Chamaecyparin – a Rare Biflavone from *Selaginella* Species

Barbara Meurer-Grimes[a,*], Jin Yu[a] and Iván A. Valdespino Q.[b,*]

[a] Amrad Discovery Technologies Pty. Ltd., Natural Products Chemistry, 576 Swan Street, Richmond, Victoria 3121, Australia. Fax: +61 3 9208 4126. E-mail: bmeurer-grimes@amrad.com.au

[b] Unidad de Manejo y Conservacion de la Biodiversidad, CATIE 7170, Turrialba, Costa Rica. Fax: +506 556 1533. E-mail: valdespi@catie.ac.cr

* Authors for correspondence and reprint requests

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Species of the genus *Selaginella* (Selaginellaceae) are reported to contain the rare 7,7″-dimethylether of hinokiflavone, chamaecyparin. The compound was identified by ^{1}H NMR spectroscopy, ESI-MS and ESI-MS/MS, and compared with data published in the recent literature.

In 1994, the structures of biflavones from the genus *Selaginella* and their occurrence in several species of the genus were reported in this journal by López-Saéz *et al.* (1994 a, b). In 1995, we received several of the compounds isolated by these authors and used them as internal standards for a chemosystematic survey of biflavones in *Selaginella* sect. Heterostachys (Valdespino, 1995; Valdespino and Meurer-Grimes, unpubl.). In 1996, Geiger and Markham reevaluated the data reported by López-Saéz *et al.*, and came to the conclusion that the biflavones isolated from *Selaginella* species were probably misidentified. Having been made aware of the problems concerning the structural identification of these isolates, we obtained ^{1}H-NMR spectra and mass spectra of the compounds that were provided to us in 1995. We report here the identification of a rare biflavone, chamaecyparin, from several *Selaginella* species.

The ^{1}H-NMR spectrum was taken in DMSO-d_6 on a 400 MHz Varian INOVA instrument at ambient temperature. Mass spectra were taken on a Finnigan LCQ ion trap mass spectrometer, via direct infusion in methanol (10 µl/min) in the negative ion mode.

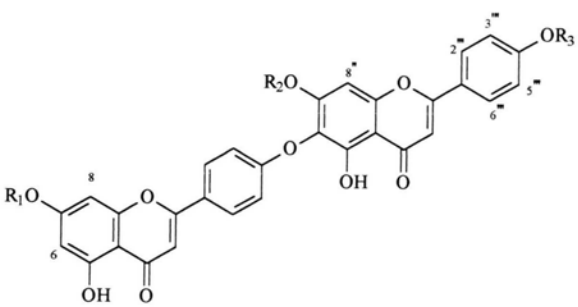
The ^{1}H NMR spectrum and the mass spectrum of the compound clearly show that this isolate represents a single pure compound. The mass spectrum showed a single signal at *m/z* 565 [M – 1]$^-$, indicating a molecular weight of MW = 566. This is the molecular weight expected for a dimethylether of hinokiflavone, such as cryptomerin B – the name under which this isolate was originally provided.

However, the chemical shifts of compound **1** (Table I) deviate from the ones reported for cryptomerin B: The signals for H-6 and H-8 are shifted downfield by 0.2 and 0.3 ppm, respectively, indicating methoxylation of the 7-position as shown in Figure 1. Isocryptomerin, another biflavone with a

Table I. ^{1}H NMR chemical shifts for chamaecyparin and comprison with reported ^{1}H NMR chemical shifts for isocryptomerin and cryptomerin B.

H	Chamaecyparin δ (ppm)	J (Hz)	Cryptomerin B**	Isocryptomerin**
3	6.92* s, 1H		6.85 s	6.84 s
6	6.39 d, 1H	2.2	6.19 d	6.19 d
8	6.77 d, 1H	2.2	6.48 d	6.47 d
2′/6′	8.01 d, 2H	9.0	8.00 d	8.00 d
3′/5′	7.04 d, 2H	9.0	7.03 d	7.02 d
3″	6.93* s, 1H		7.02 s	6.92 d
8″	7.12 s, 1H		7.15 s	7.10 d
2‴/6‴	8.03 d, 2H	9.2	8.11 d	8.01 d
3‴/5‴	6.95 d, 2H	9.2	7.15 d	6.94 d
-OCH_3	3.86 s, 3H		3.86 s	3.88 s
	3.89 s, 3H		3.89 s	

* Interchangeable signals.
** Geiger H. and Markham K. R. (1996).



Hinokiflavone, $R_1 = R_2 = R_3 = H$
Isocrytpomerin, $R_1 = H$, $R_2 = CH_3$, $R_3 = H$
Cryptomerin B, $R_1 = H$, $R_2 = R_3 = CH_3$
Chamaecyparin, $R_1 = R_2 = CH_3$, $R_3 = H$

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free 7-OH group shows the same chemical shifts and coupling patterns for H-6 and H-8 as cryptomerin B. The signals for H-2‴, H-3‴, H-5‴, and H-6‴ are shifted upfield by 0.08 and 0.2 ppm, respectively, and show exactly the same chemical shifts as observed for isocryptomerin (Table I), e.g. a biflavone with a free –OH group in 4‴-position. Methoxylation of the 4‴-position should result in a downfield shift of the signals in the aromatic ring system, as is indeed observed in the ^{1}H NMR spectrum for authentic cryptomerin B. Therefore, the isolate is most likely not methoxylated in 4‴-position. The coupling patterns, chemical shifts and the molecular weight are in accordance with the assumption that this compound is the 7,7″-dimethylether of hinokiflavone, also known as chamaecyparin.

The 7,7″-methylether of hinokiflavone was first obtained via synthesis (Miura and Kawano, 1968). Its occurrence in leaves of *Chamaecyparis pisifera* var. *squarrosa* and *C. obtusa* var. *breviramea* was demonstrated by co-chromatography using TLC, and the compound was subsequently named chamaecyparin (Miura *et al.*, 1968). The authors obtained ^{1}H NMR data from the acetylated derivative of the compound, which thus cannot be compared directly to data reported here and in the more recent literature.

The compound provided by J. López-Saéz was originally isolated from *Selaginella denticulata* (1994 b), and reported to be cryptomerin B. This interpretation was challenged by Geiger and Markham (1996). A comparison of our ^{1}H NMR data with the NMR data reported by López-Saéz *et al.* (1994 b) indicates that the compound provided to us could perhaps be the same isolate. López-Saéz *et al.* (1994 b) report chemical shifts of 6.32 (H-6), 6.71 (H-8), 6.95 (H-3‴ and H-5‴), and 7.94 (H-2‴ and H-6‴) for a compound that they called cryptomerin B. However, there are still problems with the coupling patterns that they report for the B-ring. We have shown here that this isolate is chamaecyparin instead. Two other biflavones provided to us were identified as isocryptomerin and robustaflavone using the same methods and comparison with data reported in the literature (Geiger *et al.*, 1993).

We found chamaecyparin in further three species of *Selaginella*, *S. jungermannioides* (Gaud.) Spring, *S. diffusa* (C. Presl) Spring, and *S. stellata* Spring. The compound was identified by co-chromatography using HPLC/UV (Meurer-Grimes and Stevenson, 1994; Valdespino and Meurer-Grimes, unpubl.). This is the first time that the 7,7″-dimethylether of hinokiflavone has been found in a pteridophyte.

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