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^{13}C NMR STUDIES OF SOME NATURALLY OCCURRING AMENTOFLAVONE AND HINOKIFLAVONE BIFLAVONOIDS*

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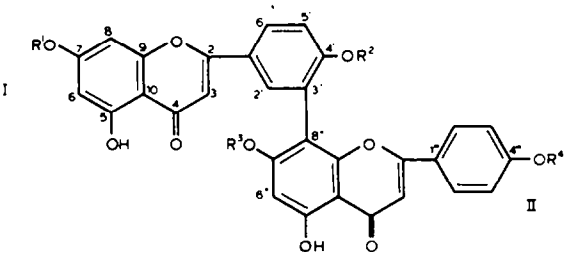
Key Word Index—^{13}C NMR; amentoflavones; hinokiflavones; methyl ethers; dihydro-derivatives.

Abstract—The ^{13}C NMR spectra of a range of amentoflavone and hinokiflavone biflavonoids are reported, most for the first time. Substituent shifts relating to the interflavonoid linkages and to specific methylation patterns are defined and appear to be of diagnostic value.

INTRODUCTION

The usefulness of ^{13}C NMR spectroscopy for the elucidation of the structure of flavonoids is well established. In the previous papers of this series [1–3] as well as in a recent review [4], the ^{13}C NMR spectra of a large number of monoflavonoids and their glycosides have been published. They provide a sound basis for the interpretation of the spectra of unknown compounds. In the biflavonoid field however reference spectra are still needed [5], the most extensive compilation to date being that of ref. [6]. The opportunity to record a range of ^{13}C NMR spectra on a series of related biflavonoids in the amentoflavone and hinokiflavone series has recently arisen with the accumulation by one of us (H.G.) of substantial quantities of these flavonoids. The presentation of these spectra, most of them previously unreported, and the deduction of a variety of spectra-structure correlations form the basis of the current note.



1	Amentoflavone	$R^1=R^2=R^3=R^4=H$
2	Bilobetin	R^2 = Me, $R^1=R^3=R^4=H$
3	Podocararpusflavone-A	R^4 = Me, $R^1=R^2=R^3=H$
4	Isoginkgetin	$R^2=R^4$ = Me, $R^1=R^3=H$
5	Ginkgetin	$R^1=R^2$ = Me, $R^3=R^4=H$
6	Tetramethylamentoflavone	$R^1=R^2=R^3=R^4$ = Me
7	2,3-Dihydroamentoflavone	$R^1=R^2=R^3=R^4=H$
8	Robustaflavone = II but with 3′-6″ interflavonoid link	

RESULTS AND DISCUSSION

Amentoflavone series—general (Table 1)

The ^{13}C NMR spectrum of amentoflavone has been assigned previously [6] and in the present communication these assignments are adopted except for those of I-1′ and II-1‴. The assignments for these two carbons have been reversed to better accommodate the spectra of bilobetin (2) and podocarpusflavone-A (3). Assignments for the spectra of the amentoflavone methyl ethers follow logically from the amentoflavone assignments as also does that of 2,3-dihydroamentoflavone (7) when the spectrum of naringenin is also used as a reference. The spectrum of the 3′,6″-isomer of amentoflavone, robustaflavone (8), was also assigned by Chari *et al.* [6] whose reported spectrum is close to that in Table 1. One important difference however is the observation of a signal at 109.3 ppm which we have assigned to carbon II-6″. This apparently was not observed by Chari *et al.* (perhaps due to the use of a shorter acquisition time) who allocated a signal at 103.5 ppm to this carbon. The revised chemical shift for carbon II-6″ brings the substitution effect of the interflavonoid linkage in robustaflavone into line with that of amentoflavone (see below).

Amentoflavone series—substituent effects

The data presented in Table 1 for the amentoflavone series of biflavonoids permit the calculation of the following (averaged) substituent effects for the interflavonoid linkage: (i) amentoflavones; I-3′ +6 ppm, II-8″ +10 ppm, (ii) robustaflavone; I-3′ +5 ppm, II-6″ +10 ppm, (iii) dihydroamentoflavone; I-3′ +4 ppm, II-8″ +9 ppm.

Substituent effects which appear to be the best indicators of specific *O*-methylation in amentoflavone include: (i) I-4′-*O*-methylation; I-5′ −4 ppm, I-1′ +2 ppm, (ii) II-4‴-*O*-methylation; II-3‴, 5‴ −1.5 ppm, II-1‴ +2 ppm, (iii) I-7-*O*-methylation; I-8 −1.5 ppm, (iv) II-7″-*O*-methylation; II-6″ −3.5 ppm.

The *O*-methylation induced shifts detailed above are in the same direction as those previously reported for monoflavonoids [3], although the magnitudes appear to differ somewhat.

* Part IV in the series '^{13}C NMR of Flavonoids', see refs [1–3].

Table 1. ^{13}C NMR spectra of amentoflavone, its derivatives and reference compounds

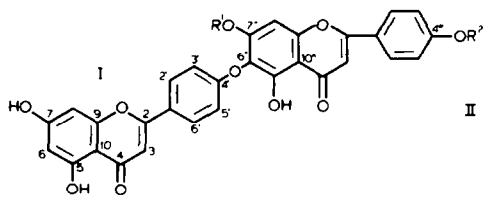
		C-2	C-3	C-4	C-5	C-6	C-7	C-8	C-9	C-10	C-1′	C-2′	C-3′	C-4′	C-5′	C-6′
Amentoflavone (1)	I	164.1[a]	103.2[b]	181.9[c]	161.6	98.8[d]	163.9[e]	94.2	157.6	104.0	120.3	127.9	121.7[f]	159.6	116.4	131.6
	II	164.3[a]	102.8[b]	182.2[c]	160.8	99.1[d]	161.9[e]	104.1	154.7	104.0	121.4[f]	128.3	116.0	161.1	116.0	128.3
Bilobetin (2)	I	163.3[a]	103.6[b]	181.7[c]	161.4[d]	98.6[e]	163.5[a]	94.1	157.4	103.8	122.5[f]	128.0	121.6[f]	160.6[d]	111.7	130.9
	II	164.2[a]	102.5[b]	182.0[c]	160.4[d]	98.9[e]	161.6[d]	103.7[b]	154.3	103.6	121.2[f]	128.0	115.8	161.0[d]	115.8	128.0
Podocarpusflavone-	I	163.9[a]	103.2[b]	181.6[c]	161.5[d]	98.9	163.3[a]	94.0	157.4	104.1	120.1[e]	127.7	121.3[e]	159.4	116.3	131.3
A (3)	II	164.1[a]	103.2[b]	182.1[c]	160.6[d]	98.9	161.9[d]	103.2[b]	154.6	103.9	123.1	127.9	114.5	162.3[d]	114.5	127.9
Isoginkgetin (4)	I	163.4[a]	103.7[c]	181.8[e]	161.9[b]	98.8[d]	163.1[a]	94.2	157.5	103.8[c]	122.6	128.2	121.7	160.7[b]	111.5	130.8
	II	164.3[a]	103.3	182.1[e]	160.5[b]	99.0[d]	161.7[b]	103.9[c]	154.4	103.8[c]	122.9	127.8	114.4	162.3[b]	114.5	127.7
Ginkgetin (5)	I	163.5[a]	103.5[e]	181.9[c]	161.5[b]	98.5[d]	165.1	92.6	157.3	104.7	122.3[f]	128.2	121.7[f]	160.6[b]	111.7	130.7
	II	163.6[a]	102.5	182.0[c]	160.4[b]	98.6[d]	161.7[b]	103.8[e]	154.3	103.5	121.2[f]	128.0	115.8	161.0[b]	115.8	128.0
7,7″,4′,4‴-Me-	I	162.5[a]	103.1[d]	181.9[c]	161.4[b]	98.0	165.1	92.7 (157)		104.7[d]	122.4[e]	128.2	121.2[e]	161.1[b]	111.7	130.8
Amentoflavone (6)	II	163.4[a]	103.1[d]	182.2[c]	160.4[b]	95.5	161.1[b]	103.9[d](153.5)		104.0[d]	122.6[e]	127.8	114.5	162.2[b]	114.5	127.8
Robustaflavone (8)	I	164.3[c]	103.2	182.1[a]	161.8[b]	99.2	164.0[c]	94.4[d]	157.7	104.1	121.2[e]	127.6	121.3[e]	159.5	116.5[f]	131.2
	II	164.5[c]	103.2	182.2[a]	160.0[b]	109.3	162.5[c]	93.6[d]	156.8	103.9	121.6[e]	128.6	116.4	161.5[b]	116.4[f]	128.6
Naringenin*		78.4	42.0	196.2	163.6	95.9	166.7	95.0	162.9	101.8	128.9	128.2	115.2	157.8	115.2	128.2
2,3-Dihydro-	I	78.8	42.5	196.5	163.6[b]	96.0	166.8	95.2	163.2[b]	101.9	128.7	127.8	119.2	156.2	115.9	131.6
amentoflavone (7)	II	163.7[b]	102.6	182.2	160.4	98.8	162.2	104.9[a]	154.5	103.7[a]	121.5	128.4	115.9	161.2	115.9	128.4

*Spectrum from ref. 4.
[a,b,c,d,e,f]Assignments bearing the same superscript in any one spectrum may be reversed.

Table 2. ^{13}C NMR spectra of Hinokiflavone, its derivatives and reference compounds.

		C-2	C-3	C-4	C-5	C-6	C-7	C-8	C-9	C-10	C-1′	C-2′	C-3′	C-4′	C-5′	C-6′
Apigenin*		164.1	102.8	181.8	161.5	98.8	163.7	94.0	157.3	103.7	121.3	128.4	116.0	161.1	116.0	128.4
Hinokiflavone (9)	I	163.1[a]	103.9[b]	181.7[c]	161.3[f]	98.9	162.2[f]	94.0	157.1[e]	103.8	124.2[g]	128.3[h]	115.3	161.4[f]	115.3	128.3[h]
	II	164.2[a]	102.6[b]	182.1[c]	153.1[d]	124.7[g]	157.3[e]	94.6	153.7[d]	104.1	121.1	128.5[h]	116.0	160.6[f]	116.0	128.5[h]
Cryptomerin A (10)	I	163.7[a]	103.9[b]	181.7[c]	160.6[f]	98.8	161.4[f]	94.0	157.3[e]	103.8	124.2[g]	128.3	115.3	162.4[f]	115.3	128.3
	II	164.2[a]	103.2[b]	182.0[c]	153.1[d]	124.7[g]	157.3[e]	94.6	153.8[d]	104.1	122.8	128.3	114.6	163.1[f]	114.6	128.3
Isocryptomerin	I	163.0[a]	104.0[b]	181.7[c]	160.5[f]	98.9	161.4[f]	94.0	157.3[e]	103.8	124.9[g]	128.4[h]	115.1	162.2	115.1	128.4[h]
Isocryptomerin	II	164.4[a]	102.8[b]	182.1[c]	152.3[d]	124.4[g]	158.1[e]	92.0	154.1[d]	105.2	121.0	128.6[h]	116.0	161.4	116.0	128.6[h]
Naringenin*		78.4	42.0	196.2	163.6	95.9	166.7	95.0	162.9	101.8	128.9	128.2	115.2	157.8	115.2	128.2
2,3-Dihydro-	I	78.1	42.0	196.0	163.4	95.9[b]	166.6	95.0[b]	162.8[a]	101.7	131.9	128.2[e]	114.6[f]	157.9[d]	114.6[f]	128.2[e]
hinokiflavone (12)	II	164.1	102.6	182.0	153.2[c]	125.1	157.4[d]	94.5	153.6[c]	104.1	121.1	128.5[e]	116.0[f]	161.2[a]	116.0[f]	128.5[e]

*Spectrum from ref. [4].
[a,b,c,d,e,f,g,h]Assignments bearing the same superscript in any one spectrum may be reversed.



9 Hinokiflavone R¹ = R² = H
10 Cryptomerin A R¹ = H, R² = Me
11 Isocryptomerin R¹ = Me, R² = H
12 2,3-Dihydrohinokiflavone R¹ = R² = H, 2,3-dihydro

Hinokiflavone series—general (Table 2)

To our knowledge, the ^{13}C NMR spectrum of hinokiflavone (9) has not been published previously. In general terms, the assignments follow logically from those for apigenin, only the signals relating to the A-ring carbons of flavonoid II requiring the application of established substituent effect rules [7] for their assignment. The spectrum of naringenin was used as a reference for assigning resonances in flavonoid I of 2,3-dihydrohinokiflavone (12).

Hinokiflavone series—substituent effects

The key effects noted in the data in Table 2 which identify the hinokiflavone skeleton, as expected, result from the *O*-linkage of flavonoid I to flavonoid II. The effect of this linkage on the resonances of flavonoid I is small whereas the effects on flavonoid II are major and distinctive: hinokiflavone (and 2,3-dihydrohinokiflavone); II-6″ +26 ppm, II-7″ −6 ppm, II-5″ −8.5 ppm.

Substituent effects indicative of the site of *O*-methylation in hinokiflavone methyl ethers are best observed at the carbon atoms *ortho*- and *para*- to the site

of methylation. The effects listed below are each calculated from only one example. II-4‴-*O*-methylation; II-1‴ + 1.7 ppm, II-3‴, 5‴ − 1.4 ppm, II-7″-*O*-methylation; II-10″ + 1.1 ppm, II-8″ − 2.6 ppm.

The correlations detailed above are clearly of diagnostic value in the recognition of new biflavones in the amentoflavone and hinokiflavone series. Further, these data provide useful guides for the location of substituents on the parent molecules.

EXPERIMENTAL

Sources of compounds. Amentoflavone (**1**) and Podocarpusflavone-A (**3**) ex *Sequoiadendron giganteum* (Lindb.) Buchholz. see ref. [8]. Cryptomerin-A (**10**), Isocryptomerin (**11**) and Bilobetin (**2**) ex *Taxodium distichum*. Rich. see ref. [9]. Ginkgetin (**5**) and Isoginkgetin (**4**) ex *Ginkgo biloba*, see ref. [10]. Hinokiflavone (**9**) and 2,3-Dihydrohinokiflavone (**12**) ex *Metasequoia glyptostroboides*. Hu et Ching. see ref. [11]. Dihydroamentoflavone (**7**) ex *Cycas revoluta*. Thumb. see ref. [12]. Robustaflavone (**8**) prepared by Wessely–Moser rearrangement of amentoflavone with constant boiling HBr. Amentoflavone-7,7″,4′,4‴-tetramethyl ether (**6**) prepared by partial demethylation of amentoflavone hexamethyl ether with dry $AlCl_3$ in nitrobenzene.

Proton noise decoupled spectra were recorded at 20 MHz on a Varian FT80A spectrometer. Spectra of 14–20 mg samples in DMSO-d_6 were measured in 5 mm tubes at 30° using acquisition times of *ca* 0.8 sec. Protonated carbon signals in the spectrum of robustaflavone were identified using the DEPT pulse sequence [13] (on a Bruker AM400 spectrometer), and the GASPE technique was used to identify multiplicities in the spectrum of isoginkgetin (on the FT80A spectrometer).

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