Pergamon

PII: S0031-9422(97)00629-9

Phytochemistry, Vol. 47, No. 5, pp. 865–874, 1998

Printed in Great Britain
0031-9422/98 $19.00+0.00

^{13}C NMR SPECTRAL ASSIGNMENT OF THE A-RING OF POLYOXYGENATED FLAVONES

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Midori Kagaku Co., Ltd., Minami-Ikebukuro 2-27-8, Toshima-ku, Tokyo 171, Japan; †Department of Chemical Science and Technology, Faculty of Engineering, The University of Tokushima, Minamijousanjima-cho, Tokushima 770, Japan

(*Received in revised form* 30 *June* 1997)

Key Word Index—^{13}C NMR; 5,6,7-trioxygenated flavones; 5,7,8-trioxygenated flavones; 5,6,8-trioxygenated flavones; 5,6,7,8-tetraoxygenated flavones; flavonols; *Scutellaria baicalensis*; *Zanthoxylum bungeanum*; *Gymnosperma glutinosum*.

Abstract—The ^{13}C NMR spectra of polyhydroxyflavones were systematically examined by using about 70 examples which have a 4-methoxyphenyl B-ring, such as 5,6,7-, 5,7,8 and 5,6,8-trioxygenated and 5,6,7,8-tetraoxygenated flavones and flavonols, and the following results were obtained. In the ^{13}C NMR spectra of flavones, the signals at the 2-, 3- and 4-positions in the C-ring were hardly affected by the substituents on the A-ring except for those at the 5-position and shifted regularly by introduction of a methoxy or hydroxy group at the 3-position. The signals at the 5- to 10-positions in the A-ring were greatly affected by the substituents and oxygenated patterns on the A-ring and exhibited the respective characteristic pattern reflecting the A-ring substituents without the effects of the substituents on the B- and C-rings. The substituent effects on the A-ring were estimated from the spectral data. Consequently, it is found that the structures of polyhydroxyflavones can be correctly defined by their ^{13}C NMR spectral assignment. Additionally, we examined the structures of some natural flavones previously reported.

INTRODUCTION

A large number of polyhydroxyflavones have been isolated from numerous plant sources and the structures have been proposed on the basis of their spectral data so far [1–4], but their general properties are not always clear and some errors in structural elucidation have occurred [5]. Generally, UV, ^{1}H NMR, and mass spectra for isomeric polyhydroxyflavones are similar to each other and their differentiation must be carried out with scrupulous care in the elucidation of the A-ring substituents. The ^{13}C NMR spectra which exhibit a diagnostic spectral pattern reflecting the respective substituents are a most suitable method for the structural elucidation and the studies on the assignment of the ^{13}C NMR spectra of flavones have already been carried out [6, 7]. The assignment of ^{13}C NMR spectra of polyhydroxyflavones, however, is generally difficult because of no detailed elucidation of the substituent effects on the A-ring: the assignment of the reported data is not consistent with each other in most cases [8] and the results are not always reflected in the structural elucidation as a result. Roitman and James [9] have examined the ^{13}C NMR spectral assignments of 20 polyhydroxyflavonols isolated from *Gutierrezia microcephala* and found that the chemical shifts can be estimated from the substituent effects. The result suggests that the chemical shifts are more accurately estimated when the substituent effects are fully clarified. Hence, we systematically examined the substituent effects in the ^{13}C NMR spectra of about 70 different flavones and flavonols, which were previously synthesized by us and had a 4-methoxyphenyl B-ring, and found that the structures of polyhydroxyflavones and flavonols could be correctly proposed by estimating the substituent effects. Additionally, we examined on the structures of some natural flavones previously reported and the structures of two natural flavones were revised.

* Author to whom correspondence should be addressed.

RESULTS AND DISCUSSION

The ^{13}C NMR spectral assignments of 5,6,7- (**A**), 5,7,8- (**B**), and 5,6,8-trioxygenated (**D**), and 5,6,7,8-tetraoxygenated (**C**) flavones and the corresponding flavonols (3-methoxyflavones and 3-hydroxyflavones), which have a 4-methoxyphenyl B-ring, are

shown in Tables 1–4. It is well known that the carbon signals at the 2-, 3- and 4-positions in the C-ring of flavones are observed in a narrow range, respectively, and the signals are diagnostically shifted by introduction of a methoxy or hydroxy group at the 3-position, or by conversion of the 5-methoxy group to a hydroxy group [8] (Tables 1–4). In the spectral comparison of the flavones, **A**, **B**, and **C**, having the same substituents in the A-ring (Tables 1–3), the A-ring carbon signals in flavones (**a** series) are slightly affected by the introduction of a methoxy (**b** series) or hydroxy (**c** series) group at the 3-position and shifted diagnostically without the effects of the substituents on the B-ring. The shift ranges of the A- and C-ring carbon signals are summarized in Table 5, in the two series of 5-methoxyflavones and 5-hydroxyflavones. The result shows that the shift ranges of the A- and C-ring signals are not affected by the other substituents except for the 5-position and are widely usable for the ^{13}C NMR spectral assignment.

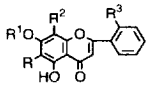
On the other hand, the conversion of methoxy groups on the A-ring to hydroxy groups causes also a diagnostic shift of the A-ring carbon signals, but the shift ranges are greatly affected by the oxygenation pattern on the A-ring. The shift ranges in the flavones **A**, **B**, and **C** (Tables 1–3) are summarized in Table 6. The conversion of the 5-methoxy group to a hydroxy group causes large upfield shift of the carbon signals at the 6- and 10-positions (*o*-positions), and the 8-position (*p*-position), although the carbon signals at the 10-position are also affected by the substituent at the 3-position. In contrast to this result, the signals of the A-ring carbons with no substituent are shifted downfield by conversion of the adjacent methoxy group (*o*-position) to a hydroxy group, as seen in the variation of the carbon signals at the 6- or 8-position in the flavones **B** and **A** by conversion of the 5- or 7-methoxy group to a hydroxy group (Table 6). In the conversion of the 6-, 7- or 8-methoxy group to a hydroxy group, the A-ring carbon signals are also shifted regularly as shown in Table 6. The shifts are available for the assignment of other types of flavones. The ^{13}C NMR spectra of 5,6,8-trioxygenated flavones, which are unusual flavones and have yet to be isolated from natural sources, are also assigned by using the substituent effects as shown in Table 4.

The data in Tables 1–6 are useful for the structural elucidation of naturally occurring flavones and flavonols, and the chemical shifts in ^{13}C NMR spectra of unknown flavones may be correctly estimated from the data for analogous compounds and the shift ranges shown in Tables 5 and 6. For example, the chemical shifts of the A-ring in ^{13}C NMR spectra of 5,7,8,3′,4′-pentahydroxy-3,6-dimethoxyflavone and **A9c** are estimated from the shift ranges shown in Tables 5 and 6, and the data for **C7b** and **C8b**, or **A9b**, **A7c**, and **A6c**, as the demethylated products of these compounds, respectively. The respective calculated data are consistent with each other as shown in Table 7, and the values are also consistent with the data observed by Roitman and James [9] and **A9c**, respectively.

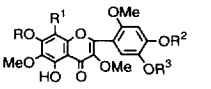
These results show that the A-ring substitution pattern in polyhydroxyflavones and flavonols is clearly confirmed by the ^{13}C NMR spectra. Actually, the A-ring carbon signals of synthetic 5,6,7,8-tetraoxygenated flavones previously reported by us are consistent with the ones of the flavones having the same substituents on the A-ring, respectively, when the reported data are rearranged [5]. Only the spectra of 5,6-dihydroxy-7,8-dimethoxyflavone (**11**) and 5,8-dihydroxy-6,7-dimethoxyflavone (**12**), however, are similar to each other; albeit, the other spectral data (UV and ^{1}H NMR) exhibit the characteristic properties corresponding to the respective structures [5]. Therefore, we re-examined the two isomeric flavones and it was found that only **11** previously synthesized has been perfectly converted into **12**. The results suggests that the compound is very slowly isomerized in a crystal form, although the conditions are not clear at all. Thus, we revised the ^{13}C NMR data for **11** and **12** as shown in Table 8. The A-ring carbon signals of **11** and **12** are consistent with those of the corresponding 5,6-dihydroxy-7,8-dimethoxyflavones, **C6a** and **13**, and 5,8-dihydroxy-6,7-dimethoxyflavones, **C8a** and **14** [5], respectively, and it shows that the two isomeric flavones are correctly differentiated by 13C NMR spectra.

Furthermore, we examined the reported ^{13}C NMR data for some natural flavones. The A-ring carbon signals in the ^{13}C NMR data for a natural flavone, isolated from *Scutellaria baicalensis* Georgi and proposed as 2′,5,8-trihydroxy-7-methoxyflavone (**15**) by Takagi *et al.* [30], are not consistent with those for **B8a**, but those for the isomeric **A7a**. The B- and C-ring carbon signals are consistent with those for the 2′-hydroxyflavones, **13** and **14**, as shown in Table 8. The result shows that the structure of the natural flavone is 2′,5,7-trihydroxy-6-methoxyflavone (**16**), an isomer of **15**. Actually, the ^{13}C NMR spectrum of the synthesized flavone **16** was consistent with that of the natural flavone and it was demonstrated that the structure of the flavone was **16**.

The ^{13}C NMR data for a natural flavone, isolated from *Zanthoxylum bungeanum* and proposed as 3,5,6-trihydroxy-4′,7-dimethoxyflavone (**A6c**) by Xiong *et al.* [31], are not consistent with those for the synthetic **A6c**, but match well those for the isomeric 3,5,8-trihydroxy-7,4′-dimethoxyflavone (**B8c**). Although the other properties are not reported, the result clearly shows that the structure of the natural flavone is **B8c**.



11 R=OH, R^1=Me, R^2=OMe, R^3=H
12 R=OMe, R^1=Me, R^2=OH, R^3=H
13 R=R^3=OH, R^1=Me, R^2=OMe
14 R=OMe, R^1=Me, R^2=R^3=OH
15 R=H, R^1=Me, R^2=R^3=OH
16 R=OMe, R^1=R^2=H, R^3=OH



17 R=H, R^1=OMe, R^2=R^3=Me
18 R=R^2=H, R^1=OMe, R^3=Me
19 R=R^2=R^3=H, R^1=OMe
20 R=Me, R^1=OMe, R^2=R^3=H
21 R=R^1=R^2=R^3=H

Table 1. ^{13}C NMR data for 5,6,7-trioxygenated 4′-methoxyflavones in DMSO-d_6

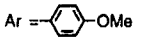
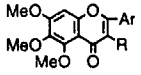
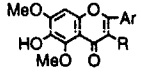
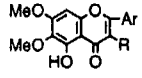
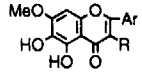
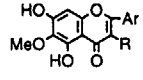
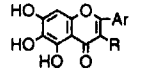
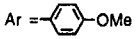
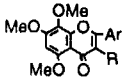
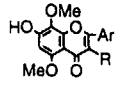
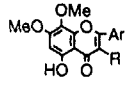
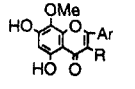
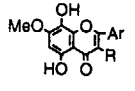
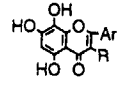
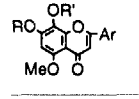
Ar = 		R	C_2	C_3	C_4	C_5	C_6	C_7	C_8	C_9	C_{10}	$C_{1'}$	$C_{2',6'}$	$C_{3',5'}$	$C_{4'}$	OMe				
																C_3	C_5	C_6	C_7	$C_{4'}$
	**A1a** [10]	H	160.2	106.1	175.6	151.6	139.7	157.4	97.3	153.9	112.0	123.0	127.8	114.4	161.8		61.8	61.0	56.4	55.5
		$\Delta\delta$	−7.9	+33.7	−3.5	−0.3	−0.2	0	−0.4	−0.9	+0.1	−0.6	+1.8	−0.4	−0.9					
	A1b [11]	OMe	152.3	139.8	172.1	151.3	139.5	157.4	96.9	153.0	112.1	122.4	129.6	114.0	160.9	59.2	61.8	60.9	56.4	55.3
		$\Delta\delta$	−9.7	−2.2	−1.2	−0.3	−0.3	0	−0.1	−0.1	−2.2	+1.0	−0.8	−0.1	−0.8					
	A1c [11]	OH	142.6	137.6	170.9	151.0	139.2	157.4	96.8	152.9	109.9	123.4	128.8	113.9	160.1		61.9	61.0	56.4	55.2
	**A2a** [10]	H	160.0	105.9	175.7	144.2	137.5	153.2	96.6	150.7	111.9	123.2	127.6	114.4	161.7		61.2		56.2	55.4
		$\Delta\delta$	−7.8	+33.9	−3.6	−0.3	−0.2	+0.2	−0.3	−0.8	+0.2	−0.5	+1.9	−0.4	−0.9					
	A2b [11]	OMe	152.2	139.8	172.1	143.9	137.3	153.4	96.3	149.9	112.1	122.7	129.5	114.0	160.8	59.2	61.2		56.2	55.3
		$\Delta\delta$	−9.8	−2.3	−1.2	−0.5	−0.3	+0.2	−0.1	0	−2.3	+0.9	−0.8	−0.1	−0.8					
	A2c [11]	OH	142.4	137.5	170.9	143.4	137.0	153.6	96.2	149.9	109.8	123.6	128.7	113.9	160.0		61.2		56.2	55.2
	**A5a** [10]	H	163.5	103.2	182.1	152.0	131.8	158.6	91.5	152.5	105.0	122.6	128.2	114.5	162.3			59.9	56.3	55.5
		$\Delta\delta$	−8.1	+34.7	−3.9	−0.4	−0.2	0	−0.1	−0.7	+0.6	−0.6	+1.8	−0.3	−0.9					
	A5b [11]	OMe	155.4	137.9	178.2	151.6	131.6	158.6	91.4	151.8	105.6	122.0	130.0	114.2	161.4	59.7		60.0	56.4	55.4
		$\Delta\delta$	−8.8	−1.8	−2.0	−0.6	−0.4	−0.1	−0.3	−0.3	−1.3	+1.1	−0.7	−0.3	−0.9					
	A5c [11]	OH	146.6	136.1	176.2	151.0	131.2	158.5	91.1	151.5	104.3	123.1	129.3	113.9	160.5			60.0	56.3	55.3
	**A6a** [12]	H	163.2	103.0	182.1	146.1	129.9	154.3	91.1	149.6	105.0	122.9	128.1	114.4	162.2				56.2	55.4
		$\Delta\delta$	−8.0	+34.7	−4.0	−0.5	−0.3	+0.2	−0.2	−0.8	+0.6	−0.6	+1.8	−0.3	−1.0					
	A6b [11]	OMe	155.2	137.7	178.1	145.6	129.6	154.5	90.9	148.8	105.6	122.3	129.9	114.1	161.2	59.6			56.2	55.4
		$\Delta\delta$	−8.8	−1.8	−2.1	−0.7	−0.4	+0.1	−0.2	0	−1.3	+1.0	−0.7	−0.2	−0.8					
	A6c [13]	OH	146.4	135.9	176.0	144.9	129.2	154.6	90.7	148.8	104.3	123.3	129.2	113.9	160.4				56.2	55.3
	**A7a** [14]	H	163.3	103.0	182.1	152.7	131.3	157.3	94.2	152.4	104.1	122.8	128.2	114.5	162.2			59.9		55.5
		$\Delta\delta$	−8.1	+34.5	−3.9	−0.4	−0.2	+0.1	−0.2	−0.9	+0.5	−0.7	+1.7	−0.3	−0.9					
	A7b [11]	OMe	155.2	137.5	178.2	152.3	131.1	157.4	94.0	151.5	104.6	122.1	129.9	114.2	161.3	59.7		59.9		55.4
		$\Delta\delta$	−8.9	−1.8	−2.0	−0.6	−0.3	−0.2	−0.2	−0.1	−1.2	+1.1	−0.6	−0.3	−0.9					
	A7c [11]	OH	146.3	135.7	176.2	151.7	130.8	157.2	93.8	151.4	103.4	123.2	129.3	113.9	160.4			59.9		55.3
	**A9a** [12]	H	163.0	102.8	181.9	147.0	129.1	153.3	93.8	149.6	104.0	123.0	128.1	114.4	162.1					55.4
		$\Delta\delta$	−8.1	+34.7	−3.9	−0.5	−0.2	+0.2	−0.2	−0.7	+0.6	−0.6	+1.7	−0.3	−0.9					
	A9b [11]	OMe	154.9	137.5	178.0	146.5	128.9	153.5	93.6	148.9	104.6	122.4	129.8	114.1	161.2	59.7				55.3
		$\Delta\delta$	−8.9	−1.9	−2.1	−0.7	−0.4	+0.1	−0.3	−0.1	−1.3	+1.0	−0.6	−0.2	−0.9					
	A9c [15]	OH	146.0	135.6	175.9	145.8	128.5	153.6	93.3	148.8	103.3	123.4	129.2	113.9	160.3					55.3

Table 2. ^{13}C NMR data for 5,7,8-trioxygenated 4′-methoxyflavones in DMSO-d_6

Ar = 		R	C_2	C_3	C_4	C_5	C_6	C_7	C_8	C_9	C_{10}	$C_{1'}$	$C_{2',6'}$	$C_{3',5'}$	$C_{4'}$	OMe C_3	OMe C_5	OMe C_6	OMe C_7	OMe C_8	OMe $C_{4'}$
	**B1a** [16]	H	159.4	106.2	175.8	155.6	93.5	156.2	129.8	150.9	107.8	123.1	127.5	114.5	161.7		56.3		56.1	60.9	55.4
		Δδ	−7.9	+33.6	−3.5	0	−0.4	−0.1	−0.3	−0.9	+0.3	−0.5	+1.8	−0.3	−0.9						
	B1b [17]	OMe	151.5	139.8	172.3	155.6	93.1	156.1	129.5	150.0	108.1	122.6	129.3	114.2	160.8	59.2	56.1		56.3	60.9	55.3
		Δδ	−9.8	−2.4	−1.1	−0.1	−0.3	−0.1	0	−0.1	−2.1	+1.0	−0.9	−0.1	−0.8						
	B1c [17]	OH	141.7	137.4	171.2	155.5	92.8	156.0	129.5	149.9	106.0	123.6	128.4	114.1	160.0		56.3		56.3	60.8	55.2
	**B3a** [18]	H	159.2	106.3	175.7	154.9	96.6	155.4	128.7	151.7	107.3	123.2	127.4	114.5	161.7		55.7			60.9	55.4
		Δδ	−7.9	+33.6	−3.5	−0.2	−0.2	0	−0.2	−1.0	+0.3	−0.5	+1.9	−0.3	−0.9						
	B3b [17]	OMe	151.3	139.9	172.2	154.7	96.4	155.4	128.5	150.7	107.6	122.7	129.3	114.2	160.8	59.2	55.8			60.8	55.3
		Δδ	−10.0	−2.5	−1.1	−0.1	−0.4	−0.1	−0.1	−0.1	−2.2	+1.0	−0.9	−0.1	−0.9						
	B3c [17]	OH	141.3	137.4	171.1	154.6	96.0	155.3	128.4	150.6	105.4	123.7	128.4	114.1	159.9		55.9			60.8	55.2
	**B5a*** [16]	H	163.9	103.8	182.7	157.5	95.7	158.5	128.9	149.4	104.8	123.6	128.1	114.6	162.7				56.3	61.7	55.5
		Δδ	−8.5	+34.0	−4.4	−1.2	0	−0.3	−0.6	−1.6	−0.3	−1.4	+1.7	−0.3	−1.3						
	B5b [17]	OMe	155.4	137.8	178.3	156.3	95.7	158.2	128.3	147.8	104.5	122.2	129.8	114.3	161.4	59.7			56.5	61.0	55.4
		Δδ	−8.9	−1.8	−1.9	−0.4	−0.5	−0.4	−0.1	−0.2	−1.1	+1.1	−0.6	−0.2	−0.8						
	B5c [19]	OH	146.5	136.0	176.4	155.9	95.2	157.8	128.2	147.6	103.4	123.3	129.2	114.1	160.6				56.4	60.9	55.3
	**B7a** [18]	H	163.0	103.3	181.8	156.2	98.9	157.1	127.6	149.4	103.5	122.9	128.0	114.6	162.3					60.9	55.5
		Δδ	−8.0	+34.6	−3.7	−0.3	−0.1	−0.2	−0.1	−0.8	+0.6	−0.6	+1.8	−0.3	−1.0						
	B7b [17]	OMe	155.0	137.9	178.1	155.9	98.8	156.9	127.5	148.6	104.1	122.3	129.8	114.3	161.3	59.7				60.9	55.4
		Δδ	−8.9	−1.8	−2.0	−0.5	−0.4	−0.4	−0.1	−0.2	−1.1	+1.1	−0.7	−0.2	−0.8						
	B7c [20]	OH	146.1	136.1	176.1	155.4	98.4	156.5	127.4	148.4	103.0	123.4	129.1	114.1	160.5					60.8	55.3
	**B8a** [21]	H	163.4	102.9	182.3	153.0	95.6	154.2	126.1	144.3	103.8	122.9	128.4	114.4	162.3				56.2		55.5
		Δδ	−8.1	+34.7	−3.8	−0.3	−0.2	−0.2	−0.1	−0.6	+0.6	−0.6	+1.7	−0.2	−1.0						
	B8b [13]	OMe	155.3	137.6	178.5	152.7	95.4	154.0	126.0	143.7	104.4	122.3	130.1	114.2	161.3	59.7			56.3		55.4
		Δδ	−8.8	−1.8	−2.0	−0.5	−0.5	−0.4	0	+0.1	−1.0	+1.2	−0.6	−0.3	−0.8						
	B8c [13]	OH	146.5	135.8	176.5	152.2	94.9	153.6	126.0	143.8	103.4	123.5	129.5	113.9	160.5				56.3		55.3
	**B9a** [21]	H	163.1	102.9	182.1	153.0	98.6	153.4	125.0	145.4	103.3	123.0	128.4	114.4	162.2						55.4
		Δδ	−8.2	+34.7	−3.9	−0.2	−0.2	−0.1	−0.2	−0.6	+0.6	−0.6	+1.6	−0.3	−0.9						
	B9b	OMe	154.9	137.6	178.2	152.8	98.4	153.3	124.8	144.8	103.9	122.4	130.0	114.1	161.3	59.6					55.4
	**B4a** [21]	R = Me, R′ = H	159.6	105.9	176.2	151.8	94.0	152.0	127.9	146.8	108.0	123.2	127.8	114.3	161.7		56.3		56.2		55.4
	B10a [22]	R = R′ = H	159.3	105.9	176.0	150.9	96.7	152.2	126.2	148.0	107.1	123.3	127.8	114.3	161.7		55.8				55.3

* Measured in $CDCl_3$, since the flavone hardly dissolved in DMSO-d_6.

Table 3. ^{13}C NMR data for 5,6,7,8-tetraoxygenated 4′-methoxyflavones in DMSO-d_6

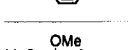
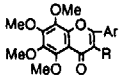
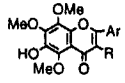
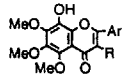
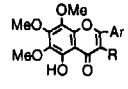
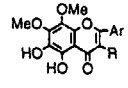
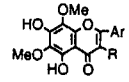
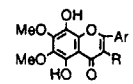
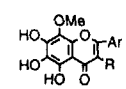
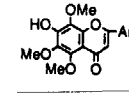
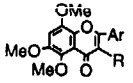
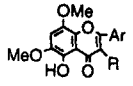
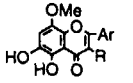
Ar = 		R	C_2	C_3	C_4	C_5	C_6	C_7	C_8	C_9	C_{10}	$C_{1'}$	$C_{2',6'}$	$C_{3',5'}$	$C_{4'}$	OMe C_3	OMe C_5	OMe C_6	OMe C_7	OMe C_8	OMe C_4
	**C1a** [23]	H	160.3	106.0	175.7	147.4	143.5	150.9	137.7	147.1	114.2	123.0	127.7	114.6	161.9		61.8	61.3	61.8	61.4	55.4
		Δδ	−7.8	+33.8	−3.5	−0.2	−0.3	−0.1	−0.3	−0.9	+0.2	−0.5	+1.8	−0.3	−0.9						
	C1b [24]	OMe	152.5	139.8	172.2	147.2	143.2	150.8	137.4	146.2	114.4	122.5	129.5	114.3	161.0	59.2	61.7	61.3	61.8	61.5	55.3
		Δδ	−9.7	−2.1	−1.0	−0.3	−0.2	−0.1	0	−0.1	−2.2	+1.0	−0.8	−0.2	−0.8						
	C1c [24]	OH	142.8	137.7	171.2	146.9	143.0	150.7	137.4	146.1	112.2	123.5	128.7	114.1	160.2		61.7	61.3	61.9	61.5	55.3
	**C2a** [5]	H	160.1	105.7	175.9	141.2	140.7	146.3	137.8	143.6	114.1	123.2	127.6	114.5	161.8		61.7		61.4	60.8	55.4
		Δδ	−7.6	+34.0	−3.6	−0.2	−0.3	+0.1	−0.2	0.8	+0.3	−0.5	+1.9	0.3	−0.9						
	C2b [24]	OMe	152.5	139.7	172.3	141.0	140.4	146.4	137.6	142.8	114.4	122.7	129.5	114.2	160.9	59.2	61.4		61.7	60.8	55.3
		Δδ	−9.6	−2.2	−1.1	−0.4	−0.5	+0.1	−0.1	0	−2.3	+1.0	−0.8	−0.1	−0.8						
	C2c [24]	OH	142.9	137.5	171.2	140.6	139.9	146.5	137.5	142.8	112.1	123.7	128.7	114.1	160.1		61.4		61.6	60.8	55.2
	**C4a** [5]	H	160.4	105.8	176.0	143.8	143.1	145.7	135.9	143.3	114.4*	123.1	128.0	114.4*	161.8		61.3	61.0	61.8		55.4
		Δδ	−18.0	+31.8	−4.6	−0.7	−0.1	−0.4	−0.2	−0.6	−2.1	+0.5	+1.0	−0.5	−1.7						
	C4c [24]	OH	142.4	137.6	171.4	143.1	143.0	145.3	135.7	142.7	112.3	123.6	129.0	113.9	160.1		61.4	61.0	61.9		55.2
	**C5a** [23]	H	163.6	103.2	182.5	148.5	135.8	152.4	132.6	145.2	106.1	122.6	128.2	114.6	162.4			60.5	61.8	61.4	55.5
		Δδ	−7.9	+34.6	−3.9	−0.4	−0.4	−0.1	−0.1	−0.8	+0.6	−0.5	+1.7	−0.2	−0.9						
	C5b [24]	OMe	155.7	137.8	178.6	148.1	135.4	152.3	132.5	144.4	106.7	122.1	129.9	114.4	161.5	59.7		60.5	61.8	61.4	55.4
		Δδ	−8.6	−1.6	−2.0	−0.7	−0.5	−0.2	−0.1	−0.2	−1.1	+1.1	−0.6	−0.3	−0.8						
	C5c [24]	OH	147.1	136.2	176.6	147.4	134.9	152.1	132.4	144.2	105.6	123.2	129.3	114.1	160.7			60.5	61.7	61.4	55.3
	**C6a** [5]	H	163.4	102.9	182.5	143.0	134.2	147.9	132.8	141.7	106.1	122.9	128.1	114.6	162.3				61.8	60.9	55.5
		Δδ	−7.8	+34.8	−4.0	−0.5	−0.4	+0.1	−0.1	−0.7	+0.6	−0.6	+1.7	−0.3	−0.9						
	C6b [24]	OMe	155.6	137.7	178.5	142.5	133.8	148.0	132.7	141.0	106.7	122.3	129.8	114.3	161.4	59.7			61.7	60.9	55.4
		Δδ	−8.7	−1.8	−2.0	−0.7	−0.6	0	0	0	−1.2	+1.1	−0.5	−0.2	−0.8						
	C6c [24]	OH	146.9	135.9	176.5	141.8	133.2	148.0	132.7	141.0	105.5	123.4	129.3	114.1	160.6				61.7	60.9	55.3
	**C7a** [25]	H	163.0	103.0	182.2	148.3	131.5	150.8	127.9	145.3	102.9	122.9	128.1	114.6	162.3			60.1		61.2	55.5
		Δδ	−8.0	+34.5	−3.8	−0.4	−0.2	0	−0.2	−0.7	+0.6	−0.6	+1.7	−0.3	−1.0						
	C7b [26]	OMe	155.0	137.5	178.4	147.9	131.3	150.8	127.7	144.6	103.5	122.3	129.8	114.3	161.3	59.7		60.1		61.1	55.4
		Δδ	−8.8	−1.7	−2.0	0.6	0.4	−0.3	−0.1	−0.2	−1.2	+1.1	−0.7	−0.2	−0.8						
	C7c [26]	OH	146.2	135.8	176.4	147.3	130.9	150.5	127.6	144.4	102.3	123.4	129.1	114.1	160.5			60.1		61.0	55.3
	**C8a** [5]	H	163.7	103.0	182.8	144.6	136.1	148.0	130.6	141.3	106.3	122.7	128.5	114.5	162.4			60.4	61.0		55.5
		Δδ	−8.0	+34.7	−4.0	−0.4	−0.3	−0.2	−0.1	−0.6	+0.6	−0.5	+1.7	−0.3	−1.0						
	C8b [24]	OMe	155.7	137.7	178.8	144.2	135.8	147.8	130.5	140.7	106.9	122.2	130.2	114.2	161.4	59.7		60.4	61.1		55.4
		Δδ	−8.7	−1.7	−2.1	−0.7	−0.5	−0.3	−0.1	0	−1.2	+1.1	−0.6	−0.2	−0.8						
	C8c [24]	OH	147.0	136.0	176.7	143.5	135.3	147.5	130.4	140.7	105.7	123.3	129.6	114.0	160.6			60.4	61.1		55.3
	**C9a**	H	162.7	102.8	182.1	142.8	129.5	147.1	127.9	142.4	102.8	123.1	127.9	114.6	162.1					61.1	55.4
		Δδ	−7.9	+34.7	−3.9	−0.6	−0.2	+0.1	−0.2	−0.7	+0.6	−0.6	+1.8	−0.3	−0.9						
	C9b [24]	OMe	154.8	137.5	178.2	142.2	129.3	147.2	127.7	141.7	103.4	122.5	129.7	114.3	161.2	59.7				61.1	55.4
		Δδ	−8.9	−1.9	−2.1	−0.7	−0.5	−0.1	−0.1	0	−1.2	+1.0	−0.7	−0.2	−0.9						
	C9c [24]	OH	145.9	135.6	176.1	141.5	128.8	147.1	127.6	141.7	102.2	123.5	129.0	114.1	160.3					61.0	55.3
	**C3a** [26]		159.8	105.8	175.7	147.3	139.4	149.1	132.5	147.5	110.6	123.2	127.6	114.6	161.8		61.7	61.1		61.1	55.4

Table 4. ^{13}C NMR data for 5,6,8-trioxygenated 4′-methoxyflavones in DMSO-d_6 and the A-ring substituent effects

Ar =		R	C_2	C_3	C_4	C_5	C_6	C_7	C_8	C_9	C_{10}	$C_{1'}$	$C_{2',6'}$	$C_{3',5'}$	$C_{4'}$	OMe C_3	OMe C_5	OMe C_6	OMe C_7	OMe C_8	OMe $C_{4'}$
	**D1a** [27]	H	160.3	105.8	176.4	138.6	149.1	103.5	140.1	145.1	118.6	123.1	127.7	114.5	161.9		56.8†	56.8†		61.0	55.4
		Δδ	−7.8	+33.6	−3.5	−0.2	−0.3	−0.1	−0.2	−0.1	+0.3	−0.5	+1.9	−0.3	−0.9						
	D1b [28]	OMe	152.5	139.4	172.9	138.4	148.8	103.4	139.9	145.0	118.9	122.6	129.6	114.2	161.0	59.2	56.9	56.7		61.0	55.3
		Δδ	−9.4	−1.5	−1.1	−0.3	−0.6	−0.1	−0.4	0	−2.3	+1.0	−0.7	−0.1	−0.7						
	D1c [28]	OH	143.1	137.9	171.8	138.1	148.2	103.3	139.5	145.0	116.6	123.6	128.9	114.1	160.3		57.0	56.7		61.2	55.3
	**D5a** [27]	H	163.7	103.1	183.3	139.5	141.0	106.6	138.6	142.1	110.4	122.7	128.3	114.6	162.5			56.8		57.3	55.5
		Δδ	−7.8	+34.8	−4.1	0	−0.5	+0.1	−0.7	−0.6	+0.6	−0.5	+1.7	−0.3	−1.0						
	D5b [28]	OMe	155.9	137.9†	179.2	139.5	140.5	106.7	137.9†	141.5	111.0	122.2	130.0	114.3	161.5	59.6		57.0		57.2	55.4
			−8.6	−1.6	−2.1	0	−0.6	−0.2	+0.1	−0.7	−1.2	+1.0	−0.6	−0.2	−0.8						
	D5c	OH	147.3	136.3	177.1	139.5	139.9	106.5	138.0	140.8	109.8	123.2	129.4	114.1	160.7			57.1		57.1	55.3
	D5b-OH* [29]	OMe	156.3	137.6	179.1	139.4	140.5	106.6	137.9	141.5	111.0	120.5	130.2	115.7	160.4	59.5		57.0		57.2	
	**D6a** [27]	H	163.6	103.1	183.2	139.6	137.6	108.2	138.6	140.1	110.6	122.9	128.2	114.6	162.4					56.8	55.5
		Δδ	−7.8	+34.8	−4.1	0	−0.7	0	−0.7	−0.5	+0.7	−0.6	+1.8	−0.3	−1.0						
	D6b [28]	OMe	155.8	137.9	179.1	139.6†	136.9	108.2	137.9	139.6†	111.3	122.3	130.0	114.3	161.4	59.6				56.8	55.4

Conversion of the substituent	Δδ C_5	Δδ C_6	Δδ C_7	Δδ C_8	Δδ C_9	Δδ C_{10}
5-OMe→OH	+1.1	−8.2	+3.2	−1.7	−3.3, −4.2‡	−8.0, −6.8‡
6-OMe→OH	−2.4	−1.2	+1.5	0	−2.0	+0.2

* **D5b-OH**; 5,4′-Dihydroxy-3,6,8-trimethoxyflavone.

† Overlapped signals: the ones of **D5b** were assigned by using the ^{13}C NMR data for **D5b-OH**.

‡ In the case of the 3-hydroxyflavones.

Table 5. Shift ranges of the A- and C-ring ^{13}C NMR signals of flavones by the substituent effects at the 3-position

	C_3-Substituent	Δδ							
		C_2	C_3	C_4	C_5	C_6	C_7	C_8	C_9	C_{10}
5-Methoxyflavones	H→OMe	−7.8±0.2	+33.8±0.2	−3.5±0.1	−0.1±0.1	−0.3±0.1	−0.1±0.1	−0.3±0.1	−0.9±0.1	+0.2±0.1
	OMe→OH	−9.8±0.2	−2.3±0.2	−1.0±0.1	−0.3±0.2	−0.3±0.2	0±0.2	−0.1±0.1	−0.1±0.1	−2.1±0.1
5-Hydroxyflavones	H→OMe	−8.0±0.2	+34.4±0.4	−3.9±0.2	−0.5±0.1	−0.3±0.1	0±0.2	−0.2±0.1	−0.8±0.2	+0.6±0.1
	OMe→OH	−8.8±0.1	−1.8±0.1	−2.0±0.1	−0.6±0.2	−0.5±0.2	0±0.2	−0.1±0.1	−0.1±0.2	−1.2±0.2

In the ^{13}C NMR data for five natural flavones, isolated from *Gymnosperma glutinosum* and proposed as **17**, **18**, **19**, **20**, and **21**, having the 2′,4′,5′-trioxygenated pattern, by Yu *et al.* [32], the A-ring carbon signals except for **19** are consistent with those for the corresponding flavones, **C7b**, **C5b**, and **A7b**, respectively, when the data are rearranged, and it supports well the proposed structures (Table 8). The spectrum of the flavone proposed as **19**, however, does not exhibit the diagnostic signals attributed to the A-ring having the 5,7-dihydroxy and 6,8-dimethoxy groups and to the B-ring having the 3′,4′-dihydroxy and 2′-methoxy groups such as the B-ring of **20** or **21**, so the structural elucidation of the flavone should be re-examined.

The ^{13}C NMR of other natural flavones, such as 5,6,4′-trihydroxy-7,8,3′-trimethoxyflavone (**22**) isolated from *Thymus vulgaris* [33], and 5,7,4′-trihydroxy-6,8,3′-trimethoxyflavone (**23**) and 4′-hydroxy-5,6,7,8-tetramethoxyflavone (**24**) isolated from *Citrus reticulata* [7], are consistent with those of the synthetic flavones, **22**, **23**, and **24**, and support the proposed structures, although the quaternary carbon chemical shifts underlined are greatly different from those for synthetic ones as shown in Table 8.

These results show clearly that the structures of polyhydroxyflavones and flavonols can be correctly proposed by ^{13}C NMR spectra, and the data obtained here may be widely applicable to the structural elucidation of naturally occurring flavones and flavonols.

EXPERIMENTAL

General. The ^{13}C NMR (at 100.4 MHz) spectra were recorded on a JEOL EX 400 spectrometer in DMSO-d_6, using TMS as an int. standard, and the chemical shifts are given in δ values. The compounds used in this study are the ones previously synthesized by us. 5,6,7-Trihydroxy-8,4′-dimethoxyflavone (**C9a**) was synthesized from 6-hydroxy-5,7,8,4′-tetramethoxyflavone (**C2a**) by selective demethylation with 5% anhydrous $AlBr_3$–MeCN (50°, 5 hr); mp 223–225° (from EtOAc–MeOH). 5,7,8-Trihydroxy-3,4′-dimethoxyflavone (**B9b**) was synthesized from 7-benzyloxy-5,8-dihydroxy-3,4′-dimethoxyflavone [36] by hydrogenolysis with 10% Pd-C; mp 208–209° (from aq. MeOH). 3,5-Dihydroxy-6,8,4′-trimethoxyflavone (**D5c**) was synthesized from 3-hydroxy-5,6,8,4′-tetramethoxyflavone (**D1c**) via the tosylate according to a method by our previous paper [24]; mp 189–190° (from MeOH).

Synthesis of 5,7,2′-*trihydroxy*-6-*methoxyflavone* (**16**). To a soln of 4′-benzyloxy-6′-hydroxy-3′-methoxy-2′-isopropoxyacetophenone [11] (0.56 g) and 2-benzyloxybenzaldehyde (0.38 g) in EtOH (10 ml), KOH (1.0 g) was added, the mixt. was warmed with stirring at 40–45° for 2.5 hr and then acidified with 1–2% aq. HCl. The sepd oily material was extracted with EtOAc to give a crude chalcone. A mixt. of the

Table 6. A-ring substituent effects in the ^{13}C NMR spectra of 4′-methoxyflavones when a methoxy group on the A-ring was converted into a hydroxy group

Compounds		Δδ			
		5-OMe→OH	6-OMe→OH	7-OMe→OH	8-OMe→OH
5,6,7-Tri-oxygenated (**A**)	C_5	+0.2, +1.7[a]	−7.5[f], −6.0	+0.8	
	C_6	−7.8	−2.1	−0.6	
	C_7	+1.1	−4.0	−1.1	
	C_8	−5.6	−0.5	+2.7	
	C_9	−1.2	−2.9	−0.1	
	C_{10}	−7.0[b], −6.5[c], −5.5[d]	0	−1.0	
5,7,8-Tri-oxygenated (**B**)	C_5	+0.6, +1.0[e]		−0.6	−3.6, 3.1[g]
	C_6	+2.4		+3.1	−0.2
	C_7	+1.7		−1.0	−4.2, −3.6[g]
	C_8	−1.2		−1.0	−2.2, −2.6[g]
	C_9	−2.2		+0.9	−4.0
	C_{10}	−3.8[b], −3.5[c], 2.5[d]		−0.5	0
5,6,7,8-Tetra-oxygenated (**C**)	C_5	+0.8, +1.5[a]	−5.7, −6.2[f]	−0.2	−3.8
	C_6	−7.5, −6.6[a]	−1.8, −2.9[f]	−4.3	+0.4, −0.2[f]
	C_7	+1.7	−4.2, −3.5[g]	−1.7, −0.8[a]	−4.5, −5.3[f]
	C_8	−5.0	+0.1	−5.0	−1.9
	C_9	−1.9	−3.4, −2.8[g]	+0.2, +0.7[a]	−3.6
	C_{10}	−8.0[b], −7.6[c], −6.6[d]	0	−3.3	+0.1

[a] When the flavones have a hydroxy group at the 6-position.
[b] In the case of the flavones (**a** series).
[c] In the case of the 3-methoxyflavones (**b** series).
[d] In the case of the 3-hydroxyflavones (**c** series).
[e] When the flavones have a hydroxy group at the 7-position.
[f] When the flavones have a methoxy group at the 5-position.
[g] When the flavones have two hydroxy groups at the 5- and 7-positions.

Table 7. Calculated and observed A-ring ^{13}C NMR signals of two flavones

	5,7,8(OH)/3,6(OMe)			3,5,6,7(OH)			
	Calcd from			Calcd from			
Carbon	**C7b**	**C8b**	Obs.*	**A9b**	**A7c**	**A6c**	Obs. (**A9c**)
C_5	144.1	144.0	144.5	145.9	145.7	145.7	145.8
C_6	131.7	131.5	131.1	128.4	128.7	128.6	128.5
C_7	146.3	146.1	146.7	153.5	153.2	153.5	153.6
C_8	125.8	125.5	125.2	93.5	93.3	93.4	93.3
C_9	141.0	140.9	140.7	148.8	148.5	148.7	148.8
C_{10}	103.5	103.6	103.3	103.4	103.4	103.3	103.3

* Reported data for 5,7,8,3′,4′-pentahydroxy-3,6-dimethoxyflavone isolated from *Gutierrezia microcephala* by Roitman and James [9].

chalcone and SeO_2 (1.0 g) in *n*-pentanol (10 ml) was refluxed for 5 hr and then diluted with EtOAc. After the ppt. was filtered off, the filtrate was washed with H_2O and evapd to dryness. The residue was chromatographed over silica gel with $CHCl_3$ to give a crude flavone. The flavone was deisopropylated with anhydrous $AlCl_3$ (0.6 g) in MeCN (12 ml) at room temp. (*ca* 20°) for 30 min to give 7,2′-bis(benzyloxy)-5-hydroxy-6-methoxyflavone: mp 114–115° (from EtOAc–MeOH); yield, 250 mg (31%).

The flavone (180 mg) was hydrogenolysed with 10% Pd-C in EtOAc–MeOH (1:1) to give quantitatively the desired flavone **16**: mp 261–263° (decomp.) (from EtOAc) lit [30] mp 270–272°): ^1H NMR; δ 6.60 (1 H, *s*, C_8-H), 7.06 (1H, *s*, C_3-H), 7.88 (1H, *dd J* = 7.8, 1.5 Hz, C_6′-H), 7.41 (1H, *td J* = 7.8, 1.5 Hz, C_4′-H), 7.06

Table 8. ^{13}C NMR data for some polyhydroxyflavones in DMSO-d_6

	C_2	C_3	C_4	C_5	C_6	C_7	C_8	C_9	C_{10}	$C_{1'}$	$C_{2'}$	$C_{6'}$	$C_{3'}$	$C_{5'}$	$C_{4'}$	OMe		
5,6(OH)/7,8(OMe) **11**	163.3	104.5	182.7	143.0	134.3	148.2	133.0	141.9	106.2	130.8	126.2		129.2		132.0	60.9	61.8	
5,6,2′(OH)/7,8(OMe) **13*** [5]	161.6	108.5	182.7	142.9	134.0	148.1	132.9	142.0	106.0	117.4	156.8	128.2	117.1	119.6	132.9	60.9	61.8	
5,8(OH)/6,7(OMe) **12**	163.5	104.6	183.0	144.6	136.2	148.2	130.7	141.4	106.5	130.6	126.6		129.0		132.1	60.4	61.1	
5,8,2′(OH)/6,7(OMe) **14*** [5]	161.5	108.6	183.0	144.5	136.0	148.0	130.6	141.5	106.3	117.1	156.9	128.7	117.0	119.4	132.9	60.4	61.1	
Nat. 5,8,2′(OH)/7(OMe); **15*** [30]	161.5	108.6	182.2	152.6	131.4	157.3	94.2	152.6	104.2	117.5	156.5	128.5	117.0	119.4	132.6	59.9		
5,7(OH)/4′,6(OMe) **A7a**				152.7	131.3	157.3	94.2	152.4	104.1									
Syn. **16**	161.2	108.4	182.2	152.5	131.2	157.4	94.1	152.5	104.0	117.2	156.6	128.4	117.0	119.4	132.7	59.9		
Nat. 3,5,6(OH)/7,4′(OMe); **A6c*** [31]	146.4	135.8	176.5	152.2	94.9	153.5	125.9	143.7	103.3	123.4	129.4		113.9		160.5	55.2	56.3	
B8c	146.5	135.8	176.5	152.2	94.9	153.6	126.0	143.8	103.4	123.5	129.5		113.9		160.5	55.3	56.3	
Nat. 5,7(OH)/3,6,8,2′,4′,5′(OMe); **17*** [32]	152.3	138.6	178.3	147.9	131.3	150.6	127.7	145.1	103.9	109.9	156.9	114.4	98.7	142.4	152.0			
Nat. 5,7,4′(OH)/3,6,8,2′,5′(OMe); **18*** [32]	152.0	139.4	179.2	148.6	131.9	151.2	128.4	145.9	104.7	114.4	157.0	117.3	98.6	140.5	151.2			
C7b				147.9	131.3	150.8	127.7	144.6	103.5									
Nat. 5,7,4′,5′(OH)/3,6,8,2′(OMe); **19** [32]	145.7	136.7	176.7	147.9	133.8	160.5	129.5	154.6	101.4	109.5	149.0	117.2	99.3	139.0	150.8			
Nat. 5,4′,5′(OH)/3,6,7,8,2′(OMe); **20*** [32]	151.3	138.7	178.7	148.2	135.5	152.3	132.5	145.0	107.2	108.8	157.5	117.1	101.0	138.9	149.3			
C5b				148.1	135.4	152.3	132.5	144.4	106.7									
Nat. 5,7,4′,5′(OH)/3,6,2′(OMe); **21*** [32]	152.3	138.8	178.4	152.7	131.3	157.4	94.0	151.3	105.2	109.5	156.7	117.2	101.2	138.8	149.0			
A7b				152.3	131.1	157.4	94.0	151.5	104.6									
Nat. 5,6,4′(OH)/7,8,3′(OMe); **22*** [33]	163.7	102.7	182.5	143.0	134.1	148.0	132.8	141.8	106.1	121.6	109.9	120.2	150.8	115.9	152.5	55.8	60.9	61.8
Syn. **22** [34]	163.7	102.6	182.5	143.0	134.1	147.9	132.8	141.7	106.0	121.5	109.8	120.1	150.7	115.8	147.8	55.7	60.9	61.8
Nat. 5,7,4′(OH)/6,8,3′(OMe) **23*** [7]	163.1	102.7	182.0	148.1	131.3	150.5	127.7	145.1	115.7	121.4	109.7	119.9	150.5	115.7	147.8	56.0	59.8	61.0
Syn. **23** [25]	163.3	102.7	182.2	148.3	131.4	150.7	127.8	145.3	102.9	121.5	109.8	120.1	150.7	115.8	148.0	55.7	60.6	61.1
Nat. 4′(OH)/5,6,7,8(OMe); **24*** [7]	161.4	106.0	176.3	148.1	144.1	151.4	138.4	138.4	116.7	122.0	128.5		114.9		161.4	62.4	(4 × OMe)	
Syn. **24** [35]	160.7	105.3	175.6	147.4	143.4	150.8	137.7	147.0	114.2	121.3	127.8		115.9		160.7	61.3	61.4	61.7 61.8

* The assignment of the reported carbon signals has been partly revised for the comparison.

(1H, *d* $J = 7.8$ Hz, C_3'-H), 7.00d (1H, *t* $J = 7.8$ Hz, C_5'-H), 3.77 (3H, *s*, OMe), 13.01 (1H, *s*, C_5-OH), 10.80 (2H, *br s*, OH). Anal. Found: C, 63.87: H, 4.23%. Calcd for $C_{16}H_{12}O_6$: C, 64.00; H, 4.03%.

REFERENCES

1. Gottlieb, O. R., In *The Flavonoids* ed. J. B. Harborne, T. J. Mabry and H. Mabry. Chapman and Hall, London 1975, p. 296.
2. Wollenweber, E., In *The Flavonoids: Advances in Research*, ed. J. B. Harborne and T. J. Mabry. Chapman and Hall, London, 1982, p. 189.
3. Wollenweber, E., In *The Flavonoids: Advances in Research*, ed. J. B. Harborne. Chapman and Hall, London, 1988, p. 233.
4. Wollenweber, E., In *The Flavonoids: Advances in Research*, ed. J. B. Harborne. Chapman and Hall, London, 1994, p. 259.
5. Horie, T., Kawamura, Y., Yamamoto, H., Kitou, T. and Yamashita, K. *Phytochemistry*, 1995, **39**, 1201.
6. Calvert, D. J., Cambie, R. C. and Davis, B. R., *Organic Magnetic Resonance*, 1979, **12**, 583.
7. Iinuma, M., Matsuura, S. and Kusuda, K., *Chemical and Pharmaceutical Bulletin*, 1980, **28**, 708.
8. Agrawal, P. K., Thakur, R. S. and Bansal, M. C., In *Carbon-13C NMR of Flavonoids* ed. P. K. Agrawal. Elsevier, Amsterdam, 1989, p. 95.
9. Roitman, J. N. and James, L. F., *Phytochemistry*, 1985, **24**, 835.
10. Horie, T., Kourai, H., Tsukayama, M., Masumura, M. and Nakayama, M., *Yakugaku Zasshi*, 1985, **105**, 232.
11. Horie, T., Shibata, K., Yamashita, K., Kawamura, Y. and Tsukayama, M. *Chemical and Pharmaceutical Bulletin*, 1997, **45**, 446.
12. Horie, T., Tominaga, H., Kawamura, Y. and Yamada, T., *Journal of Organic Chemistry*, 1992, **57**, 3343.
13. Tominaga, H. and Horie, T., *Bulletin of the Chemical Society of Japan*, 1993, **66**, 2668.
14. Fukui, K., Nakayama, M. and Horie, T., *Journal of Science of Hiroshima University*, A-II, 1969, **33**, 305.
15. Horie, T., Kobayashi, T., Kawamura, Y., Yoshida, I., Tominaga, H. and Yamashita, K., *Bulletin of the Chemical Society of Japan*, 1995, **68**, 2033.
16. Nakayama, M., Horie, T., Makino, M., Hayashi, S., Ganno, S. and Narita, A., *Nippon Kagaku Kaishi*, 1978, 1390.
17. Horie, T., Tsukayama, M., Kawamura, Y. and Seno, M., *Journal of Organic Chemistry*, 1987, **52**, 4702.
18. Nakayama, M., Horie, T., Lin, C.-N., Arisawa, M., Shimizu, M. and Morita, N., *Yakugaku Zasshi*, 1983, **103**, 675.
19. Horie, T., Tsukayama, M., Kawamura, Y. and Yamamoto, S., *Phytochemistry*, 1988, **27**, 1491.
20. Horie, T., Tsukayama, M., Kawamura, Y., Seno, M. and Yamamoto, S., *Bulletin of the Chemical Society of Japan*, 1988, **61**, 441.
21. Horie, T., Kourai, H. and Fujita, N., *Bulletin of the Chemical Society of Japan*, 1983, **56**, 3773.
22. Horie, T., Unpublished work.
23. Horie, T., Simoo, H., Masumura, M. and Okumura, S., *Nippon Kagaku Zasshi*, 1962, **83**, 602.
24. Horie, T., Kawamura, Y., Yamamoto, H. and Yamashita, K., *Chemical and Pharmaceutical Bulletin*, 1995, **43**, 2054.
25. Horie, T., Masumura, M., Kase, K., Fukui, K. and Nakayama, M., *Nippon Kagaku Kaishi*, 1974, 2400.
26. Horie, T., Kitou, T., Kawamura Y. and Yamashita, K., *Bulletin of the Chemical Society of Japan*, 1996, **69**, 1033.
27. Horie, T., Kourai, H. and Osaka, H., *Nippon Kagaku Kaishi*, 1982, 1270.
28. Horie, T., Kourai, H. Osaka, H. and Nakayama, M., *Bulletin of the Chemical Society of Japan*, 1982, **55**, 2933.
29. Horie, T., Kawamura, Y., Kobayashi, T. and Yamashita, K., *Phytochemistry*, 1994, **37**, 1189.
30. Takagi, S., Yamaki, M. and Inoue, K., *Yakugaku Zasshi*, 1980, **100**, 1220.
31. Xiong, Q., Shi, D. and Mizuno, M., *Phytochemistry*, 1995, **39**, 723.
32. Yu, S., Fang, N. and Mabry, T. J., *Phytochemistry*, 1988, **27**, 171.
33. Van Den Broucke, C. O., Dommisse, R. A., Esmans, E. L. and Lemli, J. A., *Phytochemistry*, 1982, **21**, 2581.
34. Horie, T., Kourai, H., Nakayama, M., Tsukayama, M. and Masumura, M., *Nippon Kagaku Kaishi*, 1980, 1397.
35. Nakayama, M., Mori, I. and Horie, T., *Nippon Kagaku Kaishi*, 1983, 161.
36. Farkas, L. and Nógrádi, M., *Tetrahedron Letters*, 1966, 3759.