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BIFLAVONOIDS FROM THE SEED TESTA OF CYCADALES

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Key Word Index—Zamiaceae; Cycadaceae; biflavones; amentoflavone; cupressuflavone; chemotaxonomy.

Abstract—Biflavonoid patterns from ethanolic extracts of the brightly coloured testa of eight species of Cycadales have been determined. Species from the genus *Macrozamia* are characterized by the occurrence of cupressuflavone- and amentoflavone-based patterns, while the *Cycas* species contain only amentoflavone-based patterns. The other species from the genera *Encephalartos*, *Lepidozamia*, and *Zamia* contain a very minor or no detectable biflavonoid component. This is the first report of the occurrence of cupressuflavone in the Cycadales.

INTRODUCTION

Extant members of the Cycadales represent an extremely ancient group of seed-plants that exhibit a number of very primitive features, both in morphology and in their life-cycles. A revision of the members of the Cycadales by Johnson[1] divided the group into three families, two of which are sampled in this study. The family Cycadaceae contains a single genus, *Cycas*, with a fairly widespread distribution from Japan to Australia. The other family sampled here, Zamiaceae, is divided into a number of tribes, within which the genera may show either wide or rather restricted distributions. Zamieae contains four general including *Zamia*, which is restricted to the tropics of the New World. The tribe Encephalarteae contains three genera, of which *Lepidozamia* and *Macrozamia* are restricted to tropical and subtropical eastern Australia, while *Encephalartos* is restricted to Central and South America. A recent study of biflavones in the leaves of 82 species of Cycadales found that generic and family relationships were reflected in the patterns of occurrence of amentoflavone and hinokiflavone and their methyl ethers[2]. This study examines the biflavonoid content of fresh material of the often brightly coloured, fleshy seed testa of eight species of Cycadales.

RESULTS AND DISCUSSION

The most startling results came from the extracts of two of the *Macrozamia* species tested, *M. communis* and *M. macdonnellii*. Both cupressuflavone and amentoflavone, as well as traces of their partial methyl ethers, were isolated (Table 1). The parent compounds were chromatographed against authentic

Table 1. Biflavonoids identified in seed testa of the Cycadales

Plant species (testa examined)	A	B	C	D	E	F	G	H	I	J	K	L	M	N
Macrozamia macdonnellii (F. Muell. ex Miq.) A.DC	++^{p}	+	+^{p}	++^{p}		m								
M. communis L. A. S. Johnson	++^{p}			++^{p}				+		m				
M. miquelii (F. Muell.) A.DC. (immature)	?			m										
Macrozamia leaves*				++		+			+		+			
Cycas armstrongii Miq.				++	+									
C. kennedyana F. Muell.				+^{p}	+									
Cycas leaves*				++			+		m			+/m	+	+
Lepidozamia peroffskyana Regel	Very minor traces, not characterized													
Encephalartos altensteinii Lehm.	Very minor traces, not characterized													
Zamia furfuracea Ait.	No traces present													

*Uniform pattern of genus, from Dossaji *et al.*[2].

A, cuppressuflavone; B, monomethylcupressuflavone; C, dimethylcupressuflavone; D, amentoflavone; E, 4‴-monomethylamentoflavone; F, 4′-monomethylamentoflavone; G, 7″-monomethylamentoflavone, H, 4‴,7″-dimethylamentoflavone; I, 7,4′-dimethylamentoflavone; J, trimethylamentoflavone; K, 7,4′,4‴-trimethylamentoflavone; L, hinokiflavone; M, 2,3-dihydrohinokiflavone; N, 2,3-dihydroamentoflavone.

++, Major presence; +, minor presence; m, trace detected and characterized; p, compound permethylated; ?, minor trace, characterized on Si gel only.

standards in two solvent systems, BN and BPF (see Experimental). Further, the isolated and pure compounds were permethylated, and the resulting hexamethyl ethers co-chromatographed with standards of the hexamethyl ethers of amentoflavone and cupressflavone. Reaction of amentoflavone and cupressuflavone compounds with $AlCl_3$ produced a difference in fluorescence under UV—yellow fluorescence for amentoflavone and orange fluorescence for cupressuflavone—that has been previously reported [3].

The eluant from testas of ripe seeds of *M. macdonnellii* contained a surprisingly large fraction of biflavonoids, whilst that obtained from unripe seeds of *M. miquelii* contained very little. It is not known the extent to which biflavonoids accumulate in the fleshy coverings of these species as the process of ripening proceeds, but the indication from these results is that there is a rapid build up in concentration in the last few weeks.

Two species of *Cycas* were tested and found to contain amentoflavone and its 4‴-monomethyl ether as the major biflavonoids (Table 1). Identifications were determined again with authentic standards and permethylation. Extracts from *Lepidozamia peroffskyana* and *Encephalartos altensteinii* contained very minor traces of biflavonoids that could not be characterized, whilst the extract of *Zamia furfuracea* did not contain any detectable biflavonoid material.

Biflavonoid patterns of the leaves of Cycadales are very uniform within each genus, consisting primarily of amentoflavone and its partial methyl ethers [2]. Extracts from *Cycas* leaves also contain hinokiflavone, 2,3-dihydroamentoflavone and 2,3-dihydrohinokiflavones. Hinokiflavone and the dihydro derivatives were not detected in the testa extracts of the *Cycas* species examined. Indeed, there was little similarity between biflavonoid patterns of testa extracts and those reported from the leaves (Table 1). Of most significance was the occurrence of the large fraction of cupressuflavone in the testa extracts of the *Macrozamia* species. Apart from emphasizing the chemical distinctiveness of the genus *Macrozamia*, the occurrence of cupressuflavone in the testa underlines the need to use strictly homologous tissues in chemotaxonomic work.

The occurrence of cupressuflavone and its derivatives in *Macrozamia* represents only the third record outside the Cupressaceae and Araucariaceae, the others being from a single species of *Casuarina* [4], and from the newly resurrected genus *Lepidothamnus* Phil. of the Podocarpaceae [3, 5]. The accumulation of cupressuflavone must be regarded as a condition that has been independently derived in both the Cycadales and Coniferales, since it is highly improbable that its presence in a small number of specialized members of two very distant orders of gymnosperms is due to the retention of a primitive character.

EXPERIMENTAL

Collecting details. Macrozamia macdonnellii (F. Muell. ex Miq.) A.DC., Wannan & Waterhouse, Standley Chasm NT., Jan. 1981. *M. communis* L. A. S. Johnson, Gadek & Quinn, Ettalong, N. S. W., April 1981. *M. miquelii* (F. Muell.) A.DC., Hind 16, Rockhampton, Qld, 30 April 1981. *Cycas armstrongii*. Miq., Wannan & Waterhouse, near Mt. Bundy, NT, 15 Jan. 1981, UNSW 11367. *C. kennedyana* F. Muell., Hind 15, Rockhampton Qld, 18 April 1981. *Lepidozamia peroffskyana* Regel, Gadek, cult. Royal Bot. Gdns. N.S.W., 22 April 1981. *Encephalartos altensteinii* Lehm., Gadek, cult. Royal Bot. Gdns. N.S.W., 22 April 1981. *Zamia furfuracea*, Ait. cult. Florida, U.S.A., exchange material sent to P. Hind, Royal Bot. Gdns. N.S.W.

Extraction and purification. The fleshy testa was removed from the seeds and extracted in 70% EtOH for at least 48 hr, washed with petrol (bp 60–80°) and concd under red. pres. The residue was re-extracted in EtOH, and chromatographed on pre-coated Si gel plates developed in C_6H_6–pyridine–HCO_2H (BPF) (100:20:7). Biflavonoid bands were initially identified as dark, UV absorbing bands, which fluoresced yellow and orange on addition of $AlCl_3$. Each band was extracted and a final separation was carried out on pre-coated cellulose plates developed in fresh BN(*n*-BuOH–2N NH_4OH, 1:1, upper layer). Initial identifications were made by co-chromatography with authentic samples in both solvent systems. Amentoflavone derivatives were separated from cupressuflavone derivatives by the colour of fluorescence under UV after reacting with $AlCl_3$ [3]. Confirmation of this distinction was obtained by partial methylation and permethylation of some common bands using CH_2N_2, and co-chromatography of the resulting hexamethyl ethers with authentic samples in BPF.

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