The ^{1}H-NMR Spectra of the Biflavones Isocryptomerin and Cryptomerin B – a Critical Comment on Two Recent Publications on the Biflavone Patterns of *Selaginella selaginoides* and *S. denticulata*

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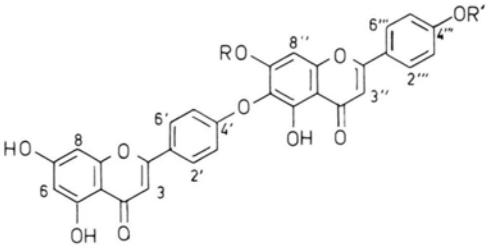
Two recent reports from another laboratory on hinokiflavone and robustaflavone in *S. selaginoides*, and hinokiflavone, isocryptomerin, cryptomerin B and amentoflavone 7-methylether in *S. denticulata* are shown to be based on ^{1}H-NMR data that are inconsistent with the biflavone structures proposed. In the current paper a comparison of the ^{1}H-nmr spectra is made both with published data and with new data derived from authentic samples. It is concluded that in both reports, only for amentoflavone the NMR data provide convincing supportive evidence. These reports are considered therefore to merely confirm an earlier identification of amentoflavone in these species.

Two consecutive papers on the biflavonoids of two *Selaginella spp* appeared in 1994 in this journal (López-Sáez *et al.*, 1994 a, b). Scrutiny of the ^{1}H-NMR data, provided in support of the biflavone structures (López-Sáez *et al.*, 1994 b) has revealed a number of inconsistencies which cast doubt upon the relationship between most of the spectra and the proposed constituents.

In the ^{1}H-NMR spectra of hinokiflavone (**1**) and its mono- and dimethylethers isocryptomerin (**2**) and cryptomerin B (**3**), signals for H-2’, 3’, 5’ and 6’ are described as exhibiting coupling patterns as follows: H-2’, doublet $J = 2$ Hz; H-3’, doublet $J = 9$ Hz; H-5’, doublet $J = 9$ Hz; and H-6’, doubledoublet, $J = 2$ and 9 Hz. These are not only incompatible with the proposed structures, but are also incompatible with any single structure since each compound exhibits an **odd** number of *ortho*-couplings, *i. e.* one *ortho*-coupled proton has no coupled partner. Further, the differences in the couplings of H-2’ and H-6’ (protons which are equivalent in a symmetrically substituted B-ring) are inexplicible. It seems likely that the spectra of mixtures have been interpreted as those of single compounds. Data for **1** – **3** obtained from authentic compounds are presented in Table I (the values of **1** are taken from Geiger *et al.* (1993), and the spectra of **2** and **3** were newly recorded on samples that had been isolated earlier from *Taxodium distichum* (Geiger and De Groot-Pfleiderer, 1973)). Signal assignments were confirmed, by cross-correlation of the ^{1}H and ^{13}C-NMR spectra. A comparison of the ^{1}H-NMR data with those in reference 2 reveals a number of chemical shift discrepancies as well as the discrepancies in the coupling pattern.



Hinokiflavone (**1**): R = R′ = H
Isocryptomerin (**2**): R = CH_3; R′ = H
Cryptomerin B (**3**): R = R′ = CH_3

The ^{1}H NMR spectrum presented in López-Sáez *et al.* (1994 b) for amentoflavone-7-methylether (the name sotetsuflavone should be avoided, because it has been used in different senses, see Ilyas *et al.*, 1978 and refs. quoted therein) is, except for a methoxyl-signal at 3.90 ppm, identical with that presented for amentoflavone. Curiously the H-6 and H-8 signals in the former spectrum do not show the relative downfield shift that would be expected from the 7-O-methylation (Markham and Geiger, 1994) (see also Table I). This biflavone is therefore most likely amentoflavone, with the signal at 3.90 ppm being produced by a contaminant. The ^{1}H NMR data cited for robustaflavone are close to, but not identical with the data presented by Geiger *et al.* (1993), thus the identity of this compound is also not beyond doubt. Only the ^{1}H-NMR data of amentoflavone are within the limits of experimental error identical with those in Geiger *et al.* (1993).

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Table I. Correlated ^{13}C and ^{1}H NMR data of the biflavones **1–3,** recorded in DMSO-d_6 at ambient temperature by the "inverse" technique at 500 MHz.**

C/H	Hinokiflavone (**1**) (Geiger *et al.*, 1993)		Isocryptomerin **2**)		Cryptomerin B (**3**)	
	^{13}C	^{1}H	^{13}C	^{1}H	^{13}C	^{1}H
3	103.9*	6.87 s*	103.8	6.84 s*	103.8*	6.85 s*
6	98.9	6.21 d^{m}	98.9	6.19 d^{m}	98.7	6.19 d^{m}
8	94.0	6.50 d^{m}	94.0	6.47 d^{m}	93.8	6.48 d^{m}
2′/6′	128.2	8.03 d^{o}	128.6	8.00 d^{o}	128.2	8.00 d^{o}
3′/5′	115.3	7.04 d^{o}	115.2	7.02 d^{o}	114.9	7.03 d^{o}
3″	102.6*	6.87 s*	102.9*	6.92 s*	103.3*	7.02 s*
8″	94.6	6.74 s	92.0	7.10 s	92.0	7.15 s
2‴/6‴	128.5	7.98 d^{o}	128.4	8.01 d^{o}	128.2	8.11 d^{o}
3‴/5‴	116.0	6.95 d^{o}	116.0	6.94 d^{o}	114.4	7.15 d^{o}
7″OMe	–	–	56.6	3.88 s	56.6	3.89 s
4‴OMe	–	–	–	–	55.3	3.86 s

* Assignments might be reversed.

** d^{m} = *meta*-coupled doublet; d^{o} = *ortho*-coupled doublet; s = singlet.

On the basis of the critical comment outlined above, it is concluded that the identification of amentoflavone only is supported by the data described by Geiger *et al.* (1993) and that isocryptomerin, cryptomerin B, amentoflavone 7-methylether and possibly robustaflavone have been misidentified. These two reports therefore merely confirm an earlier identification (Voirin, 1972) of amentoflavone in the two species referred to. (Note that in the publication of Voirin (1972) the synonymous name *S. spinulosa* A. Br. is used instead of *S. selaginoides*!).

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