Phytochemistry, Vol. 26, No. 9, pp. 2553–2555, 1987.
Printed in Great Britain.

0031-9422/87 $3.00 + 0.00
Pergamon Journals Ltd.

BIFLAVONOIDS IN THE PRIMITIVE MONOCOTS *ISOPHYSIS TASMANICA* AND *XEROPHYTA PLICATA*

CHRISTINE A. WILLIAMS, JEFFREY B. HARBORNE and FRANCISCO A. TOMAS-BARBERAN*

Phytochemical Unit, Plant Science Laboratories, University of Reading, Reading, RG6 2AS, U.K.

(*Received* 26 *February* 1987)

Key Word Index—*Isophysis tasmanica*; Iridaceae; *Xerophyta plicata*; Velloziaceae; leaves; biflavonoids; hinokiflavone; podocarpusflavone A; amentoflavone; chemotaxonomy.

Abstract—In addition to the previously characterized amentoflavone and dihydroamentoflavone, two further biflavonoids have been identified from *Isophysis tasmanica*: hinokiflavone and podocarpusflavone A. This is the first time these two biflavonoids have been found in the monocotyledons. Amentoflavone has also been discovered in *Xerophyta plicata*; this constitutes the first record of biflavonoids in Velloziaceae.

INTRODUCTION

The Tasmanian endemic *Isophysis tasmanica* is placed in the Iridaceae, although it differs from all other members in having hypogynous rather than epigynous flowers. Morphologically, it appears to be a very primitive member of the family and has been regarded as the prototype of the ancestral species [1]. Earlier phytochemical studies have shown that it is also unusual in its flavonoid constituents, in that two biflavonoids were detected in its tissues, amentoflavone and dihydroamentoflavone [2, 3]. In view of the rarity of biflavonoid reports in the monocotyledons, we decided to obtain more abundant supplies of this plant in order to examine the flavonoids in more detail.

We here report, therefore, the identification of two further biflavonoids from *Isophysis tasmanica*. We also describe the first identification of biflavonoids in another monocot family, in *Xerophyta plicata* (Velloziaceae). We also comment on the natural distribution of this class of flavonoid among the monocotyledons.

RESULTS AND DISCUSSION

Separation of the flavonoids of *Isophysis* leaf material gave, in addition to amentoflavone and dihydroamentoflavone previously reported [2], three further biflavonoid constituents 1–3. Compounds 1 and 2 were readily identified as hinokiflavone and podocarpusflavone A (amentoflavone 7-methyl ether) respectively by standard procedures, which included co-chromatography and co-HPLC with authentic markers. Co-chromatography included separations on paper and TLC on silica gel, cellulose and polyamide. R_f values for these two biflavonoids and a range of related substances are given in Table 1.

Confirmation of identification was provided by permethylation using standard procedures [4] and comparison with authentic permethyl ethers [5]. On fast atom bombardment mass spectroscopy (FAB-MS), a technique which has not been applied on any scale yet to biflavonoids, 1 and 2 gave intense molecular ions, when using the negative mode. Fragmentation occurred during FAB-MS but not all the fragment ions could easily be accounted for. With hinokiflavone (1), cleavage at the ether bridge gave predictable ions at 283, 281, 255 and 253 but two other prominent ions at 355 [M − 183] and 183 [M − 355] appeared to be fragments produced from cleavage of one of the aromatic rings. Podocarpusflavone A (2) gave a prominent ion at 375 [M − 177], presumably as a result of splitting off a phenylpropanoid moiety.

Biflavonoid 3 was contaminated with a minor component, which could only be separated on polyamide (Table 1). Permethylation gave amentoflavone hexamethyl ether and an unknown. FAB-MS gave a molecular ion corresponding to an amentoflavone monomethyl ether. Since it differs in R_f from any of the available monomethyl ethers (Table 1), 3 could be the 7′-methyl ether by elimination. An authentic specimen of this compound is not easily accessible, so confirmation of this structural assignment has not yet been possible.

These analyses show that *Isophysis tasmanica* contains at least five biflavonoids in its leaves. It is thus much richer in these components than the only other member of the Iridaceae found to have biflavonoids, namely *Patersonia glabrata* R. Br., which has amentoflavone alone [2, 3]. The accumulation of biflavonoids in these primitive members of the family is in keeping with their morphology. This is also in keeping with the idea that the accumulation of biflavonoids in isolated groups within the angiosperms represents the retention of a primitive biosynthetic pathway, inherited from an ancient ancestor among early land plants [6]. In other respects too, *Isophysis* has a simple flavonoid profile. Examination of the anthocyanin in shoot tissue showed it to be a cyanidin 3-monoside, probably the 3-glucoside. Glycosylflavones were also detected in minor amount in the aerial parts of the plant.

Previous to our finding of biflavonoids in the Iridaceae [2, 3], the only record in the monocotyledons was of a 3,8-linked biflavanone [7] in *Lophiola americana* Wood

*Permanent address: Laboratorio de Fitoquimica, CSIC, Apdo. 195, Murcia 30003, Spain.

Table 1. A comparison of R_f data for *Isophysis* biflavonoids (1–3) with authentic markers

	TLC					Paper
	Silica gel		Polyamide 11	Microcryst. polyamide nitromethane/ MeOH(4:3)	Cellulose	
Biflavonoid	TEF	TPF	T/MEK/MeOH		BN	BN
Amentoflavone	43	02	13	07	36	—
2,3-Dihydroamento flavone	39	02	05	16	16	—
1	38	06	07	14	16	—
Hinokiflavone	38	06	07	14	16	—
Amentoflavone monomethyl ethers						
2	43	07	17	19	64	48
Podocarpusflavone A	43	07	17	19	64	48
3	49	36	20, 74	24, 87	43	70
Sequoiaflavone	43	07	17	19	74	61
Bilobetin	42	08	28	40	36	—
Amentoflavone dimethyl ether						
Isoginkgetin	43	23	71	79	46	—
Amentoflavone trimethyl ethers						
Kayaflavone	48	34	73	37	48	—
Sciadopitysin	46	34	73	48	46	—

Solvent key: TEF = toluene–ethyl formate–HCOOH (5:4:1); TPF = toluene–pyridine–HCOOH (100:20:7); BN = *n*-butanol-1 N NH_4OH (1:1); T/MEK/MeOH = toluene–methylethylketone–MeOH (4:3:3).

(Melianthiaceae). This was a preliminary report which unfortunately has never been substantiated by full publication. That other families of monocots may contain this class of flavonoid became apparent to us during a preliminary survey of seven members of Velloziaceae, a family restricted to South America and some parts of Africa, and which has rarely been examined for its flavonoids. One of the seven species, *Xerophyta plicata*, yielded amentoflavone, readily identified by the same procedures described above. This constitutes the first record of a biflavonoid in this group of plants.

One of the problems of ascertaining the distribution of biflavonoids in plants is the absence of a simple screening procedure. On 2D paper chromatograms (or TLC plates) run in butanol–acetic acid–water and 15% acetic acid, they occupy a position near the front in the first solvent and near the origin in the second, which is also taken up by monomeric flavone methyl ethers. There is no clear spectral distinction, so that other procedures (e.g. permethylation [5]) have to be used to confirm the presence of biflavonoids. However, in spite of this restriction on ready ascertainment, it is our experience during surveys of all the major monocotyledonous families that biflavonoids are rarely present. Certainly, they have not yet been encountered during such surveys in any other families or during more intensive investigations of the flavonoids of individual species.

In the two families where they are now known to be present, they also seem to be uncommon. In the Iridaceae, they have so far been encountered in only two members, *Isophysis tasmanica* and *Patersonia glabrata*, in spite of a survey of 285 species [3]. We have preliminary evidence that they may be present in a few other related taxa of the tribe Aristeae and work is in progress on these species. In the case of Velloziaceae, the limited distribution of biflavonoids (only in 1 of 7 spp.) might throw light on the origin of the family, since there is controversy about whether these plants first arose in Brazil or in Africa. Our discovery of amentoflavone in a Brazilian member of the family would support de Menezes [8] in her view that the centre of origin is South America if biflavonoids are shown to be absent from African taxa.

EXPERIMENTAL

Plant material. Isophysis tasmanica (Hook. f.) T. Moore was collected and identified by A. M. Buchanan, University of Tasmania, at Cox Bight, S. W. Tasmania on 9 August 1986. A voucher specimen has been deposited in RNG. Material of *Xerophyta plicata* Mart. was taken from a herbarium specimen, collected in Minas Gerais, Brazil in 1984 by G. Hatschbach and O. Guimaraes (No. 47086) and its identity determined by L. B. Smith. This plant has also been named *Nanuza plicata* (Mart.) L. B. Smith et Ayensu, but this revision has not been accepted generally by experts in the family [R. Harley, personal communication].

Isolation and separation. Leaves of *I. tasmanica* were extracted with hot 80% MeOH and the concentrated extract run on prep. PC in BAW. The high running biflavonoid band ($R_f > 0.90$) was cut out, eluted and rerun in *n*-butanol: 1 N NH_4OH (1:1) (BN) after 24 hr equilibration, to give four bands. Band 2 was a mixture of amentoflavone and dihydroamentoflavone previously identified [2, 3]. Band 1 gave hinokiflavone (1), band 3 podocarpusflavone A (2) and band 4 an unknown amentoflavone derivative (3).

Identification of biflavonoids 1–3. R_f data for amentoflavone, 2,3-dihydroamentoflavone, compounds 1–3 and other biflavonoid markers are given in Table 1.

Compound 1 (*hinokiflavone*). 1 was identified by co-chromatography with authentic hinokiflavone on TLC in five solvents (see Table 1). UV spectral data: λ_{max} in MeOH 272, 337;

+NaOAc 276, 370; +H_3BO_3 272, 337 and +NaOH 277, 394 agreed with that of hinokiflavone. Permethylation with MeI [4] gave hexamethylhinokiflavone, identified by comparison with an authentic marker on TLC silica gel in toluene–pyridine–ethyl formate–dioxan, 5:1:2:2 (TPEFD) R_f, 40 and toluene–pyridine–HCOOH, 100:20:7 (TPF) R_f, 17. FAB-MS (negative mode): 537 [M − H], 355 [M − 183], 283 [M − 255], 281 [M − 257], 255 [M − 283], 253 [M − 285] and 183 [M − 355].

Compound **2** (*podocarpusflavone A*). **2** was identified by co-chromatography with authentic podocarpusflavone A on TLC in five solvents and BN on PC (see Table 1). UV spectral data: λ_{max} in MeOH 270, 332; +NaOAc 276, 360; +H_3BO_3 272, 325 and NaOH 277, 396 agreed with that of the marker. The low intensity Band II with NaOH suggested methylation of one of the 4′-hydroxyls. Permethylation with MeI gave hexamethylamentoflavone identified by comparison with an authentic marker on TLC silica gel in the same 2 solvents as for permethylated **1** above. HPLC of **2** on a Waters 600 using a Waters C_{18} column, initial solvent ACN 50% H_2O–HCOOH (19:1) 50% at flow rate of 2 ml/min increasing 2% of ACN per min for 10 min gave R_t of 3.8 min which agreed with that of the marker. FAB-MS (negative mode): 551 [M − H], 375 [M − 176], 311, 309, 220 and 183.

Compound **3** (*unknown amentoflavone derivative*). R_f data did not agree with any of the 3 known monomethyl ethers of amentoflavone: bilobetin, sequoiaflavone or podocarpusflavone A (see Table 1). Both R_f and UV data λ_{max} in MeOH 276, 337; NaOAc 280, 383; +H_3BO_3 276, 337 and NaOH 281, 390 (Bands I and II with same intensity) are indicative of an amentoflavone monomethyl ether. Permethylation with MeI gave a small quantity of hexamethylamentoflavone and a larger amount of a blue fluorescent cpd of much higher R_f, i.e. 40 in TPF and 65 in TPEFD compared with 8 and 19, respectively for hexamethyl amentoflavone. FAB-MS of **3** (negative mode): 551 [M − H], 325, 311 and 197.

Acknowledgements—We are very grateful to Professor Dr H. Geiger for helpful discussions and for providing authentic markers of amentoflavone, 2,3-dihydroamentoflavone, hinokiflavone, podocarpusflavone A, sequoiaflavone and bilobetin. We are also indebted to Dr C. J. Quinn for his kind advice and the provision of some permethylated biflavonoid markers. We thank R. Self (AFRC Institute of Food Research, Norwich) for determining the FAB-MS.

REFERENCES

1. Dahlgren, R. M. T., Clifford, H. T. and Yeo, P. F. (1985) *The Families of the Monocotyledons*. Springer, Berlin.
2. Williams, C. A. and Harborne, J. B. (1985) *Z. Naturforsch.* **40c**, 325.
3. Williams, C. A., Harborne, J. B. and Goldblatt, P. (1986) *Phytochemistry* **25**, 2135.
4. Ciucanu, I. and Kerek, F. (1984) *Carbohydrate Res.* **131**, 209.
5. Gadek, P. A. and Quinn, C. J. (1985) *Phytochemistry* **24**, 267.
6. Geiger, H. and Quinn, C. (1982) In *Flavonoids: Advances in Research* (Harborne, J. B. and Mabry, T. J., eds) pp. 505–534. Chapman & Hall, London.
7. Xue, L. X. and Edwards, J. M. (1980) *Planta Med.* 220.
8. De Menez, N. L. (1980) in *Petaloid Monocotyledons* (Brickell, C. D., Cutler, D. F. and Gregory, M., eds) pp. 117–138. Academic Press, London.