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BIFLAVONES OF *DACRYDIUM* SENSU LATO

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Key Word Index—*Dacrydium*; *Falcatifolium*; Podocarpaceae; chemotaxonomy; biflavones; biflavonyls; amentoflavone; cupressuflavone; hinokiflavone.

Abstract—A survey of the biflavonyls in the leaves and branchlets of all three sections of the genus *Dacrydium* s.l. revealed a complex mixture of amentoflavone and its partial methyl ethers and hinokiflavone in most species. A group of three species in section C, however, were characterised by cupressuflavone derivatives as the major biflavone constituents. The results are used to discuss proposals for revised generic boundaries.

INTRODUCTION

Dacrydium Sol. ex Lamb. comprises 27 species found within the south-west Pacific from Malaya to New Zealand, and one outlying species, *D. fonkii* Benth., from Chile [1, 2]. It is a markedly heterogenous assemblage which Florin [3] subdivided into three sections, A, B and C, while admitting that section C was quite artificial. More recently Tengnér [1], de Laubenfels [2] and Quinn [4] have questioned the naturalness of the genus, making proposals for its division into two or more genera. The group presents, therefore, a promising field for a chemical taxonomist to provide an entirely new set of character-states with which to evaluate the proposals and to help define new generic boundaries.

This paper reports a survey of the biflavonyls contained in the leaves and small branchlets of representatives of all the species groups within the genus, and a discussion of the taxonomic implications of the results.

RESULTS

The reliability of the biflavonyl pattern within species of the family was first investigated. *Podocarpus elatus* R. Br. ex Endl. from Mt. Spec, North Queensland (lat. 18° 56′), was found to contain an identical mixture of biflavonyls to that isolated from material collected on Mt. Glorious, Southern Queensland (lat. 27° 20′). Neither could any differences be found between material of *P. spinulosus* (Sm.) R. Br. ex Mirb. from Beewah, Southern Queensland (lat. 26° 51′), and Patonga, New South Wales (lat. 33° 33′). Analyses of two different collections of *Dacrydium biforme*, and separate extracts of the adult foliage and the highly distinctive juvenile foliage of *D. kirkii* were made. The remaining species are represented by a single sample only.

The results of all the analyses on *Dacrydium* s.l. are given in Table 1. Bands assigned different numbers were separable in BPF, while those distinguished only by a letter were separated subsequently in BN or by permethylation. The bands have been subjectively classed according to their relative concentrations within the extract as major (++), sub-major (+), minor (m) and trace (t). Permethylations were performed on isolates marked superscript p in Table 1. These include representative isolates of all major and some minor bands. The permethyl ether obtained from each band is given at the bottom of the table.

Identification

Permethylation showed most bands to consist of amentoflavone derivatives only (Table 1). Co-chromatography with authentic samples lead to the identification of **1B** (amentoflavone), **4B** (bilobetin), **7** (isoginkgetin), **9A** (7″,4‴-dimethyl ether), **10B** (ginkgetin) and **16** (7,4′,7″,4‴-tetramethyl ether). Partial demethylation of an authentic sample of amentoflavone 7,4′,4‴-trimethyl ether produced compounds that cochromatographed with **10B** and **7** respectively, which supports the previous identifications of these two bands as the 7,4′- and 4′,4‴-dimethyl ethers. Partial demethylation of amentoflavone 7″,4‴-dimethyl ether yielded two monomethyl ethers that cochromatographed with **3C** and **4A** respectively, suggesting that these bands are the 7″ and 4‴ monomethyl ethers, but no determination of which is which has been made. Band **13B** cochromatographed in all solvents used with sciadopitysin (the 7,4′4‴-trimethyl ether), but the latter was found to be indistinguishable in these solvents from both kayaflavone (4′,7″,4‴-trimethyl ether) and heveaflavone (7,7″,4‴-trimethyl ether) isolated from *Araucaria cunninghamii* [6] and *Hevea brasiliensis* [7] respectively. The latter compound, however, was readily distinguishable by the absence of an acetate shift in its UV absorption spectrum, while N/50 sodium methoxide gave a bathochromic shift of 65 nm in band II without greatly reduced intensity. Both kayaflavone and sciadopitysin, having free 7OH groups, showed an acetate shift and virtual elimination of band II in the presence of N/50 sodium methoxide. Examination of the spectra of

Table 1. Biflavonyl patterns in *Dacrydium* sensu lato

	1A	**1B**	**1C**	**2A**	**2B**	**2C**	**3A**	**3B**	**3C**	**3D**	**4A**	**4B**	**5A**	**5B**	**7**	**8**	**9A**	**9B**	**10A**	**10B**	**12**	**13A**	**13B**	**14**	**15**	**16**	**17**	**18A**	**18B**	**19**
R_f in BPF 100/20/7	0.17	0.17	0.17	0.25	0.25	0.25	0.33	0.33	0.33	0.33	0.45	0.35	0.55	0.55	0.59	0.60	0.61	0.61	0.67	0.67	0.72	0.75	0.75	0.79	0.83	0.86	0.89	0.92	0.92	0.95
Section B																														
D. balansae		+^{p}	+				m^p		+^{p}			m^p					+ +^{p}	m		+			+ +^{p}							
D. cupressinum		+	m			m	m		+		m	m		m	m		+ +	m		+	m		+ +	m		t			+	
D. nidulum var. *araucarioides*	m	+							+		m	m					+ +			m			+ +						m	
Section C																														
D. franklinii		m					m		+		m		m	m			+ +	m					+ +			+		m		
D. colensoi				m	+^{p}		+		+		m		m	m			+ +	m			m						t	t		
D. biforme A				t	m				+					+					m	+^{p}	+ +^{p}		+ +		t				m	
D. biforme B	t			t	m				m					+	m				m	+ +	+ +		+ +							
D. hidwillii		m			m		m			+			m	m	+^{p}					m	+ +		+							
D. kirkii (adult)		m		t	+	m	m		+^{p}		m^p		m	m^p	+				m^p	+ +^{p}	+		+ +						t	
D. kirkii (juvenile)		m		t		m	m		+		m		m	m	+				m	+ +	+		+ +							
D. intermedium				t	m^p		m^p	+^{p}			m	m	m	m		+ +				m	m	+ +^{p}	t				m	m		+ +	
D. laxifolium				t				+					m			+ +^{p}				m	m	+ +^{p}	t				m	m		+ +^{p}	
D. fonkii								+^{p}			?					+ +^{p}					m^p	+ +								+ +^{p}	
Falcatifolium (=Section A)																														
F. papuanum	m	m				m			+ +		m			+ +					m	m	+ +		m							
Permethylation product		A			A		H	C	A		A	A		?	A	C	A		A	A	A	C	A							C

Key: A—amentoflavone hexamethyl ether, H—hinokiflavone pentamethyl ether, C—cupressuflavone hexamethyl ether, p—permethylation performed. **1B** = amentoflavone, **4B** = amentoflavone 4′-monomethyl ether, **7** = amentoflavone 4′,4‴-dimethyl ether, **9A** = amentoflavone 7″, 4‴-dimethyl ether, **10B** = amentoflavone 7,4′-dimethyl ether, **16** = amentoflavone 7,4′,7″,4‴-tetramethyl ether, **3A** = hinokiflavone, **19** = cupressuflavone 7,4′,7″,4‴-tetramethyl ether.

13B derived from six of the seven species in which it was a major band revealed that all conformed to the free 7OH pattern, but the distinction between kayaflavone and sciadopitysin has not been made.

Permethylation of band **3A** yielded hinokiflavone pentamethyl ether alone, and the original compound co-chromatographed with an authentic sample of hinokiflavone. No other band that was permethylated was found to contain hinokiflavone derivatives.

Apart from amentoflavone hexamethyl ether and hinokiflavone pentamethyl ether, two other permethylation products were obtained. One, derived from bands **3B**, **8**, **13A** and **19**, proved identical with the permethyl ether obtained from the major biflavonyl constituent of *Cupressus macrocarpa* Hartweg. The same product was also obtained by permethylation of extracts of the leaves of *Araucaria araucana* (Molina) K. Koch. In view of the reported occurrence of cupressuflavone or its partial methyl ethers in all species of both these genera that have been examined for biflavonyls [5, 8] and the absence of any other common group of biflavonyl, it is concluded that this third permethyl ether is cupressuflavone hexamethyl ether.

Bands composed only or largely of cupressuflavone derivatives, when sprayed with ethanolic aluminium chloride, fluoresced dark orange under long wave UV in contrast to the light to dark yellow fluorescence of all other bands. Bands **3B**, **8** and **19** yielded only cupressuflavone hexamethyl ether, while **13A** also gave a trace of amentoflavone hexamethyl ether. Since **13A** and **B** could not be separated chromatographically, it was concluded that **13A** includes a trace of **13B**.

Partial methylation of **8** yielded a large amount of a fast moving compound that absorbed long-wave UV, and possessed a UV absorption spectrum that showed no acetate or methoxide shift, but a marked bathochromic shift in bands I (21 nm) and II (8 nm) with $AlCl_3$. This compound was found to cochromatograph with **19**, which showed the same spectral features. This supports the identification of **19** as cupressuflavone 7,4′,7″,4‴-tetramethyl ether. Again a small amount of a slower moving compound was also formed, and this cochromatographed with **13A**. This band showed a bathochromic shift in band II (10 nm) with increased intensity and virtual elimination of band I in N/50 sodium methoxide, and a 15 nm bathochromic acetate shift in band II, which suggest that both 4′ positions are methylated and a 7 position is free. The original **8** appears to be a dimethyl ether. It shows a bathochromic shift of 40 nm in band I with N/50 sodium methoxide, and no shift in band II with sodium acetate, suggesting that both 7 positions are methylated and both 4′ positions are free. Support for this identification comes from the isolation of a cupressuflavone derivative from *Araucaria araucana* which co-chromatographed with **8**. Although there is no report in the literature on the biflavones of this species, cupressuflavone 7,7″-dimethyl ether has been found in three of the four species of *Araucaria* that have been investigated, including *A. bidwillii*, which belongs to the same section (Colymbea) as *A. araucana* [8]. The fourth permethylation product, which was obtained from **5B**, has not been identified.

Only a very small amount of material of *D. fonkii* was available, so that the major bands alone could be detected. The occurrence of cupressuflavone derivatives, however, in bands **3B**, **8**, **13A** and **19** was apparent from the fluorescence after spraying with $AlCl_3$, and was confirmed by permethylation of **3B**, **8** and **19**.

Chemotaxonomy

It can be seen from Table 1 that there are only minor differences in the biflavonyl pattern obtained from the two different collections of *Dacrydium biforme*, and at least some of these differences may be due to lack of detection of compounds that were present in only trace amounts. There is also a very close similarity between the pattern obtained from the juvenile foliage of *D. kirkii* and that from the adult foliage, the most notable difference being the absence of **2B** in the former. In neither case is there any variation in the major constituents. It appears, therefore, that the biflavonyl pattern is a fairly stable feature of a species, particularly as far as the major bands are concerned. This conclusion is supported by the comparisons of the biflavonyl patterns obtained from widely separated populations of two Australian species of the related genus *Podocarpus*, there being no variations in the major biflavonyl bands in either case.

Only one species of *Dacrydium* has previously been investigated for biflavones, *D. cupressinum* [9], for which the main biflavone was reported as amentoflavone 7,4′,7″,4‴-tetramethyl ether. The material of this species used in this study, however, contained only a trace of the tetramethyl ether, the major constituents being the 7″,4‴-dimethyl ether **9A** and a trimethyl ether **13B** of amentoflavone.

An examination of Table 1 reveals that the most outstanding discontinuity in the biflavonyl patterns is the presence of cupressuflavone derivatives as the major bands in only three species, *D. laxifolium*, *D. intermedium* and *D. fonkii*. This is the first report of this group of biflavones in the Podocarpaceae. Several amentoflavone derivatives are also present, but only in minor or trace amounts. On the basis of their biflavonyl pattern, then, these three species are closely related, and clearly stand apart from the other species examined. It is of particular interest that the sole Chilean species should appear so closely related to two of the New Zealand species. Although the closeness of this relationship has not been remarked previously, it is also apparent on the basis of cone structure and vegetative anatomy.

Tengner [1] placed *D. fonkii* in a separate subgroup, but an examination of his table shows that the three species (his groups II, 1a and II, 1b) are separated only by the presence of phloem fibres in *D. intermedium* and *D. laxifolium*, but not in *D. fonkii*. However, all three species alone among the species in section C possess cupressoid pits in the cross fields and adult leaves devoid of resin ducts.

The three species examined from section B (*D. cupressinum* from New Zealand, *D. balansae* from New Caledonia and *D. nidulum* var. *araucarioides* from New Guinea) show a high degree of similarity in their biflavonyl pattern. A major amount of amentoflavone 7″,4‴-dimethyl ether **9A** and a trimethyl ether **13B**, and a submajor concentration of amentoflavone **1B** and its monomethyl ether **3C** characterise all three. This supports the view based on morphological and anatomical criteria that this section is a highly uniform and natural grouping [3, 4].

Dacrydium biforme, *D. bidwillii* and *D. kirkii* all possess **13B** and **12** as major or sub-major constituents, and **5B**, **7** (isoginkgetin) and **10B** (ginkgetin) were isolated from all

three. Again, this supports the close relationship postulated for these species on the basis of other characters [4]. The presence of a major amount of **12** and a minor amount of **10A** separates them from all but *Falcatifolium papuanum*, while the absence of **9A** separates them from all the species listed higher in Table 1.

Dacrydium franklinii and *D. colensoi* show some similarity in biflavonyl pattern, although the latter is distinguished by the absence of **13B**, and **16**. Although *D. colensoi* does possess the wholly metacentric karyotype that appears to characterise section B [10], there is nothing to support such a relationship in the characters of cone morphology [4] or wood and leaf anatomy [1].

Falcatifolium papuanum is distinguished by having only a minor amount of **13B**, its major biflavonyls being **3C**, **5B** and **12**. This combination, which includes less highly methylated compounds as major bands than any other species examined, sets this species apart, and supports the recent separation of section A to form the genus *Falcatifolium* [2].

In conclusion, it can be stated that the biflavonyl patterns obtained from the leaves and branchlets of a representative selection of species of the genus *Dacrydium* sensu lato reveals marked discontinuities which allow the recognition of the following groups:

(1) *D. bidwillii*, *D. biforme* and *D. kirkii* (bidwillii group);

(2) *D. laxifolium*, *D. intermedium* and *D. fonkii* (laxifolium group);

(3) *D. balansae*, *D. nidulum* var. *araucarioides* and *D. cupressinum* (section B);

(4) *Falcatifolium papuanum* (*Falcatifolium* = section A).

The position of the remaining two species is not so clear, but they appear to be more closely related to one another than to any of the other species studied. There is strong support for these groupings in the distribution of character-states derived from previous studies of vegetative and reproductive morphology and anatomy, as well as from the karyotypes of the species. This study adds further support to the view that these groups should be recognised at the generic level. The formalisation of this taxonomic treatment will be dealt with elsewhere [11].

EXPERIMENTAL

Except for the New Caledonian and Chilean species, all plant material was collected specifically for this study from natural populations. Locations of voucher specimens and details of all collections are given in the Appendix.

Dried material of leaves and green twigs (since some species have scale leaves the two could not always be separated) were extracted in 70% EtOH at room temp. for at least 48 hr. Comparisons between initial and repeat extracts of the same material revealed no differences in relative concentrations of the various biflavonyls. The extractant was extracted with petrol (bp 60–80°) to remove excess oils, and chromatographed on paper in *n*-BuOH–HOAc–H_2O (4:1:5). The biflavonyls, which appeared under UV as a single absorbing band immediately behind the solvent front, were eluted with 1% HOAc in 70% EtOH and rechromatographed on semi-preparative plates of silica gel in toluene–HCO_2Et–HCO_2H (5:4:1). This yielded up to 5 bands that were subsequently refined and further divided on precoated silica gel plates developed in benzene–pyridine–HCO_2H (BPF). Two forms of this solvent were employed: 100:20:7 gave a good separation of bands having lower R_fs in the previous solvent, while 100:10:5 gave a better separation of the faster moving bands. A final separation was carried out on precoated cellulose plates developed in fresh BN (*n*-BuOH–2N NH_4OH, 1:1, upper layer [5]). The last 2 systems were then used to recheck the fractions isolated, and also to compare fractions isolated from different extracts.

Permethylations were performed by refluxing with Me_2SO_4 and anhyd. K_2CO_3 in dry acetone. The permethyl ethers were isolated using BPF (100:20:7) and compared in this solvent as well as $CHCl_3$–MeOH (4:96). Identification of the permethyl ethers was made by co-chromatography with permethyl ethers produced from authentic samples of amentoflavone and its 7″,4‴-di- and 7,4′,4‴-tri-methyl ethers, hinokiflavone, cryptomerin B and isocryptomerin.

Partial methylations were performed using CH_2N_2, and demethylations using pyridinium chloride.

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APPENDIX

Details of voucher specimens, which are lodged in the University of New South Wales herbarium unless stated otherwise, are as follows:

D. balansae Brong. Gris., J. B. Hair, cult. DSIR, Christchurch, New Zealand, 8.x.71.

D. bidwillii Hook. f., J. A. Rattenbury, 3, Tongariro National Park, New Zealand, v.70.

D. biforme (Hook.) Pilger, C. J. Quinn, 2, and 42, Tongarino National Park, New Zealand, 25.i.73.

D. colensoi Hook., J. A. Rattenbury, 4, Tongariro National Park, New Zealand, 1972.

D. cupressinum Lamb., J. B. Hair, 5, South Island, New Zealand, 8.x.71.

D. fonkii (Phil.) Benth., D. M. Moore and E. Pisano TBPA 1616. Puerto Bella Vista, Chile, 11.i.77.

D. franklinii Hook. f., C. J. Quinn, 45, Pieman River, Tasmania, 10. ii. 70.

D. intermedium Kirk, P. Fletcher 6082. Forks-Okarito Rd., South Westland, New Zealand, 1977.

D. kirkii F. Muell, ex. Parl., J. A. Rattenbury, Waitakare Ra., near Auckland, New Zealand, 1972. A. adult foliage; B, juvenile foliage.

D. laxifolium Hook. f., J. A. Rattenbury, 34, Tongariro National Park, New Zealand, 1972.

D. nidulum var. *araucarioides* de Laub., C. J. Quinn 4268, Margarima, New Guinea, 28.vi.74.

Falcatifolium papuanum de Laub., C. J. Quinn, 4298, Mt. Kaindi, New Guinea, 9.vii. 74.

Hevea brasiliensis Muell. Agr., A. D. E. Elmer, 20022, Sandakan, Myburgh Province, Brit. North Borneo, 10.xii.21.

Araucaria cunninghamii D. Don, J. T. Waterhouse and M. M. Hindmarsh, 7751, Ourimbah Creek, N.S.W., 13.x.78.