5 of column D (eluted with benzene-acetone=96:4, 5 mg) was purified by preparative tlc (benzene-acetone=10:1) to give **18** (0.3 mg).

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