Biflavonoids of *Selaginella denticulata* Growing in Spain

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From *Selaginella denticulata* the following seven biflavones were isolated: amentoflavone, 7,4′,7″,4‴-tetra-O-methylamentoflavone, cryptomerin B, hinokiflavone, isocryptomerin, sotetsuflavone (7-O-methylamentoflavone) and robustaflavone.

The flavonoids and related phenolic compounds have received most attention in fern systematics (Voirin and Jay, 1978; Cooper-Driver, 1980; Cooper-Driver and Haufler, 1983). Selaginellaceae, Psilotaceae, Lycopodiaceae and Isoetaceae are considered the more primitive taxa of Pteridophyta by its flavonoid composition (Cooper-Driver and Haufler, 1983). The first report of flavonoids in *Selaginella* was given by Harada and Saiki (1955) in *S. tamariscina* and *S. pachystachys*. Kariyone (1962a) reported for the first time the presence of biflavonoids in *S. tamariscina*. At present the data reported by a number of workers indicated that the only flavonoid type accumulated by 24

Table I. Distribution of biflavonoids in *Selaginella* spp.

Species	Compound											References
	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	
S. bellula	+		+									Voirin (1972)
S. canaliculata	+		+									Voirin (1972)
S. cuspidata	+		+									Voirin (1972)
S. delicatula	+		+									Voirin (1972)
S. denticulata	+		+									Voirin (1972)
S. doederlenii	+	+			+							Lee *et al.* (1922b)
S. erythropus	+		+									Voirin (1972)
S. galeottii	+		+									Voirin (1972)
S. gracillima	+		+									Voirin (1972)
S. helvetica	+		+									Voirin (1972)
S. kraussiana	+		+			+	+					Voirin (1972); Qasim *et al.* (1985)
S. lepidophylla	+	+	+	+	+	+	+	+	+	+		Qasim *et al.* (1985)
S. martensii	+		+									Voirin (1972)
S. mutabilis	+		+									Voirin (1972)
S. nipponica	+		+	+								Okigawa *et al.* (1971)
S. pachystachys	+		+	+								Okigawa *et al.* (1971)
S. rupestris	+											Chakravarthy *et al.* (1981)
S. sanguinolenta	+											Huneck and Khaidav (1985)
S. selaginoides	+					+				+		López-Sáez *et al.* (1994)
S. spinulosa	+		+									Voirin (1972)
S. tamariscina	+		+			+	+				+	Hsu (1959); Kariyone (1962b); Miura and Kawano (1967); Nakazawa (1968); Okigawa *et al.* (1971); Shin and Kim (1991); Lee *et al.* (1992a)
S. uncinata	+		+									Voirin (1972)
S. usta	+		+									Voirin (1972)
S. watsoniana	+		+									Voirin (1972)

Compounds: **1,** amentoflavone; **2,** heveaflavone; **3,** sotetsuflavone; **4,** dimethylether of amentoflavone; **5,** tetramethylether of amentoflavone; **6,** hinokiflavone; **7,** isocryptomerin; **8,** dimethylether of hinokiflavone; **9,** trimethylether of hinokiflavone; **10,** robustaflavone; **11,** cryptomerin B.

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Selaginella species were biflavones (Table I). The genus is characterized by the presence of amentoflavone as the major biflavonoid (Voirin, 1972; Geiger and Quinn, 1988). The hinokiflavone series has been reported from *S. tamariscina* (Miura and Kawano, 1967); Nakazawa, 1968; Okigawa *et al.*, 1971), *S. kraussiana* and *S. lepidophylla* (Qasim *et al.*, 1985). Further studies (Chakravarthy *et al.*, 1981; Huneck and Khaidav, 1985; Shin and Kim, 1991; Lee *et al.*, 1992b) confirmed that these reports were only sporadic occurrence in the genus. The isolation of robustaflavone from *S. lepidophylla* (Qasim *et al.*, 1985) was the first report of this compound outside the seed plants.

In the present work we have investigated the biflavonoid composition of *S. denticulata* gathered in Spain. The only report of biflavonoids in this species was given by Voirin (1972) who mentioned the presence of amentoflavone and tentatinely sotetsuflavone.

Experimental

Plant material

Two samples of *Selaginella denticulata* (L.) Spring were collected from Natural Park of Montfragüe, Cáceres (Spain) and Villa del Prado, Ma-

Table II. Chromatographic and UV data of biflavonoids **4** and **7** from *Selaginella denticulata*.

Compound	**4**	Sotetsuflavone[1]	Sotetsuflavone[2]	**7**	TMEA[3]
Colour reactions					
UV (350 nm)	deep purple	deep purple	deep purple	purple	purple
NH_3	dark	–	–	dark	–
NA[4]	yellow	yellow	–	yellow-orange	–
TLC hRf values					
Sorbens: Silica Gel					
BPF[5] (36:9:5)	41	45	37	88	90
BPEFD[6] (5:1:2:2)	46	48	52	81	85
BEAA[7] (10:3:2)	43	44	–	85	87
BEAA[7] (8:5:2)	50	–	48	89	88
TEFF[8] (5:4:1)	55	–	53	67	63
TPA[9] (10:1:1)	13	–	16	70	66
UV Data					
MeOH	272, 290 sh 339	274, 288 sh 334	–	248 sh, 300 sh 329	246 sh, 272 328
NaOMe	269, 297 sh 387	276, 296 sh 384	–	248 sh, 298 sh 328	246 sh, 273 326
$AlCl_3$	281, 300 357, 389	280, 304 352, 390	–	249 sh, 275 305, 350, 393	248 sh, 281 301, 348, 388
$AlCl_3$/HCl	277, 299 357, 388	278, 302 352, 390	–	249 sh, 273 304, 343, 391	248 sh, 276 300, 340, 385
NaOAc	277, 321 sh 373	276, 319 sh 370	–	247 sh, 275 335	246 sh, 277 332
NaOAc/H_3BO_3	277, 330	276, 328	–	247 sh, 270 330	247 sh, 272 328

[1] From Dossaji *et al.* (1975).
[2] From Chexal *et al.* (1970).
[3] 7,4′,7″,4‴-tetra-O-methylamentoflavone (TMEA) from Chexal *et al.* (1970) and Dossaji *et al.* (1975).
[4] NA = Naturstoffreagenz A.
[5] BPF = Benzene-pyridine-formic acid.
[6] BPEED = Benzene-pyridine-ethyl formate-dioxane.
[7] BEAA = Benzene-ethyl acetate-acetic acid.
[8] TEFF = Toluene-ethyl formate-formic acid.
[9] TPA = Toluene-pyridine-acetic acid.

drid (Spain). Different specimens have been deposited in the "M.A.C.B." Herbarium (Department of Plant Biology, Faculty of Biology, Complutense University of Madrid).

Isolation and identification of biflavonoids

The plant material from *S. denticulata* (225 g) was first extracted with 100% chloroform (4 l) at room temperature for 48 h to eliminate chlorophylls, and afterwards the leaves of *S. denticulata* were extracted with 80% methanol (5 l) for 48 and 76 h. The combined alcoholic extracts were concentrated to a syrup (10 ml) under reduced pressure and then dried on a waterbath (Geiger and Quinn, 1975). The residue was sequentially washed with hexane. This was chromatographed over silica gel column and eluted with C_6H_6–$MeCO_2$ mixture of increasing polarity (Chakravarthy *et al.*, 1981). Further separation and purification was done by CC on Sephadex LH 20 with Me_2CO : H_2O : MeOH (2:1:1). The main biflavonoids were isolated from methanolic extracts, purified and identified according to standard methods: TLC, UV spectra, MS, ^1H NMR and ^{13}C NMR (Chexal *et al.*, 1970; Mabry *et al.*, 1970; Dossaji *et al.*, 1975; Lin and Chen, 1975; Chari *et al.*, 1977; Markham *et al.*, 1987) (Tables II and III).

Yields: 90 mg of amentoflavone (**1**); 45 mg of robustaflavone (**2**); 50 mg of hinokiflavone (**3**); 27 mg of sotetsuflavone (**4**); 43 mg of isocryptomerin (**5**); 12 mg of cryptomerin B (**6**); and 18 mg of amentoflavone tetra-O-methylether (**7**).

The compounds were eluted from silica gel colum as follows: **7, 6, 6 + 5, 5, 4, 4 + 3, 3 + 1, 1 + 2, 2.**

^1H NMR spectroscopy: Bruker AM 400, 400 MHz, DMSO-d_6, ambient temperature.

^{13}C NMR spectroscopy: Bruker AM 400, 100 MHz, DMSO-d_6, ambient temperature.

Mass spectra were recorded by FAB-technique (negative mode) on a Hewlett Packard 5970 (70 eV).

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Table III. PMR-spectra of **1, 2, 3, 4, 5** and **6** (DMSO-d_6, ambient temperature, 400 MH_2). In parentheses the coupling constants in [Hz].

	1	**2**	**3**	**4**	**5**	**6**
H-3	6.72 s	6.77 s	6.77 s	6.72 s	6.77 s	6.75 s
H-6	6.19 d (2)	6.20 d (2)	6.19 d (2)	6.18 d (2)	6.18 d (2)	6.32 d (2)
H-8	6.47 d (2)	6.50 d (2)	6.47 br. s	6.45 d (2)	6.47 br. s	6.71 s
H-2′	8.04 d (2)	7.88 d (2)	7.99 d (2)	8.02 d (2)	7.96 d (2)	7.94 d (2)
H-3′	–	–	7.08 d (9)	–	7.07 d (9)	6.99 d (9)
H-5′	7.19 d (9)	7.09 d (9)	7.11 d (9)	7.15 d (9)	7.07 d (9)	6.99 d (9)
H-6′	7.98 dd (2; 9)	7.90 dd (2; 9)	7.98 dd (2; 9)	7.98 dd (2; 9)	7.96 dd (2; 9)	7.94 dd (2; 9)
H-3″	6.78 s	6.77 s	6.80 s	6.76 s	6.82 s	6.85 s
H-6″	6.41 s	–	–	6.41 s	–	–
H-8″	–	6.64 s	7.04 s	–	7.04 s	7.04 s
H-2‴	7.56 d (9)	7.95 d (9)	7.97 d (9)	7.54 d (9)	7.96 d (9)	7.94 d (9)
H-3‴	6.77 d (9)	7.01 d (9)	7.02 d (9)	6.75 d (9)	6.99 d (9)	6.95 d (9)
H-5‴	6.77 d (9)	7.01 d (9)	7.02 d (9)	6.75 d (9)	6.99 d (9)	6.95 d (9)
H-6‴	7.56 d (9)	7.95 d (9)	7.97 d (9)	7.54 d (9)	7.96 d (9)	7.94 d (9)
3H 7-OCH_3	–	–	–	3.90 s	–	–
3H 7″-OCH_3	–	–	–	–	3.88 s	3.83 s
3H 4‴-OCH_3	–	–	–	–	–	3.80 s

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