BIFLAVONES OF THE SUBFAMILY CUPRESSOIDEAE, CUPRESSACEAE

P. A. GADEK and C. J. QUINN

School of Botany, University of New South Wales, Kensington, NSW, 2033, Australia

(Received 8 May 1984)

Key Word Index—Cupressoideae; Cupressaceae; leaves; chemotaxonomy; biflavones; amentoflavone; cupressuflavone; hinokiflavone; taiwaniaflavone.

Abstract—Thirty species, representing all eight genera of the subfamily Cupressoideae, were examined for biflavonoid content of the leafy twigs. The major biflavonoid constituents are based on amentoflavone, cupressuflavone and hinokiflavone. The affinities suggested by biflavonyl distribution do not correlate with the currently recognized tribal groupings. There is evidence of closer links between northern and southern hemisphere genera than would be expected on the basis of the presently recognized subfamilies.

INTRODUCTION

The subfamily Cupressoideae sensu Li [1] includes all the northern genera of the family Cupressaceae. Although Li's basic division of the family into two subfamilies is still generally followed, subsequent authors have revised the status and affinities of some species within each of the subfamilies [2, 3]. Three tribes are recognized within the northern subfamily: Cupresseae Neger, including Cupressus L. (ca 20 spp.), Chamaecyparis Spach. (6 spp.) and Fokienia Henry & Thomas (1 sp.); Thujopsideae Endlicher, including Thuja L. (5 spp.), Thujopsis (L.f.) Siebold & Zuccarini (1 sp.), Biota Endl. (1 sp.) and Calocedrus Kurz. (3 spp.); and Junipereae Neger, containing Juniperus L. (ca 60 spp.).

This paper reports on a survey of representative species of all eight genera in the Cupressoideae, and, together with a previous paper [4], completes a chemotaxonomic survey of biflavone patterns in the leaves of the Cupressaceae.

RESULTS

Investigations on many of the species were conducted on small samples (20–50 g dry wt) obtained from herbarium specimens, but comparisons with the results of larger analyses where abundant material was available indicated that such samples were adequate for the isolation and characterization of the major biflavonyl constituents. In fact, permethylation of small-scale crude extracts often allowed the detection of trace amounts of a biflavonyl series that could not be detected by TLC of a larger-scale unmethylated extract of the same material.

The biflavonyl patterns obtained are given in Table 1, and the results of analyses of permethylated leaf extracts from a broader range of species are given in Table 2.

DISCUSSION

An examination of Tables 1 and 2 shows that typically the major biflavonyls are derived from three parental structures: viz. amentoflavone, cupressuflavone and hinokiflavone. This is in agreement with previous reports for the family, although as some authors have pointed out [5-7], many early reports on the biflavonyl content of conifer leaves were obviously incomplete, as only some of the major biflavonyls were reported. Despite the small size of samples used in this study and the reliance on TLC techniques, a comparison of our data with those of previous studies on the same species gives no indication of any consequent lack of sensitivity to minor fractions.

Cupressus torulosa D. Don and C. sempervirens were the original source from which the 8,8"-linked biflavone, cupressuffavone, was first isolated [8]. The three species of Cupressus surveyed here, including a sample of C. sempervirens, contained cupressuflavone and the 3,8"-linked amentoflavone as the major biflavonyls. A total of seven species of this genus have now been examined for biflavonyl content of the leaves. All are reported to contain cupressuflavone, and all but one amentoflavone [7, 9-12]. The report of cupressuffavone alone in the leaves of C. arizonica [12] appears likely to be an incomplete report, since these authors also reported cupressuflavone alone in C. goveniana. Two subsequent analyses of the latter species [7, 11] have revealed the presence of the amentoflavone and hinokiflavone series in addition to cupressuflavone. The hinokiflavone series, however, is of variable occurrence in Cupressus species, having been reported only from C. goveniana, C. lusitanica, C. funebris and C. torulosa [7, 9, 11]. Previous reports of its absence from C. sempervirens [7, 10] were confirmed in this survey; neither was it found in C. glabra. The previous report of the presence of a minor amount of hinokiflavone in C. lusitanica [9] was also confirmed by the detection of a trace of hinokiflavone pentamethyl ether in the permethylated extract of this species. Hence, the genus Cupressus is typified by a leaf biflavonyl pattern having major amounts of amentoflavone and cupressuflavone, with hinokiflavone and some minor monomethyl ethers also often present.

Chamaecyparis, on the other hand, is chemically heterogeneous. All four species analysed contain amentoflavone and a range of its partial methyl ethers (at least some of the latter as major constituents), and also hinokiflavone. C. nootkatensis alone contains cupressuflavone. This species has been reported to be phytochemically atypical of the

Table 1	1.	Biflavonyl	derivatives	detected	in	leaf	extracts
---------	----	------------	-------------	----------	----	------	----------

								Inte	rflavony	l link						
					3'8"	_			88"	6-0	-4‴	3′:	3″		3′6″	
Tribe	Taxon	1*	2	3	4	5	6	7	8	9	10	11	12	13	14	15
C	Cupressus sempervirens	+	m						+							
C	C. lusitanica	+	m						+							
J	Juniperus drupacea	+							+	+						
J	J. communis	+							m							
J	J. oxycedrus	+							+							
J	J. virginiana	+	t			t			+	m						
J	J. excelsa	+							+	+						
J	J. procera	+	m						+							t
J	J. bermudiana	+				m			m	+				m		t
T	Calocedrus decurrens	+	m				m		+	m		+	m			
T	Biota orientalis	+	m						m	+						
T	Thuja occidentalis	+	m			m		m	m	+						
C	Chamaecyparis nootkatensis	+	+		m	m			m	+				m		
T	Thujopsis dolobrata	m	t	m		+		+		+	t			m	m	
C	Fokienia hodginsii	+	m		+	+				+				t		
C	Chamaecyparis formosansis	+	+		m	+		m		m						
C	C. thyoides	+		+		m		m		+						
C	C. lawsoniana 'Erecta'	+	+			m		m		m						
T	Thuja koraiensis	m	m			m		m		m						
Т	T. standishii	m				t										
T	T. plicata	+	m													

*Compound 1, amentoflavone; 2, 4"-monomethylamentoflavone; 3, 4'-monomethylamentoflavone; 4, 4'4"-dimethylamentoflavone; 5, 7,4'-dimethylamentoflavone; 6, 7",4"-dimethylamentoflavone; 7, trimethylamentoflavone; 8, cupressuflavone; 9, hinokiflavone; 10, 7"-monomethylhinokiflavone; 11, taiwaniaflavone; 12, monomethyltaiwaniaflavone; 13, robustaflavone; 14, monomethylrobustaflavone; 15, dimethylrobustaflavone. +, Major band; m, minor band; t, trace detected by TLC only; C, Cupresseae; J, Junipereae; T, Thujopsideae.

genus Chamaecyparis, particularly in being rich in modified leaf oil terpenes, a feature that links it with Cupressus and Juniperus rather than Chamaecyparis [13-15]. The occurrence of the cultivated hybrid × Cupressocyparis leylandii (Jack. & Dallim.) Dallim., a 'spontaneous' hybrid between Cupressus macrocarpa Hartw. and Chamaecyparis nootkatensis [2], also supports a close affinity between C. nootkatensis and Cupressus. C. nootkatensis is also distinctive within the genus Chamaecyparis in characters of the leaf cuticle [16], wood anatomy [17, 18] and leaf and seed morphology [2, 3].

Chamaecyparis thyoides is distinguished by the presence of a major band of the 4'-methyl ether of amentoflavone, rather than the 4"-methyl ether found in the remaining three species. This difference again correlates with a discontinuity in wood, leaf and seed characters between this species and the remainder of the genus [2, 3, 17].

The third genus of the tribe Cupresseae, the monotypic Fokienia, most closely resembles Chamaecyparis formosansis in its biflavonyl pattern, having major amounts of amentoflavone and its 4',4"- and 7,4'-dimethyl ethers, and hinokiflavone.

Within the tribe Thujopsideae, the genera again show marked heterogeneity. Of the four species of *Thuja* examined, only *T. occidentalis* contains cupressuflavone, and data on leaf characters and wood anatomy [2, 3, 17] lend support to this discontinuity within the genus.

T. koraiensis contains hinokiflavone, amentoflavone and a range of its partial methyl ethers in more or less equal concentrations. T. standishii and T. plicata each contain detectable amounts of amentoflavone and a single monomethyl ether only, although the hinokiflavone series is clearly detectable in the permethylated extract of both (Table 2).

The monotypic genus *Biota* shows some similarity in biflavone content to *T. occidentalis*, containing the same major biflavones (amentoflavone and hinokiflavone) as well as minor amounts of cupressuflavone and of the 4"-monomethyl ether of amentoflavone.

The other monotypic genus in this tribe, *Thujopsis*, displays a distinctive biflavonyl pattern in which the more highly methylated amentoflavone derivatives are the major constituents and both robustaflavone and its monomethyl ether are detectable.

Calocedrus decurrens, the only member of this genus to be examined, is distinguished by the presence of major amounts of cupressuflavone and the 3',3"-linked taiwania-flavone, as well as a minor constituent, the 7",4"'-dimethyl ether of amentoflavone, a unique combination in the family and also in the order. The taiwaniaflavone series was first identified from Taiwania cryptomerioides (Taxodiaceae) [19, 20], and its occurrence in Calocedrus may lend some support to the views of previous workers that this genus is chemically atypical of the Cupressaceae

Table 2. Permethyl ethers detected in permethylated leaf extract of Cupressaceae

Tribe	Taxon	Am	Cu	Hi	Tw	Ro	Ag
C	Cupressus sempervirens	+	+				
C	C. lusitanica	+	+	t			
C	C. glabra	+	+				
J	Juniperus drupacea	+	+	+		t	
J	J. communis	+	+	+		t	
J	J. oxycedrus	+	+	m		t	
J	J. virginiana	+	+	+		t	t
J	J. excelsa	+	+	m		t	
J	J. procera	.+	+	+		t	t
J	J. bermudiana	+	+	+		t	
J	J. conferta	+	+	+		t	
J	J. deppeana	+	+				
J	J. monosperma	+	+	+		t	t
J	J. chinensis	+	+	+		t	
J	J. californica	+	+	+			
J	J. foetidissima	+	m	m			
T	Calocedrus decurrens	+	+	+	+	t	
T	Biota orientalis	+	m	+		t	
T	Thuja occidentalis	+	m	+		t	
С	Chamaecyparis nootkatensis	+	m	+		m	
T	Thujopsis dolobrata	+		+		m	
C	Fokienia hodginsii	+		+		t	
C	Chamaecyparis formosansis	+		+		t	
C	C. thyoides	+		+		t	
C	C. lawsoniana 'Erecta'	+		+		t	
C	C. obtusa	+		+			
С	C. pisifera 'Squarrosa'	+		+		t	
T	Thuja koraiensis	+		+			
T	T. standishii	+		+			
T	T. plicata	+		+		t	

Am, Amentoflavone hexamethyl ether; Cu, cupressuflavone hexamethyl ether; Hi, hinokiflavone pentamethyl ether; Tw, taiwaniaflavone hexamethyl ether; Ro, robustaflavone hexamethyl ether; Ag, agathisflavone hexamethyl ether; +, major band; m, minor band; t, trace detected by TLC only; C, Cupresseae; J, Junipereae; T, Thujopsideae.

[13, 15]. However, we have now determined that the unidentified permethyl ether (U2) previously reported in Neocallitropsis pancheri (Carr.) de Laub. [4] was also taiwaniaflavone hexamethyl ether. The occurrence of this rare series of biflavonyls in three such widely placed species poses a problem of interpretation.

All the species of Juniperus surveyed contained a major amount of amentoflavone as well as some cupressuflavone; hinokiflavone derivatives were detected, at least in the permethylated extract, in all but one. Few partial methyl ethers were detected. Twelve species of Juniperus have previously been examined for leaf biflavones, in several cases by more than one worker [10, 21-26]; apparent contradictions in these reports appear to be mainly due to incomplete analyses. Thus, for J. communis, Lamer-Zarawska [21] records cupressuflavone, amentoflavone and 4'-monomethylamentoflavone, while Pascual Teresa et al. [26] record cupressuflavone and hinokiflavone. Our own study of this species reveals the presence of all three series of biflavones. Amentoflavone has been reported in all 12 species, and cupressuflavone in all but four of them: viz. J. sabina L., J. squamata Buch. Ham., J. occidentalis Hook. f. and J. virginiana [21]. The last species was included in our survey, and was found to contain major amounts of both cupressuflavone and amentoflavone. Since the data for the other three species are drawn from the same report [21], it seems probable that a careful re-examination of these species would also reveal cupressuflavone as a major biflavonyl. Typically, cupressuflavone is present unmethylated, and there is only one report, for *J. recurva*, of a partial methyl ether (7,7"-dimethylcupressuflavone) constituting the major cupressuflavone component [23]. Interestingly, this dimethyl ether also constitutes the major cupressuflavone component in several callitroid genera [4].

J. drupacea is morphologically separated from other Juniperus species by its broader leaves and larger cones [2, 3], and has sometimes been separated from the remaining species at either the generic [1] or subgeneric [3] level. Its biflavonyl pattern is, however, indistinguishable from those of the other Juniperus species surveyed, which favours its placement with those species.

The presence of traces of other biflavonyls, particularly robustaflavone, was detectable in some species of all the cupressoid genera except *Cupressus*; a wider survey of that genus would be needed before any significance can be

attached to this distribution. The occurrence of a trace of the agathisflavone series in three species of *Juniperus* also appears to us to be of little systematic significance.

The data in Tables 1 and 2 have been used to group the taxa as follows:

- Cupressus and Juniperus—The biflavonyl pattern of these genera comprises major amounts of amentoflavone and cupressuflavone; the more highly methylated biflavones are typically absent.
- Calocedrus—Assuming that the single species examined is typical of the other two, this genus is distinguished by the presence of taiwaniaflavone and 7",4"-dimethylamentoflavone, both unique amongst the cupressoid genera, as well as a major amount of cupressuflavone.
- Biota orientalis, Thuja occidentalis and Chamaecyparis nootkatensis—These are characterized by the presence of minor amounts of cupressuflavone in combination with hinokiflavone and amentoflavone and its partial methyl ethers.
- 4. The remaining taxa constitute a rather heterogeneous group that is distinguished by the absence of cupressuflavone. All are characterized by the presence of amentoflavone and varying numbers of its partial methyl ethers, while hinokiflavone or its partial methyl ethers are detectable at least in the permethylated extracts. Thujopsis dolobrata and Chamaecyparis thyoides are characterized within the group by the presence of the 4'-monomethylamentoflavone in contrast to the more usually occurring 4"'-monomethyl ether, and the former species is further characterized by

robustaflavone and its monomethyl ether. Fokienia hodginsii and Chamaecyparis formosansis share the 4',4"'-dimethylamentoflavone (as also does C. nootkatensis) in addition to the more usual 7,4'-dimethyl ether.

The biflavonyl data presented in this and the previous survey [4] of the Cupressaceae show that the chemical discontinuities do not correlate closely with the existing taxonomy, particularly at the tribal and subfamilial levels. There is a marked similarity between some of the biflavonyl patterns in both subfamilies, suggesting closer affinities than is indicated by the present division into northern and southern genera. A summary of groupings suggested by biflavonyl pattern is set out in Table 3.

The presence of the group 1 biflavonyl pattern in both Cupressus and Juniperus suggests an affinity between these genera that is not indicated by the present tribal groupings. Tetraclinus articulata displays an identical pattern to that of the group 1 genera, having major amounts of cupressuflavone and amentoflavone, together with a minor amount of 4"-monomethylamentoflavone and a trace of a hinokiflavone derivative [4].

The pattern in Fitzroya, Diselma and Wadringtonia is distinct from the above group, cupressuffavone being replaced by its dimethyl ether, and the latter two genera being typified by major amounts of hinokiflavone and/or amentoflavone partial methyl ethers. Austrocedrus, Libocedrus, Papuacedrus and Pilgerodendron show a close similarity to the group 4 pattern, having a major amount of amentoflavone and trace amounts of hinokiflavone derivatives, but being devoid of cupressuffavone.

The presence of taiwaniaflavone and its partial methyl

Table 3. Taxa grouped by biflavonyl patter	Table	3.	Taxa	grouped	by	biflavonyl	patter
--------------------------------------------	-------	----	------	---------	----	------------	--------

Group	Tribe	Taxa	Pattern
1	С	Cupressus	Major amounts of amentoflavone
	J	Juniperus	and cupressuffavone; methylated
	Te	Tetraclinus	biflavonyls mostly absent
2	A	Fitzroya	Partially methylated biflavones con-
	L	Diselma	spicuous; major amounts of amen-
	L	Widdringtonia	toflavone and 7,7"-dimethyl- cupressuflavone
3	Tj	Biota	Major amounts of amentoflavone
	Tj	Thuja occidentalis	and hinokiflavone, plus a minor
	C	Chamaecyparis nootkatensis	amount of cupressuflavone
4	Tj	Thujopsis and remaining spp. of Thuja	Amentoflavone partial methyl ethers conspicuous; variable
	С	Fokienia and remaining spp. of Chamaecyparis	amounts of hinokiflavone; cup- ressuflavone absent
	L	Libocedrus	
	L	Papuacedrus	
	L	Austrocedrus	
	L	Pilgerodendron	
5	A	Actinostrobus	Amentoflavone only, with cup-
	A	Callitris	ressuflavone and hinokiflavone undetectable
6	Tj	Calocedrus	Characterized by the presence of
	L	Neocallitropsis	taiwaniaflavone

A, Actinostrobeae; C, Cupresseae; J, Junipereae; L, Libocedreae; Te, Tetraclineae; Tj, Thujopsideae.

Table 4	Chromatographic and	spectral data of permethylated biflayones
I ADIC 4.	Chromatographic and	i soccurat dala of defidentifiated dillavones

	1	R₅s*		****		
	BPF	BPEFD	Fluoresc.†	UV spectra (λ _{max} ^{MeOH})‡	Emission (nm)§	
Hexa-O-methylamentoflavone	0.37	0.40	Yellow	266, 328	460	
Hexa-O-methylcupressuflavone	0.41	0.45	Orange	268, 324	470	
Hexa-O-methyltaiwaniaflavone	0.42	0.58	Lt. blue	264, 324	427	
Hexa-O-methylagathisflavone	0.46	0.51	Yellow	266, 322	_	
Hexa-O-methylrobustaflavone	0.50	0.68	Lt. blue	263, 324	_	
Penta-O-methylhinokiflavone	0.53	0.75	Lt. blue	265, 323	437	

 $[*]R_f$ values are variable, but the relative positions of the permethyl ethers in each solvent are characteristic.

ethers as minor fractions in Neocallitropsis suggests an affinity with Calocedrus, though the absence of even trace amounts of cupressuflavone from the former clearly distinguishes it. Indeed, the atypical phyllotaxis and leaf morphology of Neocallitropsis leads one to question whether it is correctly placed in the Cupressaceae. There is certainly nothing in the biflavonyl patterns to support the contention of de Laubenfels that this monotypic genus is closely related to Callitris neocaledonica [27]. The fact that taiwaniaflavone also occurs in the Taxodiaceae raises the suggestion of a close relationship between the Cupressaceae and Taxodiaceae that has been commented on by several authors [28-30]. This compound is not, however, a common constituent in either family. We have been unable to detect it in leaf extracts of Athrotaxis selaginoides, Sequoia sempervirens or Cunninghamia lanceolata. Nor has cupressuflavone, which occurs in a number of cupressaceous genera, been detected or reported in any taxodiaceous species [5, 6]. Hence the taxonomic significance of the distribution of taiwaniaflavone in these three diverse genera will only be revealed by a detailed analysis of other data sources in order to reassess their affinities properly.

It is apparent that the affinities of species and genera suggested by their biflavonyl patterns highlight many inconsistencies in the present tribal and possibly familial groupings, and underlie a need noted by other authors [2, 3] for a critical reappraisal of the taxonomy of the family. A re-definition of the taxa, based on a reassessment of a broad range of character-states, is currently under way in this laboratory and will be reported elsewhere.

EXPERIMENTAL

Details of voucher specimens are given in the Appendix. Extraction and identification of biflavonyls were carried out by the methods described previously [4, 31]. Standards of agathis-flavone and its hexamethyl ether were prepared from Araucaria bidwillii Hook.; standards of robustaflavone and its hexamethyl ether were prepared from Agathis robusta (C. Moore ex F. Muell.) F. M. Bail. [32]. Taiwaniaflavone was extracted from Taiwania cryptomerioides Hayata [19, 20]. Details of the chromatographic behaviour and UV spectra of the permethylated biflavones, repurified using an RP C-18 HPLC column, are given in Table 4.

Acknowledgements—We thank Mr. D. Symon of the Waite Institute, University of Adelaide, South Australia for assistance in obtaining plant material; Dr. L. A. S. Johnson, Director, for permission to sample specimens held by the National Herbarium of N.S.W. and the Royal Botanic Gardens; Dr. G. D. McPherson, Herbarium, Missouri Botanical Gardens, for specimens of Neocallitropsis pancheri; and Dr. I. McFarlane, School of Biochemistry, University of New South Wales, for assistance with HPLC equipment.

REFERENCES

- 1. Li, H. (1953) J. Arnold Arbor. Harv. Univ. 34, 17.
- Dallimore, W. and Jackson, A. B. (1966) A Handbook of Coniferae and Ginkgoaceae (Revised by Harrison, S. G.) 4th edn Edward Arnold, London.
- Gaussen, H. (1968) Trav. Lab. Forest. Toulouse Pt 2, Fasc. 10, 13, 1.
- 4. Gadek, P. A. and Quinn, C. J. (1983) Phytochemistry 22, 969.
- Geiger, H. and Quinn, C. J. (1975) The Flavonoids (Harborne, J. B., Mabry T. J., and Mabry, H., eds.), pp. 692-742. Chapman & Hall, London.
- Geiger, H. and Quinn, C. J. (1982) The Flavonoids: Advances in Research (Harborne, J. B. and Mabry, T. J., eds.), pp. 505-534. Chapman & Hall, London.
- Natarajan, S., Murti, V. V. S. and Seshadri, T. R. (1970) Phytochemistry 9, 575.
- Murti, V. V. S., Raman, P. V. and Seshadri, T. R. (1964) Tetrahedron Letters 2995.
- Taufeeq, H. M., Fatma, W., Ilyas, M., Rahman, W. and Kawano, N. (1978) Indian J. Chem. Sect. B 16, 655.
- Lebreton, P., Boutard, B. and Sartre, J. (1978) Bull. Inst. Sci. 155.
- Taufeeq, H. M., Mohd, F. and Ilyas, M. (1979) Indian J. Chem. Sect. B 17, 535.
- Miura, H. and Kawano, N. (1968) J. Pharm. Soc. Jpn. 88, 1459.
- 13. Lebreton, P. (1982) Candollea 37, 243.
- Erdtman, H. and Norin, T. (1966) Fortschr. Chem. Org. Naturst. 24, 206.
- 15. von Rudloff, E. (1975) Biochem. Syst. Ecol. 2, 131.
- Alvin, K. L., Dalby, D. H. and Oladele, F. A. (1981) The Plant Cuticle (Cutler, D. F., Alvin, A. L. and Price, C. E., eds.), pp. 379-396. Academic Press, London.
- 17. Young, D. J. and Watson, L. (1969) New Phytol. 68, 427.
- Bauch, J., Liese, W. and Schultze, R. (1972) Wood Sci. Technol. 6, 165.

[†] As observed on plates run in BPF and dried in a hood for ca 0.5 hr. The residual formic acid which remains on the plate will partially affect the fluorescence colour (see §).

[‡]Compounds purified using an RP C-18 HPLC column (updating the previously reported data [4]).

[§]Emission maximum at 320 nm (uncorrected), in MeOH, characterizing the fluorescence of the free compounds (as opposed to the fluorescence of the partially protonated form illustrated by †).

- Kamil, M., Ilyas, M., Rahman, W., Hasaka, N., Okigawa, M. and Kawano, N. (1977) Chem. Ind. 160.
- Kamil, M., Ilyas, M., Rahman, W., Hasaka, N., Okigawa, M. and Kawano, N. (1981) J. Chem. Soc. Perkin Trans. 1, 553.
- 21. Lamer-Zarawska, E. (1975) Pol. J. Pharmacol. Pharm. 27, 81.
- Fatma, W., Taufeeq, H. M., Shaida, W. A. and Rahman, W. (1979) Indian J. Chem. Sect. B 17, 193.
- Hameed, N., Ilyas, M., Rahman, W., Okigawa, M. and Kawano, N. (1973) Phytochemistry 12, 1494.
- Ilyas, M., Ilyas, N. and Wagner, H. (1977) Phytochemistry 16, 1456.
- Pelter, A., Warren, R., Hameed, N., Ilyas, M. and Rahman, W. (1971) J. Indian Chem. Soc. 48, 204.
- Pascual Teresa, J. de, Barrero, A. F., Muriel, L., San Feliciano, A. and Grande, M. (1980) Phytochemistry 19, 1153.
- de Laubenfels, D. J. (1972) in Flora de le Nouvelle Caledonie et Dependences, (Aubreville, A. and Leroy, J., eds.), Vol. 4, pp. 144-164. Museum National d'Histoire Naturelle, Paris.
- 28. Keng, H. (1975) Taxon 24, 289.
- 29. Eckenwalder, J. E. (1976) Madrono 23, 237.
- 30. Eckenwalder, J. E. (1976) Taxon 25, 337.
- 31. Gadek, P. A. and Quinn, C. J. (1982) Phytochemistry 21, 248.
- Wannan, B., Waterhouse, J. T., Gadek, P. A. and Quinn, C. J., Biochem. Syst. Ecol. (in press).
- Stafleu, F. A. (1981) Index Herbariorum Part 1. Dr. W. Junk, The Hague.

APPENDIX

Location and collecting details of voucher specimens are given below. Abbreviations of herbaria follow Index Herbariorum [33]. Cupressus sempervirens L. UNSW 10339, Gadek 13. viii. 1980, cult. RBG, NSW. C. lusitanica Mill. UNSW 10336, Gadek 13. viii. 1980, cult. RBG, NSW. C. glabra Sudworth UNSW 10340, Gadek 13. viii. 1980, cult. RBG, NSW. Juniperus drupacea Labillardiere NSW, Hartfield ii. 1899, cult. RBG, NSW. J. communis L. UNSW 10343, Martin 7. vi. 1961, Kamloops, British Colombia, Canada. J. communis L. UNSW, Symon 1. ii. 1982, cult. Waite Arboretum 744, SA. J. oxycedrus L. NSW, Ferguson 2924 11. ii. 1971, Sierra des Mos., Spain. J. oxycedrus L. UNSW, Symon 1. ii. 1982, cult. Waite Arboretum 740, SA. J. virginiana L. UNSW, Symon 1. ii. 1982, cult. Waite Arboretum 570, SA. J. virginiana L. UNSW, Symon 1. ii. 1982, cult. Waite Arboretum 617, SA. J. excelsa Bieb. UNSW, Symon 1. ii. 1982, cult. Waite Arboretum 1272, SA. J. procera Hochst. UNSW,

Symon 1. ii. 82, cult. Waite Arboretum 742, SA. J. bermudiana L. UNSW 10338, Gadek 13. viii. 1980, cult. RBG, NSW. J. conferta Parl. UNSW 10335, Gadek 13. viii. 1980, cult. RBG, NSW. J. deppeana Steud. UNSW, Symon 1. ii. 1982, cult. Waite Arboretum 1273, SA. J. monosperma (Engel.) Sarg. UNSW, Symon 1. ii. 1982, cult. Waite Arboretum 1278A, SA. J. chinensis L. UNSW, Symon 1. ii. 1982, cult. Waite Arboretum 1273A, SA. J. californica Carr. NSW, Clokey 7823 27. vii. 1938, Charleston Mts., Nevada, U.S.A. J. foetidissima Wild. NSW, Waleres i. 1947, Troodes Forest, Cyprus. Calocedrus decurrens (Torr.) Florin NSW, Parks 24251 viii. 1943, Darlington, Del Norte County, U.S.A. C. decurrens (Torr.) Florin UNSW, Symon 1. ii. 1982, cult. Waite Arboretum 1242. SA. Biota orientalis (L.) Endl. UNSW 10337, Gadek 13. viii. 1980, cult. RBG, NSW. B. orientalis (L.) Endl. NSW 12.i. 1922, cult. Beauport Park, U.K. Thuja occidentalis L. UNSW 10334, Gadek 13. viii. 1980, cult. RBG, NSW. T. occidentalis L. UNSW, Symon 1. ii. 1982, cult. Waite Arboretum 551, SA. T. koraiensis Nakai NSW Headfort 15. viii. 1940, cult. Kells. Co. Meath, Ireland. T. standishii (Gordon) Carr. NSW O'Bytne 6 23. ix. 1949, cult. RBG Kew, U.K. T. plicata D. Don NSW Metcalf viii. 1920, Nelson, British Columbia, Canada. T. plicata D. Don NSW Calder, Parmelee & Taylor 18552 7. vii. 1956, Bella Coota, British Columbia, Canada. Thujopsis dolobrata (L.f.) Sieb. et Zucc. NSW, Togasi 24. viii. 1956, Mt. Zaozan, Echigo, Japan. Fokienia hodginsii (Dunn.) Henry and Thomas NSW, McIndoe 21.ii. 1963, cult. RBG, NSW. Chamaecyparis nootkatensis (D. Don) Spach NSW Calder, Parmelee & Taylor 19471 26. vi. 1956, Mt. Arrowsmith, Vancouver Isl., Canada. C. formosansis Matsumura NSW Wilson 9764 2.ii. 1918 Gisan, Prov. Kagi, Formosa. C. thyoides (L.) Britten, Sterns and Poppenberg NSW, Lawrence and Dress 295 20. v. 1948, Penn State Forest, New Jersey, U.S.A. C. lawsoniana (A. Murray) Parl. 'Erecta' UNSW 7164 Quinn 26. ii. 1980, cult. Sydney, NSW. C. obtusa (Sieb. et Zucc.) Endl. UNSW 10341 Gadek 13. viii. 1980, cult. RBG, NSW. C. pisifera (Sieb. et Zucc.) Endl. 'Squarrosa' UNSW 10342 Gadek 13. viii. 1980, cult. RBG, NSW. Taiwania cryptomerioides Hay. NSW Wilson 9836 4. ii. 1918, Arisan, Prov. Kagi, Formosa. Athrotaxis cupressoides D. Don. UNSW 4312, Quinn 2. i. 1975, Pine Lake, Tas. Sequoia sempervirens (Lamb.) Endl. UNSW, Evans, cult. RBG, NSW. Cunninghamia konishii Hay. UNSW 14294, Gadek 18.x. 1983, cult. RBG, NSW. Abbreviations: RBG, Royal Botanic Gardens; NSW, New South Wales, Australia; SA, South Australia, Australia; Tas, Tasmania, Australia; U.S.A., United States of