

The Biflavonoid Pattern of *Selaginella selaginoides*

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From *Selaginella selaginoides* the following three biflavonoids were isolated: amentoflavone, hinokiflavone and robustaflavone. The compounds were identified by means of spectroscopic methods.

As part of our research on the biflavonoid pattern of *Selaginellaceae* growing in the Iberian Peninsula, the methanol extract of *Selaginella selaginoides* was analyzed by two dimensional TLC (López-Sáez, 1992). Only one spot was detected with the chromatographic behaviour of amentoflavone (**1**). We used column chromatography on silica gel and Sephadex LH 20 (Chakravarthy *et al.*, 1981) for further separation and identification. Amentoflavone (**1**), hinokiflavone (**2**) and robustaflavone (**3**) were characterized. These compounds were identified by their spectroscopic data (^1H NMR, ^{13}C NMR, FAB-MS) (Chari *et al.*, 1977; Chakravarthy *et al.*, 1981; Markham *et al.*, 1987; Shin and Kim, 1991) and by TLC with authentic standards.

The genus *Selaginella* is characterized by the presence of amentoflavone as the major biflavonoid (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). The isolation of robustaflavone (**3**) from *S. selaginoides* is the second report of this com-

pound outside the seed plants, after its identification in *S. lepidophylla* (Qasim *et al.*, 1985).

The biflavonoid pattern of *Selaginella selaginoides* is studied here for the first time.

Experimental

Plant material

Sporophytic material of *Selaginella selaginoides* (L.) PB. ex Schrank & C.F.P. Mart. was collected in Borleña, Santander (Spain), 4. 11. 1991, leg. et det. J. A. López-Sáez. Voucher specimens are deposited in the Herbarium of the Department of Plant Biology, Faculty of Biology, Complutense University of Madrid ("MACB", No. 42188 and 42189).

Extraction and isolation

185 g thoroughly cleaned air-dried plant material was ground and defatted with CHCl_3 . Afterwards the biflavonoids were exhaustively extracted by 80% aq. methanol. To eliminate chlorophylls the combined extracts were evaporated and the residue subjected to a four step Craig distribution between the upper and lower phases of $\text{DMF}/\text{H}_2\text{O}/\text{Et}_2\text{O}$ (4:1:8). The combined lower phases were reduced *in vacuo* to a thin syrup (about 75 ml). This was chromatographed over silica gel column and eluted with C_6H_6 – Me_2CO mixture of increasing polarity. The compounds were eluted as follows: **2**, **2+1**, **1+3**, **3**. Further separation and purification was done by CC on Sephadex LH 20 with $\text{Me}_2\text{CO}:\text{H}_2\text{O}:\text{MeOH}$ (2:1:1).

Yields: 65 mg of **1**; 20 mg of **2**; 45 mg of **3**.

^1H NMR spectroscopy: Bruker AM 400, 400 MHz, $\text{DMSO}-d_6$, ambient temperature.

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

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

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