Contribution to the phylogeny of Philonotis Brid. (Bartramiaceae Schwaegr.): Flavonoids of sections Catenularia (C. Müll.) Par. and Euphilonotis Limpr.

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Abstract:

LÓPEZ-SÁEZ, J.A., Mª. JOSÉ PÉREZ-ALONSO & ARTURO VELASCO NEGUERUELA. 1996. Contribution to the phylogeny of Philonotis Brid. (Bartramiaceae Schwaegr.): Flavonoids of sections Catenularia (C. Müll.) Par. and Euphilonotis Limpr. *Bot. Complutensis* 21: 51-58.

Five biflavonoids, four biflavones (philonotisflavone, dicranolomin, 5',3'''-diOH-amentoflavone, 5',3'''-diOH-robustaflavone) and the flavone-flavanone dimer 2,3-dihydro-philonotisflavone were investigated from sects. *Catenularia* (C. Müll.) Par. and *Euphilonotis* Limpr. of *Philonotis* Brid. genus. Phylogenetic position of both sections is analysed according to their biflavonoid composition.

Key Words: *Philonotis, Bartramiaceae,* biflavonoids, chemosystematics.

Resumen:

LÓPEZ-SÁEZ, J.A., Mª. JOSÉ PÉREZ-ALONSO & ARTURO VELASCO NEGUERUELA. 1996. Contribución a la filogenia de Philonotis Brid. (Bartramiaceae Schwaegr.): Flavonoides de las secciones Catenularia (C. Müll.) Par. y Euphilonotis Limpr. *Bot. Complutensis* 21: 51-58.

Cinco biflavonoides, entre ellos cuatro biflavonas (filonotisflavona, dicranolomina, 5',3'''-diOH-amentoflavona y 5',3'''-diOH-robustaflavona) y el dímero flavona-flavanona 2,3-dihidro-filonotisflavona fueron identificados en especies de las secciones *Catenularia* (C. Müll.) Par. y *Euphilonotis* Limpr. del género *Philonotis* Brid. Se comenta la posición filogenética de ambas secciones de acuerdo a su composición biflavonoídica.

Palabras clave: *Philonotis, Bartramiaceae,* biflavonoides, quimiosistemática.

INTRODUCTION

The *Bartramiaceae* Schwaegr. is one of the most distinctive of moss families (GRIFFIN III & BUCK, 1989). In the last years many papers dealing with the identification of flavonoids including unusual macrocyclic derivatives from *Bartramiaceae* species have been published (GEIGER & BOKEL, 1989; GEIGER & al., 1995; LÓPEZ-SÁEZ, 1994; LÓPEZ-SÁEZ, & al., 1995; SALM & al., 1993; SEEGER & al., 1991, 1992, 1993). In addition, flavonoids proved to be significant chemical characters for chemotaxonomic studies (GEIGER, 1990; GEIGER & QUINN, 1988; GEIGER & al., 1988; LÓPEZ-SÁEZ, 1994; MARKHAM, 1990). For these reasons, a study of the presence of biflavonoids in selected members of the *Bartramiaceae* has been carried out, in order to study taxonomic and phylogenetic implications of comparative flavonoid chemistry of species in the sects. *Catenularia* (C. Müll.) Par. and *Euphilonotis* Limpr. of *Philonotis* Brid. genus.

MATERIAL STUDIED

Voucher specimens are deposited in the MACB Herbarium (Department of Plant Biology, Faculty of Biology, Complutense University of Madrid):

P. andina (Mitt.) Jaeg.: **COLOMBIA**: Cundinamarca, Páramo de Palacio, Lagunas de Buitrago, alt. 3600 m, *Antoine M. Cleef & T. van der Hammer* 4973. **PERU**: Department of Cajamarca, Quebrada Cavilan, alt. 3150 m, *E. Hegewald.*

P. australis (Mitt.) Par.: **MAURICE ISLAND**: Le Pouce, roches volcaniques ensoleillés, alt. 550 m, *M. Onraedt.*

P. caespitosa Jur.: **GERMANY**: Erlenbruuch bei Reimsbach am Wegrand, Kreis Merzig, 9.IV.1986, *R. Mües.* **SPAIN**: Guadalajara, Sierra del Bulejo, 17.III.1986, *Ayala, Herguido, Mazimpaka & Ron.*

P. calcarea (B. & S.) Schimp.: **BELGIUM**: prov. Luxembourg, Forges de Buzenol, au bord de l'eau, *M. Onraedt.* **NORWAY**: Nordland, env. Narvik sól au bord d' un lac, *M. Onraedt* 11687. **SPAIN**: Toledo, Castillo de Bayuela, 9.III.1982, *E. Fuertes.*

P. capillaris Lindb.: **NORWAY**: environs de Srjotli, Mocher suintant, alt. 1200 m, *M. Onraedt* 7647.

P. fontana (Hedw.) Brid.: **FRANCE**: Jura, Col de Rothenbach, rocher calcaire suintant, *M. Onraedt* 11900; Jura, Lac d'Ilcy, sol humide, *M. Onraedt* 10573. **SPAIN**: Segovia, subida al puerto de la Quesera, en hayedo, 19.IV.1992, *López-Sáez & Tapia Ariza*; Toledo, Navahermosa, fuentes del río Estena, 26.IX.1989, *Ron, Velasco, Jover & Buades.*

P. lancifolia C. Müll.: **SRI LANKA**: Nuwara tliya, Lover's Leaf Talls, alt. 1900 m, sur granit humide, *M. Onraedt* 2858.

P. marchica (Hedw.) Brid.: **BELGIUM**: prov. Luxembourg, Carlsbourg, alt. 440 m, rivière á eau limpide et froide, *M. Onraedt* 7650. **ICELAND**: W-Island, Snaefellness, Miklholtshreppur, 19.VIII.1987, *M. Neitzke*.

P. scabrifolia (Hook. f. & Wils.) Braithw.: **PERU**: Pasco, along road from Cerro de Pascoto Lima via Canta, north of Huayllay, km. 224.4 on soil, alt. 4160 m, *Vitt* 21734.

P. seriata Mitt.: **FRANCE**: Mont Aigual, 30.VII.1983, *R. Mües*. **SWITZERLAND**: Canton des Srisons, Col de la Bermica, pré marécagluse, alt. 2300 m, *M. Onraedt* 11580.

P. turneriana (Schwaegr.) Mitt.: **U.S.A.**: Hawaii, Oahu, Waianag Mount, Metrosideros-Cheirodendron moss forest, very wet roadside areas, alt. 4000 ft., 22.II.1975, *W.J. Hoe* 3350-O.

METHODS

Air-dried plant material (5-10 g by population) were extracted with MeOH:H_2O (8:2) and with Me_2CO:H_2O (8:2) at room temperature. To eliminate chlorophylls, the combined extracts were evaporated and the residue was subjected to a four step Craig distribution between the upper and lower phases of DMF:H_2O:Et_2O (4:1:8) (GEIGER & al. 1988, GEIGER, 1990). The combined lower phases were reduced *in vacuo* to a thin syrup (about 10 ml) which was stored at -20°C until further analysis.

The flavonoids were identified by co-chromatography with authentical standards recently isolated from *Bartramia ithyphylla* Brid. (LÓPEZ-SÁEZ & al., 1995), *Bartramia stricta* Brid. (GEIGER & al., 1995) and *Bartramia pomiformis* Hedw. (LÓPEZ-SÁEZ, 1994). Compounds were separated by TLC (GEIGER, 1990) and HPLC on a Nucleosil C-18 column (4 mm x 250 mm i.d., Hewlett Packard) at a flow rate of 1 ml min^{-1} using a linear gradient (5% acetic acid, 0 min: 30% methanol, 40 min: 70% methanol). The injection volume was 50 µl. All separations were monitored with a photodiode array detector at 22-440 nm (MEIER & STICHER, 1986). As the constituents are all known, their ^{1}H-NMR, ^{13}C-NMR and MS data have been published elsewhere (GEIGER & al. 1993; LÓPEZ-SÁEZ & al., 1995; MARKHAM & al., 1988; SEEGER & al., 1992).

RESULTS AND DISCUSSION

For this study we have looked particulary at selected characters. Those which we have used in developing our concepts of phylogeny in the *Bartramiaceae* included rhizoid ornamentation (HIROHAMA & IWATSUKI, 1980), stem anatomy (HABERLAND, 1886; KAWAI, 1982, 1989), spore ornamentation (GRIFFIN III &

ACUÑA, 1983; HIROHAMA, 1977), morfology of axillary hairs (GRIFFIN III & BUCK, 1989), and chromosome numbers (FRITSCH, 1982). The character that we found of most interest and use, and which have been ignored previously in the *Bartramiaceae*, is the flavonoid composition. Earlier work has established the flavonoid composition of *Philonotis fontana* (GEIGER & BOKEL, 1989).

Twenty samples representing 11 *Philonotis* species were analysed regarding their biflavonoid profiles (Figure 1). Comparison of populations within the same species revealed no significant differences in flavonoid composition. This is in line with observations in other mosses (FREITAG & al., 1986; LÓPEZ-SÁEZ, 1992, 1994; SIEGEL & al., 1989). The results as presented in Table 1 therefore refer only to the respective species name.

Table 1: *Distribution of flavonoids in 11 species of Philonotis. Concentration: +++ high, ++ middle, + low, - not detected. Numeration of compouds: **1** 2,3-dihydro-philonotisflavone; **2** 5',3'''-dihydroxi-amentoflavone; **3** 5',3'''-dihydroxi-robustaflavone; **4** philonotisflavone; **5** dicranolomin*

Taxon	Flavonoid Number				
	1	2	3	4	5
section *Euphilonotis*					
P. andina	+	+	-	++	+
P. australis	+	+	-	++	+
P. caespitosa	++	++	+	+	+
P. calcarea	+	+	+	+	+
P. capillaris	+	+	+	+++	+
P. fontana	+	+	+	+++	+
P. lancifolia	+	++	+	++	-
P. marchica	+	-	-	+	+
P. seriata	+	-	-	-	-
P. turneriana	+	+	+	++	-
section *Catenularia*					
P. scabrifolia	++	+	-	-	-

The genus *Philonotis* is mainly characterized by the presence of biflavones, particulary biluteolins and 2,3-dihydrobiluteolins Among the isolated compounds were the biflavones 5',3"-dihydroxi-amentoflavone (5',8"-biluteolin); 5',3'''-dihydroxi-robustaflavone (5',6"-biluteolin); dicranolomin (2',6"-biluteolin); philonotisflavone (2',8"-biluteolin) and the flavanone-flavone 2,3-dihydro-philonotisflavone (eriodyctiol-(2',8")-luteolin) (Figure 2). While the biflavones accumulated by these species are relatively diverse, the flavone-flavanone components are essentially identical. Other flavonoids were also present in the crude extracts, awaiting further identification. The flavonoids were characterized as already described; data are presented in Table 2.

Table 2: Chromatographic and spectral characteristics of flavonoids identified from crude extracts of 11 *Philonotis* species. * colour, TLC-system: acetic acid (15%), cellulose F_{254}; ** NA = Naturstoffreagenz A; *** red with the time; sh = shoulder

Flavonoids	TLC*	UV_{366} + NA**	R_f	HPLC R_t min.	UV-spectra (λmax/nm)
1 2,3-dihydrophilonotisflavone	deep purple	orange***	37	23,08	254sh-273-288sh-344
2 5',3'''-diOH-amentoflavone	deep purple	yellow	25	26,29	256-288sh-353
3 5',3'''-diOH-robustaflavone	deep purple	yellow	12	29,88	223sh-266sh-360
4 philonotisflavone	deep purple	yellow	60	21,51	255sh-345
5 *dicranolomin*	*deep purple*	*yellow*	47	25,77	254-288sh-344

In the section *Catenularia* the papillae are centric over the lumina on both sides or only on the abaxial side. While most species of *Philonotis* laminal cell papillae emerge from the anterior end on the adaxial side, in *P. fontana* (section *Euphilonotis*) there is a shift basally in the leaf with the cells in the lower half bearing papillae from the posterior end (GRIFFIN III & BUCK, 1989). These anatomic and morfologic differences (BROTHERUS, 1924) can also be appreciated in the biflavonoid pattern of both sections. The section *Catenularia* contains low concentrations of 2,3-dihydro-philonotisflavone. Philonotisflavone and dicranolomin are not present. The species of the section *Euphilonotis* are poor in 2,3-dihydro-philonotisflavone (excepting *P. caespitosa*), apomorphic character (LÓPEZ-SÁEZ, 1994); and rich in philonotisflavone and dicranolomin. 5',3'''-dihydroxi-biflavones also been found in less amount.

According to its flavonoid composition, the section *Euphilonotis* could be considered as the least evolved of the genus *Philonotis*, closed to sect. *Leiocarpus* and *Philonotula* (GRIFFIN III & BUCK, 1989). The absence of philonotisflavone and dicranolim and also the reduction of biflavonoid synthesis make us consider section *Catenularia* as the most evolved of the genus *Philonotis*. The distribution of the different biflavonoids appears to be well suited for biochemical systematics or chemotaxonomy, and may also be applied to *Bartramiaceae* flavonoids. An important factor to consider in trying to judge relationships in the family on the basis of chemical data is that of whether assignments of evolutionary advancement may be made. However, as in many phylogenectic exercises the mode of assigning a weight to a chemical character is largely subjective.

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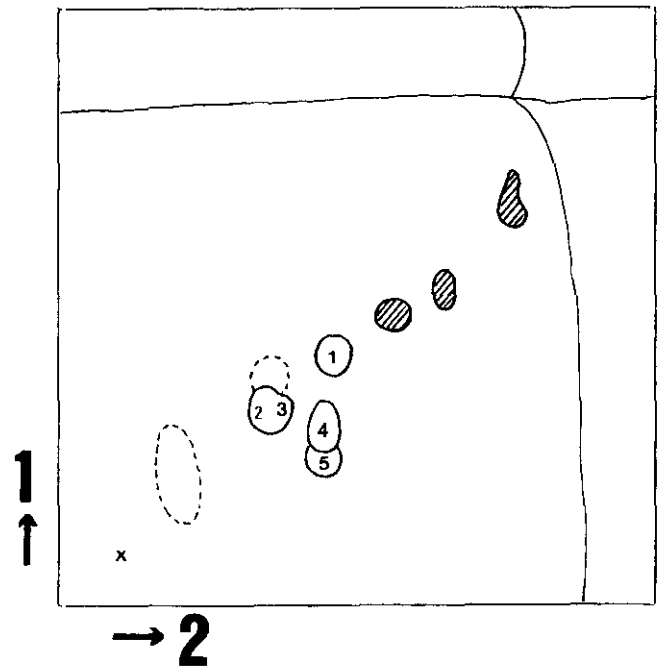

Fig. 1: *Type chromatogram of the phenolics obtained from airdried gametophytic material of* Philonotis fontana. *Adsorbnet: polyamide-6, sol ent: 1) ethyol acetate-methylethylcetone-formic acid-H_2o (5:3:1:1), 2) Me_2CO-H_2O-HOAc (:2:2). Detection: UV (350 nm) with and without NA, dotted lines indicate minor componets, hatched spots show blue fluorescence.*

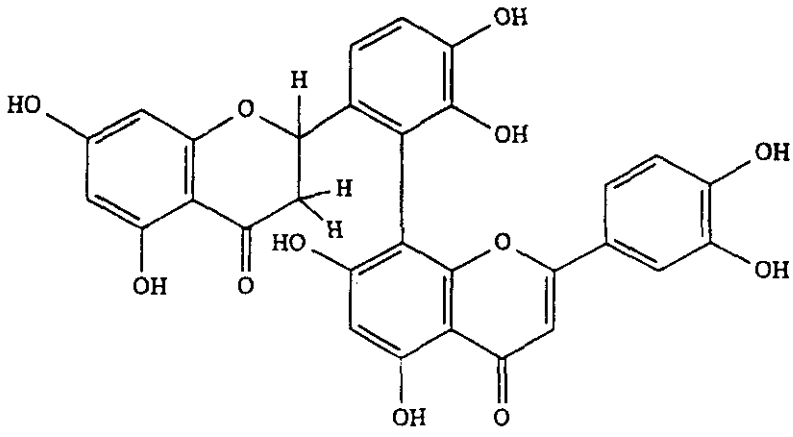

Fig. 2: *Major component of* Philonotis *bifla onoids: 2, 3-dihydro-philonotisfla one, a fla anone-fla one dimer.*

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