

Flavonoid Glycosides from the Pinnae of *Lunathyrium japonicum*

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ABSTRAK

Penyelidikan semula terhadap flavonoid glikosida dari spesies *L. japonicum* telah menunjukkan kehadiran kuersetin 3-O-rutinosida, vicesin-2, kaempferol glikosida dan vitexin. Oleh yang demikian flavonoid data dari kajian ini kelihatan berbeza (kecuali penemuan vitexin) dengan data yang diperolehi oleh Hiraoka (1978). Perbezaan corak flavonoid di antara *L. japonicum* dari Jepun dengan *L. japonicum* dari Semenanjung Malaysia, mencadangkan variasi geografi. Lanjutan dari pengestrakan flavonoid dari pinna-pinna *L. japonicum* dalam kajian ini juga mendapati kuersetin 3-O-rutinosida dan vicesin-2 dilaporkan pertama kalinya dijumpai dalam famili Athyriaceae.

ABSTRACT

Reinvestigation of the flavonoid glycosides of the species *L. japonicum* indicated the presence of quercetin 3-O-rutinoside, vicesin-2, kaempferol O-glycosides and vitexin. Thus, the flavonoid data at present seem to be different except for the presence of vitexin from those of Hiraoka (1978). The differences in flavonoid patterns between *L. japonicum* from Japan and *L. japonicum* from Peninsular Malaysia suggest geographical variation. In addition to the extraction of flavonoids in *L. japonicum* in the present study, quercetin 3-O-rutinoside and vicesin-2 were reported for the first time in the family Athyriaceae.

INTRODUCTION

Previous flavonoid studies on *Lunathyrium japonicum* (Thunb.) Kurata (Hiraoka 1978) revealed the presence of vitexin, orientin, kaempferol 3-O-glucoside and quercetin 3-O-glucoside in the pinnae. Reinvestigation of the flavonoid glycosides of the species *L. japonicum* in the present study established that the major flavonoid glycosides in this fern are quercetin 3-O-rutinoside, vicesin-2, unidentified kaempferol O-glycosides but besides vitexin.

MATERIALS AND METHODS

Plant Sources

Fern samples were collected from the natural habitat in Malaysia. A voucher specimen was deposited in the herbarium at the Botany Department, University of Reading, Berkshire, England (collector No. U174). Fresh grown samples were supplied by Kew Garden, Surrey, England. The fern samples were air-dried

before extraction. Dried pinnae (3 g) were homogeneously powdered.

Identification of Flavonoids

Two-dimensional paper chromatographic surveys of pinnae were carried out using the solvent pairs; rc-BuOH-HOAc-H₂O (4:1:5) (BAW) and 15% HOAc. R_fS, UV spectral analysis and colour reaction with and without ammonia for the compounds, run one-dimensionally by descent on Whatman No. 1 paper, are given in Table 1. Known flavonoid glycosides were identified by standard procedures (Harborne 1967) and in most cases compared directly with authentic samples. Flavonoid aglycones were identified in acid hydrolysed pinnae extracts using standard procedures (Harborne 1967) by comparison with authentic markers.

RESULTS AND DISCUSSION

On acid hydrolysis, both samples of *L. japoniam* produced cyanidin and quercetin. The alcoholic extracts produced quercetin 3-O-rutinoside but in the fresh-grown sample of *L. japoniam* unidentified kaempferol Oglycosides, vitexin and vicenin-2 were detected. However, these glycosides were not found in dried samples of the field collection. This may be due to variations in the chemistry between the two populations of *L. japonicum*, or due to differences in fresh and dried plant material. The former possibility seems more likely, since most flavonoids are stable and will not be destroyed on drying. Furthermore, Hiraoka (1978) studied the same species and found a different flavonoid pattern again. *L. japoniam* appears to show interspecific chemical variation. Hiraoka found vitexin, orientin, kaempferol 5-O-glucoside and quercetin 3-O-glucoside in the frond extracts of the species. The differences in flavonoid patterns between *L. japonicum* from Japan and Malaysia suggest geographical variation, Wollenweber (1982) also encountered this phenomenon when he analysed the flavonoids in the fronds of *Cheilanthes farinosa* (Polypodiaceae). In the samples from Africa, he found kaempferol 3,7,4-tri-O-methyl ether, kaempferol 7,4'- α -dimethyl ether, methyl ether of quercetin, kaempferol 7-O-methyl ether and apigenin 7,4'-6-kimethyl ether. In the samples from Asia, the same derivatives of kaempferol were present, in addition to kaempferol 3,7-Odimethyl ether. In *C. argentea*, there also seemed to exist a correlation between geographical and flavonoid composition. The specimens from Japan produced an unidentified diterpene and a series of new flavanones (Wollenweber *et al* 1980). Those from China produced the same diterpene but none of the new flavanones. The samples from Taiwan were distinguished very clearly by production of a different unknown diterpene and possibly one of the new flavanones (Dietz 1980). A similar observation has been made in *C. anceps*. The flavonoid pattern in samples from the northern part of India appears to be different from that of material from the south of India (Wollenweber 1982). These findings, however, are still to be confirmed by analysis of further samples from the different regions.

Quercetin 3-O-rutinoside has previously been found from the leaf of *Ruta graveolens* (Rutaceae) (Harborne 1967). Vicenin-2 has been found before in bryophytes (Mues and Zinsmeister 1976;

TABLE I
Central anion of *Lamathyrus japonicum*

Glycoside	Absorption spectrum (nm) in		R _f (x100) in				Colours in UV	
	Water	+NaOAc	+H ₃ BO ₃	BAW	H ₂ O	15%IOAc	PhH	+NH ₃
Quercetin-3-O-rutinoside	258, 354	271	410	51	47	34	dark	yellow
Vitexin	270, 388	380	39	11	20	8	yellow	yellow
Vicenin-2	271, 336	376	68	17	37	4	yellow	yellow

BAW, n-BuOH-HOAc-H₂O (4:1:5); PhH, PhOH-H₂O (4:1); sh. = shoulder.

Osterdahl 1979) and ferns (Wallace *et al.* 1981). However, these flavonoids are reported for the first time in the Athyriaceae. Further flavonoid studies on other taxa of this family are in progress.

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ABSTRACT

In a survey of 12 specimens of the Asplenoid fern for the genus *Lunathyrium*, 10 glycosides were found in 80% (specimens 22%) and present in 13% of the species studied. *Kampferol 7-O-glucuronide* was found in *A. japonicum* only. *Quercetin* and *quercetin-3-O-glucuronide* were also previously found in the fern, *Podochilus arthropoda* (L.) Nakai and, for the first time in *A. nodosum*. This is the second report of the occurrence of the type of flavone *apigenin* in ferns. *Apigenin* and *apigenin-7-O-glucuronide* in *A. japonicum* is the first report of the occurrence of these flavones in ferns. *Apigenin-7-O-glucuronide* and *apigenin-7-O-glucuronide* were also found in *A. japonicum*. *Apigenin-7-O-glucuronide* and *apigenin-7-O-glucuronide* were also found in ferns.

INTRODUCTION

The presence of flavonoids in the Asplenoid ferns, genus *Lunathyrium* was previously reported by Yano (1977). He found *quercetin-3-O-glucuronide* in *A. japonicum*. Later, Yano (1979) reported the distribution of flavonoid aglycones in 19 species of Asplenoid. He studied mainly European species and a few species from Central Asia. He found *quercetin* in 14 species, *quercetin-3-O-glucuronide* and *quercetin-3-O-glucuronide* in 12 species. Harborne *et al.* (1973) studied the flavonoid characters of plants and hybrids of Appalachian species of *Asplenium* and found a series of *quercetin* derivatives in the ferns of three parental species of the Appalachian *Asplenium* complex. *A. platyneuron* was characterized by the presence of the 7-O-glucuronide of *quercetin*.

3,4-Dihydroxy-7-ethoxy-5,7,8-trihydroxyflavone and *apigenin-7-O-glucuronide* were found in *Lunathyrium japonicum*. By contrast, *quercetin-3-O-glucuronide* and *quercetin-3-O-glucuronide* were not found in *Lunathyrium japonicum*. In addition to being the glycosyl anthones (Yano and Harborne 1977) the another two glycosyl glycosides (Harborne *et al.* 1973). Further flavonoid studies of ferns *Lunathyrium* were carried out by Harborne (1982) and Yano (1982) who found several flavonoid glycosides in the Asplenoid ferns. As part of an ongoing project on the chemistry of *Lunathyrium*, the present study aims to add to the number of reports in the flavonoid profiles of the genus. For this purpose, we examined the pinnae of the Asplenoid species collected in local habitats from Malasia and those species from England.