^{1}H NMR Assignments in Biflavonoid Spectra by Proton-Detected C-H Correlation

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Dedicated to Professor J. B. Harborne on the occasion of his 65th birthday

Biflavonoids, ^{1}H NMR, ^{13}C NMR, Proton-Detected C-H Correlation

The ^{1}H and ^{13}C NMR spectra of some representative biflavonoids have been reinvestigated by proton-detected C-H correlation. Some assignments made in earlier publications are revised. Rules for the influence of one flavonoid moiety upon the chemical shift of the other's protons are given.

Introduction

The structure of biflavonoids can be deduced in most cases from their ^{13}C and ^{1}H NMR spectra, if completely assigned spectra of the constituent monoflavones are available. A full assignment of the biflavonoid spectra however can rarely by made without a correlation of the ^{1}H and ^{13}C spectra and with small samples this can often be achieved in a reasonable period of time only by means of the new "inverse" *i.e.* proton detected C-H correlation techniques. With the recent discovery of triflavonoids in several mosses and the need to establish their structures it became important to have a set of fully assigned biflavonoid spectra. For this reason the ^{1}H and ^{13}C NMR spectra of some representative biflavonoids were reinvestigated using the "inverse" method.

Most "inverse" experiments in the literature [1] have utilized the HMQC (Heteronuclear Multiple Quantum Coherence) pulse sequence [2, 3] in which the ^{13}C heteronuclei are indirectly detected in the second time dimension (t_1) *via* zero- and double quantum coherences. An alternative "Overbodenhausen" experiment [4] (HSQC, for Heteronuclear Single Quantum Coherence) incorporating a double INEPT type sequence relies on indirect detection *via* ^{13}C single quantum coherence and offers potentially superior resolution. Comparisons of "inverse" techniques are available [5, 6], however in general a disadvantage of HSQC is that it requires more pulses than HMQC and is consequently more prone to artifacts, pulse missetting and pulse imperfections. In the present study the HMQC sequence was used throughout. Compared to the INEPT based ^{13}C detected conventional 2D sequence [7], "inverse" shift correlated 2D spectra can be acquired about 60 times faster [7].

Common usage of trivial and semisystematic names has led in the field of biflavonoids to numberings that are somewhat ambigous. Therefore all compounds in the present study, whose NMR data are given in the tables are also represented by structural formulae on which the numbering is indicated. Ambiguities are found especially in the numbering of a **B**-ring that bears an interflavonyl linkage (*e.g.* **1b, 1c, 2b, 2c**) where in some cases it differs from the numbering used for die **B**-ring of the corresponding monoflavone. Most of the ^{13}C and ^{1}H NMR data of monoflavonoids referred to in this text can be found loc. cit. [8] and [9] respectively.

Results and Discussion

The correlated ^{13}C and ^{1}H NMR data derived from "inverse" studies of biflavones with an interflavonyl linkage between the rings **IB** and **IIA** are

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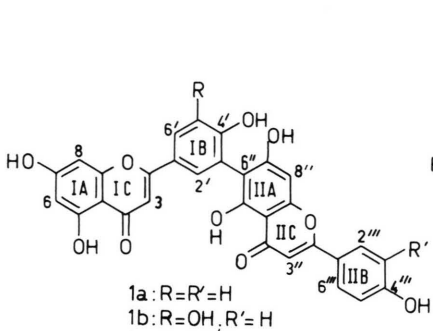
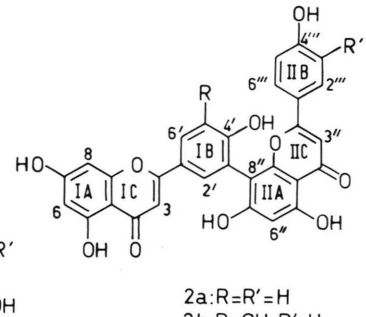
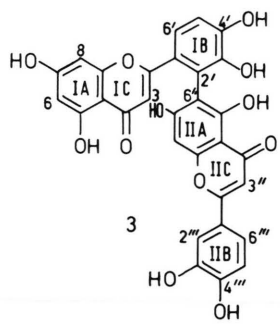
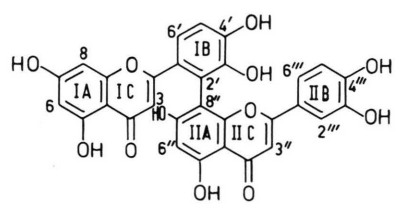








listed in Table I. It can be seen from the formulae (or better from stereo models) of these compounds that, due to steric hindrance, the preferred orientation of the rings **IB** and **IIA** is perpendicular to each other. Therefore, if as with **2a, 2b, 2c** and **4,** **IB** is linked to the 8-position of **IIA,** the protons of the freely rotating **IIB**-ring come into the shielding zone of **IB.** A **IB**-ring linked to the 6-position of **IIA** however cannot affect the **IIB** protons. This is the case with **1a, 1b, 1c** and **3,** whose **IIB**-ring protons show almost identical chemical shifts to those of the **B**-ring protons of the corresponding monoflavones. In the spectra of the **IIA**-8 linked biflavones however, the **IIB**-ring protons are shifted upfield by about 0.2–0.5 ppm (*cf.* also [9]). In contrast to this effect on the proton resonances the respective carbon signals are virtually unaffected.

Further shielding effects can be expected with biflavonoids like **3** and **4,** that have their **IIA**-ring linked to the 2′-position of **IB.** In this case the protons at **I**-3 and **I**-8 come into the shielding zone of **IIA** on rotation about the **IC-IB** bond. Thus in **3** and **4** the H-3 resonances appear at 0.6 ppm higher field than in **1c** and **2c.** The H-8 signals of **3** and **4** are shifted upfield by 0.4 and 0.6 ppm respectively relative to those in **1c** and **2c.** The larger shift in the case of **4** can be explained by a contribution of the **IIB**-ring to the overall shielding. With **3,** H-8 cannot come into the shielding zone of its **IIB**-ring.

In the ^{13}C NMR spectra of **1a–c** and **2a–c** the signals of C-3 and C-3″ are both found at about 103 ppm, whereas in the spectra of **3** and **4** only the resonances of C-3″ appear around 103 ppm. The resonances of C-3 are seen near 106 ppm, because the interflavonyl-linkage at C-2′ prevents coplanarity of the rings **IC** and **IB** thus interfering with the resonance between these two rings. Similar effects have been observed in the spectrum of hegoflavone [10].

The first compound in Table II, anhydrobartramiaflavone (**5**), is derived from philonotisflavone (**4**) by the addition of a second interflavonyl bond between C-2‴ and C-8, which produces a symmetrical macrocyclic molecule. In this compound the **B**-rings are fixed almost perpendicular to their

Table I. Correlated ^{13}C and ^{1}H NMR data of biflavones with an interflavonyl linkage between the rings **I B** and **II A.**

C/H	Robustaflavone (**1a**)		5′-Hydroxyrobusta-flavone (**1b**)		5′,3‴-Dihydroxyrobusta-flavone (**1c**)		Dicranolomin (**3**)	
3	103.2*	6.79 s*	102.7	6.59 s*	102.8*	6.68 s*	106.2	6.04 s
6	99.2	6.19 d_m	98.8	6.17 d_m	98.7	6.19 d_m	98.6	6.08 d_m^+
8	94.4	6.47 d_m	93.8	6.42 d_m	93.8	6.43 d_m	93.3	5.97 d_m^+
2′	131.2$^+$	7.77 d_m	122.3	7.34 d_m	121.9	7.27 d_m	–	–
5′	116.5	7.04 d_o	–	–	–	–	114.6	6.94 d_o
6′	127.6$^+$	7.90 dd_{om}	112.0	7.37 d_m	111.7	7.40 d_m	120.5	7.17 d_o
3″	103.2*	6.75 s*	102.7	6.78 s*	102.7	6.60 s*	103.3	6.65 s
8″	93.6	6.63 s	93.6	6.60 s	93.7	6.61 s	93.1	6.53 s
2‴	128.6	7.95 d_o	128.4	7.94 d_o	113.3	7.43 d_m	113.3	7.40 d_m
3‴	116.4	6.95 d_o	115.9	6.94 d_o	–	–	–	–
5‴	116.4	6.95 d_o	115.9	6.94 d_o	116.0	6.92 d_o	116.0	6.90 d_o
6‴	128.6	7.95 d_o	128.4	7.94 d_o	119.0	7.44 dd_{om}	118.9	7.41 dd_{om}
C/H	Amentoflavone (**2a**)		5′-Hydroxyamento-flavone (**2b**)		5′,3‴-Dihydroxyamento-flavone (**2c**)		Philonotisflavone (**4**)	
3	103.2*	6.81 s*	102.9	6.66 s*	102.8*	6.66 s*	106.4	6.03 s
6	99.1	6.19 d_m	98.6	6.18 d_m	98.7	6.18 d_m	98.6	6.07 d_m^+
8	94.2	6.46 d_m	93.8	6.40 d_m	93.8	6.42 d_m	93.0	5.76 d_m^+
2′	131.6$^+$	7.99 d_m	122.3	7.53 d_m	122.2	7.47 d_m	–	–
5′	116.4	7.15 d_o	–	–	–	–	114.6	7.01 d_o
6′	127.9$^+$	8.00 dd_{om}	112.0	7.46 d_m	112.3	7.47 d_m	120.5	7.24 d_o
3″	102.8*	6.77 s*	102.4	6.74 s*	102.5*	6.62 s*	102.4	6.57 s
6″	98.8	6.40 s	98.8	6.36 s	98.6	6.38 s	98.3	6.27 s
2‴	128.3	7.57 d_o	128.2	7.58 d_o	113.7	7.07 d_m	113.5	6.89 d_m
3‴	116.0	6.72 d_o	115.7	6.70 d_o	–	–	–	–
5‴	116.0	6.72 d_o	115.7	6.70 d_o	115.5	6.67 d_o	115.5	6.74 d_o
6‴	128.3	7.57 d_o	128.2	7.58 d_o	118.6	7.04 dd_{om}	118.9	6.89 dd_{om}

* = assignments may be reversed.
$^+$ = assignments made previously by "best fit" had to be reversed.

Table II. Correlated ^{13}C and ^{1}H NMR data of biflavones with two interflavonyl linkages or with linkages between the rings **I A** and **II A** or **I B** or **II B**.

C/H	Anhydrobartramiaflavone (**5**)		Agathisflavone-7,7″-dimethylether (**6**) [13]		Cupressuflavone-7,7″-dimethylether (**7**) [13]		3′,3‴-Biapigenin (**8**)	
3	108.9	5.74 s	103.1*	6.93 s*	102.6	6.86 s	102.9	6.84 s
6	98.6	6.26 s	–	–	95.5	6.78 s	98.6	6.18 d_m
8	–	–	90.8	7.05 s	–	–	93.8	6.48 d_m
2′	–	–	128.0	7.53 d_o	127.9	7.46 d_o	129.7	7.88 d_m
3′	–	–	116.0	6.77 d_o	115.9	6.77 d_o	–	–
5′	113.9	6.80 d_o	116.0	6.77 d_o	115.9	6.77 d_o	116.1	7.07 d_o
6′	118.7	6.73 d_o	128.0	7.53 d_o	127.9	7.46 d_o	127.2	7.93 dd_{om}
3″	108.9	5.74 s	102.5*	6.82 s*	102.6	6.86 s	102.9	6.84 s
6″	98.6	6.26 s	95.5	6.64 s	95.5	6.78 s	98.6	6.18 d_m
8″	–	–	–	–	–	–	93.8	6.48 d_m
2‴	–	–	128.6	8.04 d_o	127.9	7.46 d_o	129.7	7.88 d_m
3‴	–	–	116.0	6.98 d_o	115.9	6.77 d_o	–	–
5‴	113.9	6.80 d_o	116.0	6.98 d_o	115.9	6.77 d_o	116.1	7.07 d_o
6‴	118.7	6.73 d_o	128.6	8.04 d_o	127.9	7.46 d_o	127.2	7.93 dd_{om}

* = assignments may be reversed.

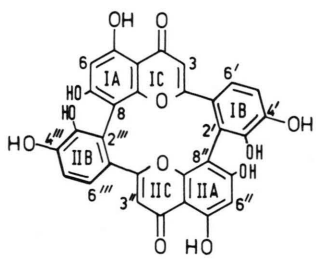
respective **C**-rings which causes a downfield shift of the C-3 resonances to 108.9 ppm. The H-3 signals in contrast are shifted upfield to 5.74 ppm by the shielding effect of the **A**-ring of the other flavone moiety. The overall highfield shift of the **B**-ring protons is presumably due to the mutual shielding of the two **B**-rings as well as to the elimination of resonance with the **C**-rings by virtue of their almost perpendicular orientation to the **A**- and **C**-rings.

Compounds **6** and **7** in Table II are characterized by an interflavonyl-linkage between the rings **IA** and **IIA.** Agathisflavone-7,7″-dimethylether (**6**) has its interflavonyl-linkage between C-6 and C-8″. The **IB**-ring protons are therefore not shielded by the **IIA**-ring, whereas its **IIB**-ring protons are shielded by the **IA**-ring to much the same extend as in **2a.**

The symmetrical cupressuflavone-7,7″-dimethylether (**7**) with its interflavonyl-linkage between C-8 and C-8″ has the protons of the **IB** and **IIB** rings shielded by the **IIA** and **IA** rings respectively.

3′,3‴-Biapigenin (**8**) is also symmetrical and its ^{1}H NMR spectrum is very close to that of the **I**-moiety of **1a.** The 0.1 ppm difference between the H-2′ resonances is not surprising, since the substituents at C-3′, the **IIB**-ring with **8** and the **IIA**-ring with **1a,** are quite different.

NMR spectral data for aulacomnium-biaureusidin (**9**), campylopusaurone (**10**) and hinokiflavone (**11**) are presented in Table III. The interflavonyl linkage of **9** and **10** is analogous to that of robustaflavone (**1a**) and its derivatives **1b,c;** thus the **IIB**-ring protons show no sign of shielding by the **IB**-rings. The structure of the biaureusidin **9** can in fact be deduced easily by comparing its ^{1}H and ^{13}C NMR spectra with those of aureusidin itself [11], the absence of the above shielding being of no structural importance. In the case of the aurone-flavanone biflavonoid **10** however the absence of shielding on the protons of the equatorially oriented **IIB**-ring by the **IB**-ring provides the essential logic for excluding the alternative 5′→8″ interflavonyl linkage, because with flavanones the chemical shifts of C-6 and C-8 are almost identical and therefore cannot be used to distinguish the 5′–6″ from the 5′–8″ interflavonoid linkages. 6- and 8-substitution in flavanones have been distinguished in the past by dehydrogenation of the flavanone to the corresponding flavone, although the possibility of a Wessely-Moser type rearrangement occurring during this reaction can never be excluded.



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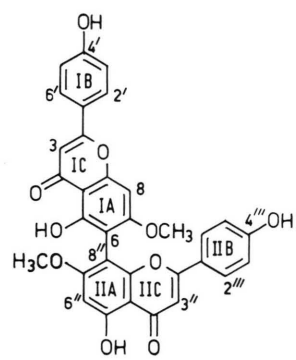

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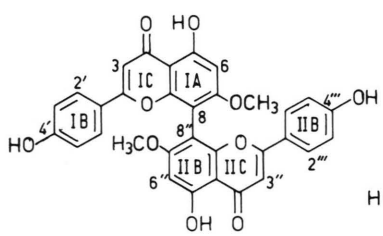

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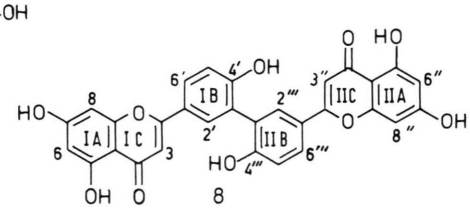

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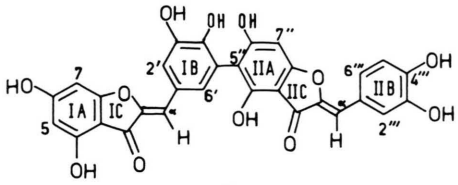

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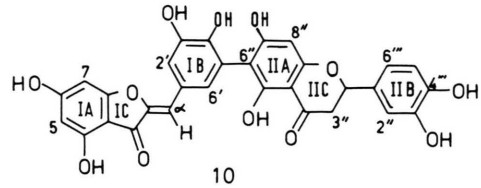

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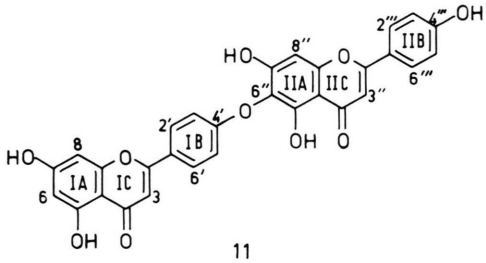

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Table III. Correlated ^{13}C and ^{1}H NMR data of two aureusidin based biflavonoids and an ether-linked biflavone.

C/H	Aulacomnium-Biaureusidin (**9**) [14]		C/H	Campylopusaurone (**10**) [11]		C/H	Hinokiflavone (**11**)	
α	109.9*	6.45 s*	α	109.9	6.45 s	3	102.6*	6.87 s*
5	97.6	6.06 d_m	5	97.6	6.06 d_m	6	98.9	6.21 d_m
7	90.2	6.15 d_m	7	90.2	6.14 d_m	8	94.0	6.50 d_m
2′	115.8	7.41 d_m	2′	115.9	7.42 d_m	2′/6′	128.2	8.03 d_o
6′	127.3	7.03 d_m	6′	127.4	6.98 d_m	3′/5′	115.3	7.04 d_o
α″	109.5*	6.50 s*	2″	78.4	5.42 dd	3″	103.9*	6.87 s*
5″	–	–	3″	42.2	3.21 dd/2.71 dd	6″	–	–
7″	90.5	6.36 s	8″	94.5	6.05 s	8″	94.6	6.74 s
2‴	117.5	7.44 d_m	2‴	114.3	6.91 s	2‴	128.5	7.98 d_o
3‴	–	–	3‴	–	–	3‴	116.0	6.95 d_o
5‴	115.8	6.83 d_o	5‴	115.3	6.77 s*	5‴	116.0	6.95 d_o
6‴	124.0	7.20 dd_{om}	6‴	117.9	6.77 s*	6‴	128.5	7.98 d_o

* = assignments may be reversed.

Hinokiflavone (**11**) is a diarylether type biflavonoid. Due to the wide separation of the two monoflavones by the 4′−6″ oxygen bridge, its ^{1}H and ^{13}C NMR data are very close of those of its constituent monomers. The corresponding 4′-O→8″ linked biapigenin, Lanaroflavone, has been discovered only recently and it was not available for a detailed study. Data in the literature [12] however are suggesting that there is a considerable interaction between the **I B**- and **II B**-rings.

Experimental

Most of the inverse, shift correlated 2D spectra were recorded on a Varian Unity 500 in DMSO-d_6 with a 5 mm inverse probe at 23 °C, using the

standard supplied "HMQC" pulse sequence with ^{13}C WALTZ decoupling during acquisition. Samples were typically 2–5 mg and run for at least 12 h each, although this was usually not necessary as an excellent signal to noise ratio was normally observed.

For each HMQC spectrum, a total of 512 FID's, each of 2k complex data points were collected in "hypercomplex" mode and zero filled twice in t_1 before a phase sensitive 2D fourier transform with non-shifted gaussian weighting in both dimensions was carried out.

Sources of compounds

Robustaflavone (**1a**) was prepared by Wessely-Moser rearrangement of **2a** [15], anhydrobartramiaflavone (**5**) by acid-treatment of bartramiaflavone [16], and 3′,3‴-biapigenin (**8**) by dehydrogenation of 3′,3‴-binaringenin [17]. All other compounds have been isolated from plants: **1b** and **2b** from *Rhytidiadelphus squarrosus* [18], **2a** from *Sequoiadendron giganteum* [19], **1c** and **2c** from *Racomitrium lanuginosum* [20], **3** and **4** from *Bartramia pomiformis* [21], **6** and **7** from *Agathis australis* [13], **9** from *Aulacomnium palustre* [14], **10** from *Campylopus clavatus* [11], and **11** from *Metasequoia glyptostroboides* [22].

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