Phytochemistry, 1973, Vol. 12, pp. 371 to 373. Pergamon Press. Printed in England

BIFLAVONES OF *DIOON*

S. F. Dossaji and E. A. Bell*
Department of Botany, University of Texas, Austin, TX 78712, U.S.A.
and
J. W. Wallace
Department of Biology, Western Carolina University, Cullowhee, NC 28723, U.S.A.

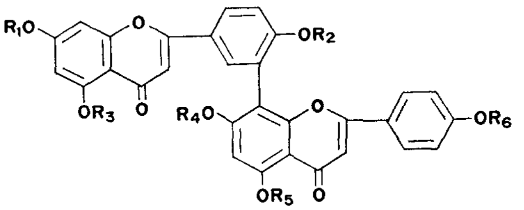
(*Received* 1 *August* 1972. *Accepted* 1 *September* 1972)

Key Word Index—*Dioon*; Zamiaceae; biflavones; amentoflavonehexamethyl ether; chemotaxonomy.

Abstract—Seven biflavones, amentoflavone, bilobetin, sequoiaflavone, ginkgetin, sciadopitysin, 7,4′,7″,4‴-tetra-*O*-methylamentoflavone, and diooflavone (amentoflavone hexamethyl ether), were identified from extracts of the cycad genus *Dioon*. The biflavones were identified by direct comparison with authentic samples using m.m.p., co-chromatography in 3 solvents, and NMR studies of the acetates. This is the first time amentoflavone hexamethyl ether has been identified as a natural product. After surveying numerous species of the Cycadales, no evidence could be obtained for the occurrence of biflavone glycosides or of biflavones based upon any other nucleus than apigenin.

INTRODUCTION

Dioon, one of the ten extant genera of the cycads, is a small genus of approximately 5 species that is limited in distribution to Mexico. Although several papers have been published on the toxic chemistry of the various cycads, very little information is available concerning the phenolics, especially the flavonoids. Wallace has described the hydroxybenzoic and hydroxycinnamic acids of the Cycadales[1] and has identified the *C*-glycosylflavones, orientin and vitexin, from *Dioon spinulosum* Dyer ex Eichl.[2] In addition, the biflavones, amentoflavone (I), bilobetin (II), sequoiaflavone (III), ginkgetin (V), and 7,4′,7″,4‴-tetra-*O*-methylamentoflavone (VII), have been identified in *Zamia angustifolia* Facg. (*Z. floridana* A. DC),[3] sotetsuflavone (IV), 2,3-dihydroamentoflavone, 2,3-dihydrohinokiflavone, and amentoflavone (I) have been identified from *Cycas revoluta* Thunb.[4,5] The present report concerns the identification of amentoflavone (I), bilobetin (II), sequoia-



		R_1	R_2	R_3	R_4	R_5	R_6
(I)	Amentoflavone	H	H	H	H	H	H
(II)	Bilobetin	H	Me	H	H	H	H
(III)	Sequioaflavone	Me	H	H	H	H	H
(IV)	Sotetsuflavone	H	H	H	Me	H	H
(V)	Ginkgetin	Me	Me	H	H	H	H
(VI)	Sciadopitysin	Me	Me	H	H	H	Me
(VII)	7,7″,4′,4‴-Tetra-O-methylamentoflavone	Me	Me	H	Me	H	Me
(VIII)	Diooflavone	Me	Me	Me	Me	Me	Me

---

* Present address: Department of Botany, King's College, London, England.

[1] J. W. Wallace, *Am. J. Bot.* **59**, 1 (1972).

[2] J. L. Carson and J. W. Wallace, *Phytochem.* **11**, 842 (1971).

[3] B. K. Handa, K. K. Chexal, W. Rahman, M. Okigawa and N. Kawano, *Phytochem.* **10**, 436 (1971).

[4] N. Kawano and M. Yamada, *J. Pharm. Soc. Japan* **80**, 1576 (1960).

[5] H. Geiger and W. De G. Pfleiderer, *Phytochem.* **10**, 1936 (1971).

flavone (III), ginkgetin (V), sciadoptysin (VI), 7,4′,7″,4‴-tetra-*O*-methylamentoflavone (VII), and diooflavone (5,7,4′,5″,7″,4‴-hexa-*O*-methylamentoflavone) (VIII) from species of the genus *Dioon*.

RESULTS AND DISCUSSION

The distribution of biflavones in the cycad genus *Dioon* is summarized in Table 1. The compounds were found in all the plants which were examined. The major biflavone in each species was amentoflavone (I). Except for the absence of diooflavone (VII) in *D. purpusii*, *D. mejea*, *D. imbricata*, and *D. san blas* and the lack of sequoiaflavone (III) in the latter, the biflavone distributional pattern is rather uniform and does not appear to be useful taxonomically, at least at the specific level.

Table 1. Distribution of biflavones in *Dioon*

Species	I	II	III	IV	V	VI	VII	VIII*
1 *Dioon edule*	+++	+	+	–	++	+	+	+
2 *D. spinulosum*	+++	+	+	–	++	+	+	+
3 *D. purpusii*	+++	+	+	–	++	+	+	+
4 *D. san blas*	+++	+	–	–	+	+	trace	–
5 *D. mejea*	+++	+	+	–	++	+	trace	–
6 *D. imbricata*	+++	+	+	–	++	+	trace	–

* For key, see text or formulae.

However, the biflavone chemistry of *Dioon* is very similar to that of *Zamia*.[3] Preliminary results (unpublished) indicate that the remaining cycad genera, except *Stangeria*, have biflavones similar to those identified from *Dioon* and *Zamia*; in addition, *Cycas* species appear to have biflavones presently unknown to the rest of the Order. Hinokiflavone derivatives, which have been identified in *Cycas*,[5] have not been detected in *Dioon*.

Table 2. R_fs for biflavones isolated from *Dioon* species

Compound	R_f in solvent* BPF	 BPEFD	 BEAA
Amentoflavone (I)	0·25	0·33	0·29
Bilobetin (II)	0·45	0·48	0·44
Sequioflavone (III)	0·45	0·48	0·44
Ginkgetin (V)	0·69	0·65	0·59
Sciadopitysin (VI)	0·71	0·78	0·72
7,4′,7″,4‴-Tetra-*O*-methyl amentoflavone (VII)	0·90	0·85	0·87
Diooflavone (VIII)	0·50	0·19	0·34

* BPF = C_6H_6–pyridine–HCO_2H (36:9:5); BPEFD = C_6H_6–pyridine–HCO_2Et–dioxan (5:1:2:2); BEAA = C_6H_6–EtOAC–HOAC (10:3:2). R_fs are averages of three determinations. All the solvents distinguish between the fully methylated derivatives of amentoflavone and hinokiflavone.

Since the cycads are a primitive group of plants, the present results strengthens the view that biflavone biogenesis a primitive metabolic character.[8] It is interesting that there is no evidence for the natural occurrence of biflavone glycosides. It may well be that the coupling of flavone nuclei, i.e. the formation of biflavones, serves the same metabolic or physiological function as glycosylation in other species. This hypothesis is presently being investigated.

EXPERIMENTAL

Plant material. Dioon edule Lindl., *D. spinulosum* Dyer, and *D. purpusii* Rose were obtained both from the cycad collection of the University of Texas, Austin, and from Fairchild Tropical Gardens, Miami, Florida, U.S.A. (accession Nos. 6127; 58545 and 59958; and 651318). *D. mejea* Stand., *D. san blas*, *D. tenoi*, and *D. imbricata* Dyer were obtained from a private collection of Dr. M. R. Birdsey, Miami, Florida.

Extraction and identification procedures. Dried leaf material was exhaustively extracted with boiling 95% EtOH as previously described[2] and/or by extracting with (90/10) MeOH–pyridine. Column chromatography on Polyclar AT and elution with $CHCl_3$–MeOH–MeCOEt (4:2:1) gave excellent separation of the biflavonyl mixture. Individual compounds were identified by direct comparison with authentic samples using m.m.p., co-chromatography (TLC in 3 solvents, see Table 2)[6] with the original compounds and their hexamethyl derivatives, and by NMR spectra of their acetates.[7] Considerable difficulty was experienced in separating sciadopitysin and amentoflavone 7,4′,7″,4‴-tetra-*O*-methyl ether.

Acknowledgements—The authors thank Drs. J. B. Harborne, N. Kawano and B. S. Joshi for furnishing standard compounds and J. L. Carson and K. E. Herman for technical assistance. Two of us (E. A. B. and S. F. D.) thank the National Institutes of Health (P.H.S. Grant No. R01 ES00442) and the Robert A. Welch Foundation (Grant No. F 375) for financial support. S. F. D. thanks the African American Institute (AAI), New York, for support as a Graduate Fellow through the AFGRAD Fellowship Program.

---

[6] K. K. Chexal, B. K. Handa and W. Rahman. *J. Chromatog.* **48,** 484 (1970).

[7] H. Miura, T. Kihara and N. Kawano, *Tetrahedron Letters* **19,** 2339 (1968).

[8] A. S. Murti, *Bull. Nat. Inst. Sci. India* **34,** 161 (1967).