Phytochemistry, Vol. 29, No. 2, pp. 501–507, 1990.
Printed in Great Britain.

0031-9422/90 $3.00+0.00

FLAVONOID PROFILES OF NEW ZEALAND *LIBOCEDRUS* AND RELATED GENERA

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(*Received* 26 *May* 1989)

Key Word Index—*Libocedrus bidwillii*; *L. plumosa*; *Austrocedrus*; *Papuacedrus*; *Neocallitropsis*; *Calocedrus*; Cupressaceae; flavonoids; biflavonoids; 8-hydroxyluteolin and 8-hydroxyapigenin 7-xylosides; 7-*O*-methyl-2,3-dihydroamentoflavone; quercetin 3-*O*-α-[2-*p*-hydroxybenzoyl-4-*O*-*p*-coumaroylrhamnopyranoside].

Abstract—Flavonoids common to both *Libocedrus bidwillii* and *L. plumosa*, which were sampled throughout New Zealand, are: kaempferol and quercetin 3-rhamnoside, kaempferol and quercetin 3-rhamnoside-7-glucoside, quercetin 3-glucoside, apigenin and luteolin 7-glucoside, luteolin 7-di- (and tri)-glucosides, amentoflavone, 7-*O*-methylamentoflavone, 2,3-dihydroamentoflavone, and the new flavonoids, 8-hydroxyapigenin and 8-hydroxyluteolin 7-*O*-xylosides and 7-*O*-methyl-2,3-dihydroamentoflavone. *Libocedrus plumosa* is distinguished by the additional accumulation of myricetin 3-rhamnoside, and *L. bidwillii* by the presence of quercetin 3-*O*-α-[2-*p*-hydroxybenzoyl-4-*O*-*p*-coumaroylrhamnopyranoside] which was found amongst the biflavones. A chromatographic survey of some related non-New Zealand species and genera is also reported.

INTRODUCTION

An extensive chemotaxonomic investigation of the flavonoids of New Zealand conifers has been underway now for several years. The New Zealand representatives of the Podocarpaceae have received most attention with published reports on *Podocarpus sensu lato* [1] and associated hybrids [2], *Phyllocladus* [3] and *Dacrydium sensu lato* [4, 5]. These studies have supported proposed subdivisions of both *Podocarpus* and *Dacrydium* and have been used to define individual species and genera, and the origins of natural hybrids. Of the other New Zealand conifers, only representatives of the Cupressaceae and the Araucariaceae remain to be investigated. The present paper reports on the former group.

The family Cupressaceae is presently divided into two subfamilies *sensu* Li [6]: Cupressoideae, with eight Northern Hemisphere genera (including *Calocedrus*) divided among three tribes (Cupresseae, Thujopsideae, Juniperae); and Callitroideae, including the monotypic Northern Hemisphere genus *Tetraclinus* and 10 Southern Hemisphere genera divided among three tribes (Actinostrobeae, Libocedreae Tetraclineae). The New Zealand representatives of the family, *Libocedrus bidwillii* and *L. plumosa*, are placed in the tribe Libocedreae, along with their close relatives *L. austro-caledonica*, *L. chevalieri* and *L. yateensis* of New Caledonia, and the related genera *Papuacedrus*, *Austrocedrus*, *Pilgerodendron*, *Neocallitropsis*, *Widdringtonia* and *Diselma*.

Previous studies of the flavonoids of the Cupressaceae have tended to concentrate on Northern Hemisphere genera of the subfamily Cupressoideae [7]. However, an early survey of biflavonoids [8] covered several species and three genera from each subfamily, including *Libocedrus plumosa* of New Zealand. More recently, Gadek and Quinn reported on the biflavones of representatives of all the genera in both subfamilies [9,10]. Their evidence casts doubt on the validity of the present classification, doubt previously expressed by de Laubenfels [11] and others on botanical grounds, underlying the need for a critical reassessment of the taxonomy of the family. No previous studies of the flavonoid glycoside chemistry of the two New Zealand representatives of the family, *Libocedrus bidwillii* and *L. plumosa* have been undertaken.

RESULTS AND DISCUSSION

Two-dimensional-PC analyses of the two New Zealand *Libocedrus* species, *L. bidwillii* ("Kaikawaka") and *L. plumosa* ('Kawaka') revealed that they have similar flavonoid glycoside patterns, together with an unresolved complex of aglycone material. However, one major flavonoid glycoside clearly visible in *L. plumosa*, was absent from *L. bidwillii*.

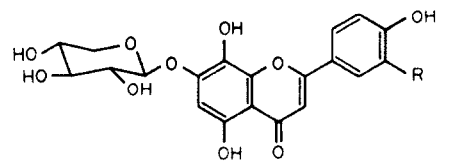
Flavonoid glycoside components

The constituent flavonoids from both species were isolated by column chromatography, polyamide being used for the *L. bidwillii* flavonoids and cellulose for *L. plumosa*. Most flavonoid glycosides from *L. bidwillii* were isolated from the 60–80% methanol fraction and were separated and purified by PC prior to the final clean-up on a reversed phase column. Standard analytical techniques [12] and direct comparison with authentic material, permitted the identification of the following major (ma) and minor (mi) glycosides: kaempferol 3-rhamnoside (ma), quercetin 3-rhamnoside (ma), quercetin 3-glucoside (mi), apigenin 7-glucoside (mi) and luteolin 7-glucoside (mi). Additional flavonoids isolated from *L. plumosa* and identified by standard analytical techniques were myricetin 3-rhamnoside (ma), kaempferol and quercetin 3-rhamnoside-7-glucoside (mi), luteolin 7-diglucoside (mi) and luteolin 7-triglucoside (mi).

A 2D-PC survey of *L. bidwillii* and *L. plumosa* sampled over the full range of their geographic occurrence revealed that the presence of myricetin 3-rhamnoside consistently distinguished *L. plumosa* from *L. bidwillii*. The lower level di- and tri-glucosides of luteolin, quercetin 3-glucoside and the flavonol diglycosides were all found to be of sporadic occurrence, but more often visible in *L. plumosa* than in *L. bidwillii*.

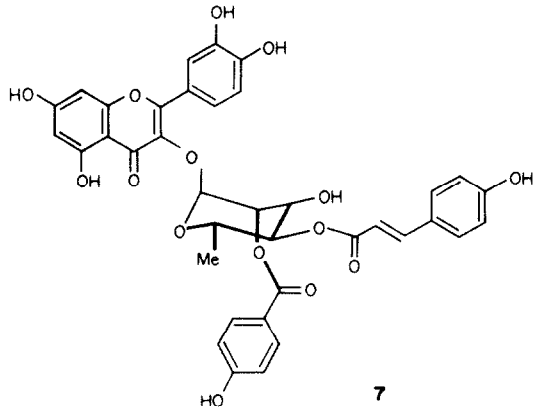
Compounds **1** and **2**, found in both species, were isolated from *L. bidwillii*. They were eluted from the polyamide column with 100% methanol and were separated from the accompanying aglycone material by 1D-PC in TBA. Compound **1** on acid hydrolysis with TFA gave xylose and 6-hydroxyluteolin, but on treatment with a mixture of pectinase and β-glucosidase, xylose and 8-hydroxyluteolin (hypolaetin) were produced. Hypolaetin was identified by direct comparison with authentic material from *Marchantia berteroana* [13]. The ^{1}H NMR spectrum of **1** confirmed this oxygenation pattern and defined **1** as a mono-xyloside which is β-linked ($J_{H\text{-}1''/H\text{-}2''}$ = 7.5 Hz) to the aglycone and in the pyranose form [14, 15]. The effect of shift reagents on the absorption spectrum of **1** required that the 5-, 3′- and 4′-hydroxyls are unsubstituted and thus that **1** be the 7- or 8-*O*-xyloside of 8-hydroxyluteolin. The spectra in methanol and the effects of sodium acetate and sodium methoxide on that spectrum readily distinguished between these two possibilities [16], and compound **1** was accordingly defined as the 7-glycosylated alternative, 8-hydroxyluteolin-7-*O*-β-xylopyranoside, which is a new natural product.

Compound **2** was identified by similar means, as the 7-*O*-xyloside of 8-hydroxyapigenin (isoscutellarein). Acid hydrolysis produced 6-hydroxyapigenin plus xylose, while enzymes yielded 8-hydroxyapigenin which was identified by comparison with an authentic standard from *M. berteroana* [13]. The absorption spectra of **2** are quite unlike those of 8-hydroxyapigenin 8-*O*-glycosides, but approximate closely to those of 8-hydroxyapigenin 7-*O*-glycosides [16]. Compound **2** is therefore identified as 8-hydroxyapigenin 7-*O*-β-xylopyranoside, a new natural product.



2 R = H

1 R = OH



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Biflavonoid components

Aglycone material from *L. bidwillii* which eluted from the polyamide column with methanol, was separated from accompanying **1** and **2** by 1D-PC in TBA. TLC analysis and comparison with standards indicated that most of the six major components (**3**–**7**) in this fraction, were biflavonoids. Individual components were isolated by various combinations of column chromatography, TLC and HPLC.

Compound **3** gave absorption spectra (Table 1) and an ^{1}H NMR spectrum (Table 2) identical with those of authentic amentoflavone (3′,8″-biapigenin), and on permethylation gave hexamethylamentoflavone. In addition it was shown to isomerize to robustaflavone (3′,6″-biapigenin) in hydrogen iodide–acetic anhydride, thereby confirming its identity, and its distinction from the 3′6″-linked isomer.

Compound **4** also gave hexamethylamentoflavone on permethylation and its ^{1}H NMR spectrum (Table 2) indicated it to be a monomethylamentoflavone. The effects of various standard shift reagents on its absorption spectrum (Table 1) suggested that the 4′,4‴, 5 and 5″-hydroxyls are unsubstituted. The methoxyl group therefore must reside at C-7 or C-7″. Compound **4** was proved to be 7-*O*-methylamentoflavone by its production from the co-occurring 7-*O*-methyl-2,3-dihydroamentoflavone (**6**) by dehydrogenation (see below). This biflavone is new to the Cupressaceae [9, 10].

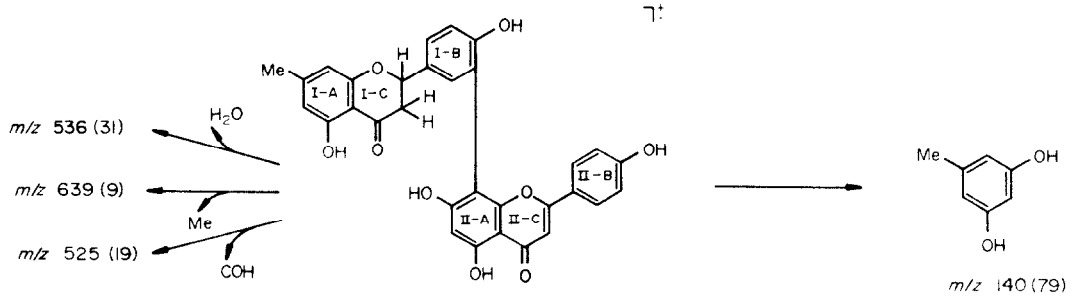

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Table 1. UV spectral data (λ_{max}; nm) for *Libocedrus* biflavonoids **3–6**.

	3	**4**	**5**	**6**
MeOH	271, 295 sh, 335	272, 294 sh, 335	278 sh, 288, 325	280 sh, 288, 325 sh
NaOMe	276, 295 sh, 310 sh, 386	270 sh, 274, 295 sh, 325 sh, 388	275 sh, 292 sh, 325, 390	270 sh, 276 sh, 289, 328 sh, 395
$AlCl_3$	280, 303 sh, 348, 386	263 sh, 279, 302, 350, 385 sh	286, 308, 346, 390 sh	263 sh, 286, 309, 354, 393 sh
$AlCl_3$/HCl	281, 303 sh, 344, 386	263 sh, 281, 302, 346, 385 sh	287, 307, 343, 390 sh	261 sh, 286, 308, 350, 386 sh
NaOAc	274, 293 sh, 312 sh, 365	274 sh, 291, 335, 390 sh	292, 316, 385	290, 320 sh, 333 sh, 390 sh
NaOAc/H_2BO_3	272, 332	274 sh, 288, 335, 390 sh	290, 323	288, 333 sh

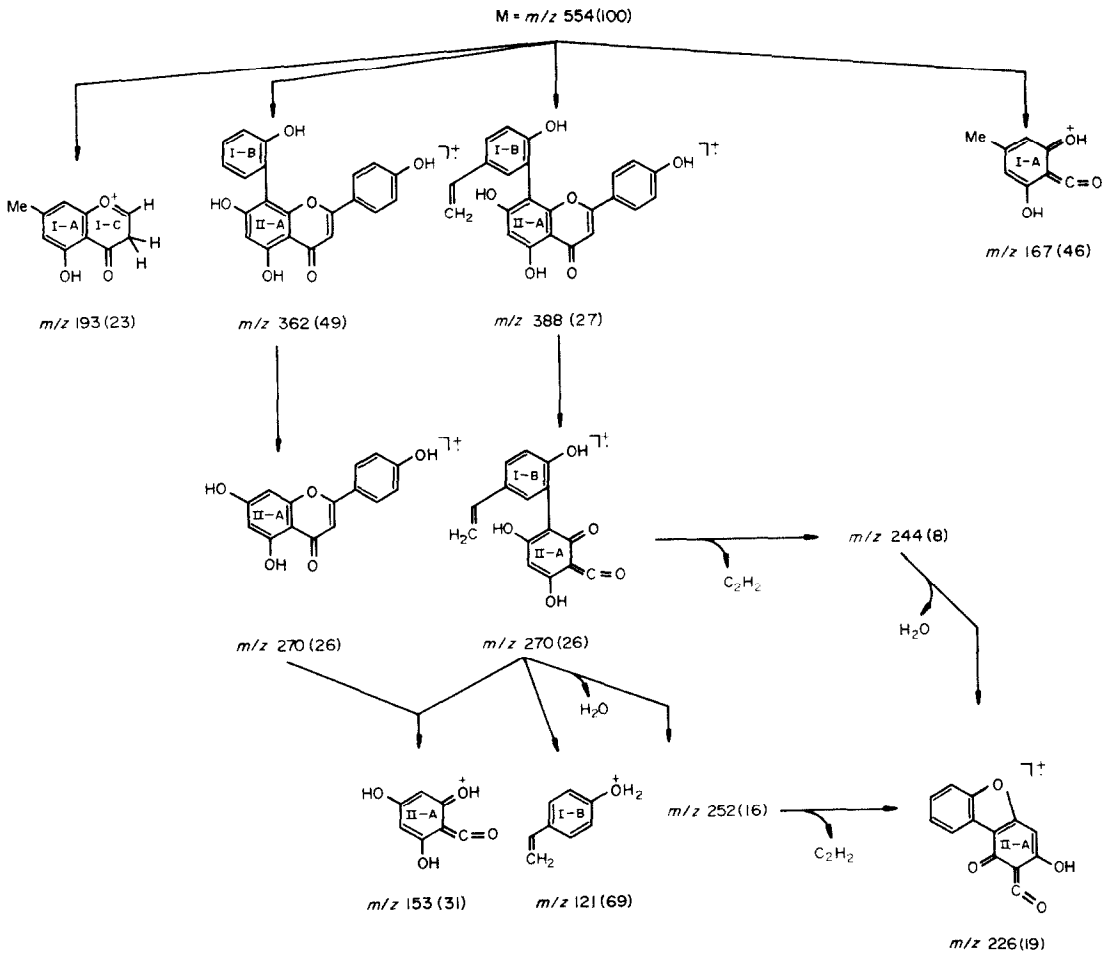
Table 2. ^{1}H NMR data* for *Libocedrus* biflavonoids **3–6**

	3[a]	**4**[a]	**5**[a]	**6**[a]	**6**[b]
H-2	—	—	5.39 *dd* (2.1; 13.3)	5.47 *dd* (2.0; 14.1)	5.58 *dd* (2.6; 13.1)
H-3	6.80 *s*	6.78 *s*	3.45 *m*	3.31 *dd* (14; 16)	3.3 *m*
			2.55 *dd* (2.1; 16.0)	2.65 *dd* (2.0; 16.5)	obscured
H-6	6.20 *d* (1.9)	6.35 *d* (2.1)	5.80 *d*[c] (2.0)	6.02 *br s*[d]	6.03 *d* (2.5)
H-8	6.48 *d* (1.9)	6.73 *d* (2.1)	5.87 *bs*[c]	5.82 *s*[d]	6.03 *d* (2.5)
H-2′	7.99 *bs*	8.11 *d* (2.0)	7.57 *bs*	7.61 *s*	7.56 *d* (1.9)
H-5′	7.17 *d* (9.0)	7.09 *d* (8.6)	6.83 *d* (8.1)	6.81 *d* (8.1)	7.17 *d* (8.2)
H-6′	8.00 *dd* (2.0; 9.0)	8.01 *dd* (2.0; 8.6)	7.33 *dd* (2.0; 8.1)	7.30 *br d* (8.1)	7.52 *br d* (8.2)
H-3″	6.84 *s*	6.90 *s*	6.81 *s*	6.83 *s*	6.67 *s*
H-6″	6.44 *s*	6.31 *s*	6.61 *s*	6.60 *s*	6.42 *s*
H-2‴/-6‴	7.57 *d* (8.6)	7.59 *d* (8.8)	6.67 *d* (8.6)	7.69 *d* (8.4)	7.68 *d* (8.4)
H-3‴/-5‴	6.73 *d* (8.6)	6.68 *d* (8.8)	6.79 *d* (8.6)	6.81 *d* (8.4)	6.91 *d* (8.4)
OH-5	13.10 *s*	13.11 *s*	12.20 *br s*	12.20 *br s*	12.13 *s*
OH-5″	12.97 *s*	12.99 *s*	13.15 *s*	13.14 *s*	13.14 *s*
OH-7	10.92 *s*	—	8.43 *br s*	8.5 *br s*	9.5 *s*
OH-7″	10.36 *s*	10.28 *s*	8.43 *br s*	8.5 *br s*	9.5 *s*
OMe	—	3.81 *s*	—	obscured	3.82 *s*

[a] In DMSO-d_6 at room temperature; reference: DMSO = 2.503 ppm.
[b] In $(CD_3)_2CO$ at room temperature; reference: acetone = 2.05 ppm.
* Chemical shifts in ppm; coupling constants in parantheses, in Hz.
[c,d] Assignments may be interchanged.
— Non-existent.

Permethylation of the crude aglycone mixture converted two further components, **5** and **6**, to a permethyl ether different from hexamethylamentoflavone. The absorption and ^{1}H NMR spectra of **5** and **6** (Tables 1, 2) indicated both compounds to be of the dihydromentoflavone type, and indeed, the permethyl ether proved to be chromatographically identical with authentic hexamethyl-2,3-dihydroamentoflavone. The ^{1}H NMR spectrum of **5** contained no methoxyl signal and was identical with that of 2,3-dihydroamentoflavone ex *Cycas revoluta* [17]. Microscale dehydrogenation to amentoflavone and co-chromatography with authentic material confirmed the identity of **5** as 2,3-dihydroamentoflavone.

The ^{1}H NMR spectrum of **6** in acetone-d_6 (Table 2) revealed the presence of one, three proton methoxyl signal, and the mass spectrum gave a molecular ion at *m/z* 554, consistent with a monomethyl-2,3-dihydroamentoflavone formulation. Further mass spectral breakdown



Scheme I. EIMS fragmentation of 7-*O*-methyl-2,3-dihydroamentoflavone (**6**).

(Scheme 1) produced major fragments at *m/z* 193, 362 and 388, the presence of which indicated the site of *O*-methylation to be the 7-hydroxyl group. Methylation at the 7-hydroxyl rather than the 7″-hydroxyl was confirmed by the absorption spectra. The spectra of 7-hydroxy-2,3-dihydroflavones possess strong band II absorption at about 290 nm which is shifted to about 320 nm with sodium methoxide or acetate [12]. This shift however is not seen in the spectra of **6** (Table 1). Accordingly compound **6** lacks a free 7-hydroxyl function on its 2,3-dihydroflavone moiety and its structure is defined as 7-*O*-methyl-2,3-dihydroamentoflavone. This is not only new to the Cupressaceae [9, 10] but is also a new natural product. Microdehydrogenation of **6** produced compound **4** in sufficient yield, to confirm the structure of **4** (above).

Structural studies on the one remaining aglycone, **7**, from the 100% methanol elution of the polyamide column, have been reported elsewhere [18]. Compound **7** is highly distinctive on TLC in that it turns orange in NA in contrast to the yellow-green coloration of the accompanying biflavonoids. Originally thought to be a luteolin-containing biflavone, **7** was ultimately shown to be quercetin 3-*O*-α-[2-*O*-*p*-hydroxybenzoyl-4-*O*-*p*-coumaroylrhamnopyranoside]. This flavonol glycoside, because of its unusual aglycone-like chromatographic properties, was originally missed in the standard 2D-PC screening of *Libocedrus* for flavonoid glycosides.

A TLC comparison of the aglycone fraction constituents from *L. plumosa*, with compounds **3**–**7** from *L. bidwillii*, revealed that the major components in *L. plumosa* are **3**–**6**. Compound **7** was not found in *L. plumosa*.

In summary, the two *Libocedrus* species endemic to New Zealand, although accumulating similar flavonoid types, are still clearly distinct. Thus *L. plumosa* is distinguished from *L. bidwillii* by the presence of myricetin 3-rhamnoside and the absence of the di-acylated quercetin 3-rhamnoside (**7**). These two species are the sole representatives of the Cupressaceae endemic to New Zealand and as a group are clearly distinguished by their flavonoid profiles from all other groups of New Zealand conifers, i.e. *Podocarpus s.l.* [1, 2], *Dacrydium s.l.* [4], *Phyllocladus* [3] and *Agathis* (Markham, K. R. and Vilain, C., unpublished results).

Survey of non New Zealand Libocedrus *species and related genera*

The New Caledonian species *Libocedrus austro-caledonica*, *L. chevalieri* and *L. yateensis* were surveyed to determine their relationship with *L. bidwillii* and *L. plumosa* of New Zealand. Also, because the relationship

of other genera in the tribe Libocedreae is still unresolved [6, 9, 10, 11], it seemed appropriate to survey a selection of these as well. Those available included *Papuacedrus papuana* (New Guinea), *Austrocedrus chilensis* (S. America), and *Neocallitropsis pancheri* (New Caledonia). In addition, two species of the Northern Hemisphere genus *Calocedrus* (subfamily Cupressoideae, tribe Thujopsideae) were surveyed as fresh cultivated material was readily available.

Flavonoid glycosides were surveyed by 2D-PC followed by spraying of the PCs with NA, and spectroscopy and hydrolyses of selected components. Biflavonoids and compound **7** were screened for by subsequent TLC analysis of the 'aglycone' spot from these PCs.

The survey results presented in Table 3 reveal that the flavonol 3-*O*-diglycosides, which are a dominant feature of the two New Zealand species of *Libocedrus*, are also present in the New Caledonian species and to a greater or lesser extent in the other genera studied. It is the presence of the 8-hydroxyflavone glycosides that perhaps best typifies *Libocedrus*. These compounds oxidise readily, and this may account for their apparent absence from *L. austro-caledonica* which was available only as a herbarium specimen. While the di-acylated glycoside **7**, is found scattered among the genera studied, and amentoflavone (**3**) and its 7-methyl ether (**4**) are ubiquitous, the dihydroamentoflavones **5** and **6** are restricted largely to *Libocedrus*, being detected in all species but one. These results indicate a close chemical relationship among all five species assigned to *Libocedrus* which is strengthened by their close morphological similarity.

Overall the flavonoid profiles of the genera selected in this study are sufficiently similar to be consistent with at least a familial relationship. There is some chemical evidence to support the current separation of *Libocedrus*, *Papuacedrus* and *Austrocedrus*, but there is nevertheless a similarity in their general patterns which would equally support a closer taxonomic relationship. *Calocedrus*, on the other hand, would seem to be chemically more distinct. In general, the results obtained in this study support the conclusions drawn by Gadek and Quinn [9, 10] in respect to the same genera. However, a redefinition of the taxa within the Cupressaceae is beyond the scope of the present work and will only be resolved by a complete reassessment of a range of character-states among the taxa concerned.

EXPERIMENTAL

Apparatus and chromatography. For general PC and survey work: Whatman 3 MM paper, solvents TBA (*t*-BuOH–HOAc–H_2O, 3:1:1) and HOAc (15–50%). CC was carried out with RP-8 'Lichroprep' Merck (40–63 μm) or 'MN-Polyamid SC-6' Macherey Nagel (0.05–0.16 mm), and TLC on cellulose (F1440) Schleicher and Schull or silica gel (60 F_{254}) Merck (foils or glass plate). Visualization of flavonoid spots: 1% diphenylboric acid 2-aminoethyl ester (NA). HPLC was carried out on a Versapack C-18 column (250 × 4.1 mm, 10 μm) using MeOH–H_2O–HCO_2H (270:230:11). Absorption spectra were measured as described by Markham [12], MS were obtained in EI mode (direct inlet, 70 eV), FDCI mode (reactant gas NH_3) and FAB mode (matrix thioglycerol).

Plant material. Libocedrus plumosa (bulk sample) was collected at DSIR, Lincoln, New Zealand on 19.7.1985 and a voucher placed in the Botany Division, DSIR herbarium (CHR 418730). Survey samples from throughout New Zealand: CHR 439788, 439789, 317778B, 322426, 124283 and 417599. *Libocedrus bidwillii* (bulk sample) was collected at the Christchurch Botanic Gardens, New Zealand (CHR 418729) on 22.7.85 and survey samples from throughout New Zealand: CHR 439790–439795, 129510, 325496, 277455 and 200153. Sources (and voucher numbers) of non New Zealand samples: *L. yateensis*, Rivière Bleue, New Caledonia (CHR 437610); *L. austro-caledonica*, Nekando, New Caledonia (NOU 5654, CHR 439797); *L. chevalieri*, Kouakoue, New Caledonia (NOU 6767); *Papuacedrus papuana*, Mt Kenive, Papua, New Guinea (CHR 343728); *Austrocedrus chilensis*, Christchurch Botanic Gardens (CHR 437871); *Calocedrus decurrens*, Christchurch Botanic Gardens (CHR 280244, 130099); *Calocedrus formosana*, Christchurch Botanic Gardens (CHR 437872); and *Neocallitropsis pancheri*, Montagne des Sources, New Caledonia (CHR 437615).

Sample extraction and work-up. Although the flavonoid components of *L. plumosa* were isolated by cellulose CC (in 2% HOAc) of a MeOH–H_2O (1:1) extract of dry plant material (30 g), the bulk of the chemical work was carried out on components from *L. bidwillii*. Dry plant material of *L. bidwillii* (100 g) was ground and extracted ×2 at room temp. with MeOH–H_2O (9:1). The extract was chromatographed on a polyamide column, monitored by 2D-PC (TBA, 15% HOAc). Fractions obtained with 20–50% MeOH in H_2O contained blue fluorescent cinnamic acid derivatives, the 60–80% MeOH fractions contained flavonoid glycosides, and the last (100% MeOH followed by Me_2CO) fractions contained **1**, **2** and the biflavonoids. Compounds **1** and **2** and the other flavonoid glycosides, were separated by prep. PC (TBA and/or 25–30% HOAc) and purified by CC on RP-8 using MeOH–H_2O (1:9 to 4:1). The biflavonoid mixture isolated by PC was separated into its components by CC in the following systems: SiO_2($CHCl_3$–MeOH–HCO_2H, 1000:10:1, 190:10:1, 40:10:1) and RP-8 (MeOH–H_2O–HCO_2H, 20:20:1 to 90:10:1). Prep. TLC on silica gel ($CHCl_3$–MeOH–HCO_2H, 90:10:1) yielded pure **5**–**7** and HPLC separated **3** from **4**.

Acetylation of biflavonoid mixture. The dry mixture (10 mg) in pyridine–Ac_2O (1:1, 2 ml) was kept at room temp. for 18 hr. The soln was then poured into H_2O and the mixture extracted with $CHCl_3$. An EtOH soln of the $CHCl_3$ solubles gave white orthorhombic crystals, mp 156–158° (authentic amentoflavone hexaacetate: 157–160°), R_f (silica gel TLC in $CHCl_3$–MeOH–HCO_2H, 290:10:1) = 0.87, λ_{max}^{MeOH} 263, 282sh, 315sh; (NaOMe) 269 *br*; ($AlCl_3$ and $AlCl_3$/HCl) 262, 285sh, 315sh, nm.

Permethylation of biflavonoids. Permethyl ethers were prepared using NaH–MeI–DMF according to ref. [12], and purified by TLC on silica gel using C_6H_6–pyridine–HCO_2H, 100:20:7. The products from **3** and **4** (EIMS, $[M]^+ = 622$ *m/z*) were identical with hexa-*O*-methylamentoflavone, appearing as yellow fluorescent spots (365 nm) on SiO_2 TLC, R_f (C_6H_6–pyridine–HCO_2H, 100:20:7) 0.35 and ($CHCl_3$–MeOH–HCO_2H, 180:20:1) 0.54, and λ_{max}^{MeOH} 248 sh, 268, 308 sh, 328 nm unchanged by shift reagents. The products from **5** and **6** were identical with hexa-*O*-methyl-2,3-dihydroamentoflavone (TLC as above but R_f values of 0.51 and 0.61 respectively, and λ_{max}^{MeOH} 262 sh, 272, 312 sh, 330 nm, unchanged by shift reagents).

Dehydrogenation of **5** *and* **6**. Using the micro-dehydrogenation technique previously developed for biflavonoids [19], followed by TLC analysis of the products (silica gel, $CHCl_3$–MeOH–HCO_2H, 90:10:1), and absorption spectroscopy, it was shown that **5** was converted to **3** and **6** was converted to **4**.

Isomerization of **3** *to robustaflavone.* Compound **3** in HI (3 ml) and Ac_2O (1 ml) was refluxed for 3 hr and poured into 5% aq. $Na_2S_2O_3$ (3 ml). The products were recovered by CC on RP-8 in H_2O–MeOH mixtures. TLC analysis (polyamide,

Table 3. Survey of flavonoid types in *Libocedrus* and related genera†

	Flavonol 3-diglycosides*	Other flavonol glycosides	8-Hydroxy-flavone glycosides*	Other flavones	Compound **7**	Biflavonoids **3** and **4**‡	Biflavonoids **5** or **6**
Libocedrus austro-caledonica++	KQ				+	+	+
Libocedrus chevalieri++	KQM		AL		+	+	+
Libocedrus yateensis	KQM		L		+	+	
Libocedrus bidwillii	KQ	w	AL	w	+	+	+
Libocedrus plumosa	KQM	w	AL	w		+	+
Papuacedrus seedlings G1088	KQM	+		+		+	
Papuacedrus papuana++	QM	+			+	+	
Austrocedrus chilensis	Q(M?)			w	+	+	+
Calocedrus decurrens	KQM	+		+		+	
Calocedrus formosana	KQM	+		+		+	
Neocallitropsis pancheri	Q	+	L?	w	+	+	

†Symbols used: +(present), w(weak), blank(not seen), ?(doubtful) K(kaempferol), Q(quercetin), M(myricetin), A(8-hydroxyapigenin), L(8-hydroxyluteolin).

*Aglycones only determined.

‡Low levels of hinokiflavone may accompany **4** in some samples. One other sporadically occurring biflavone was not identified.

++Herbarium material used, other samples were fresh plant material.

EtOAc–MEK–HCO_2H–H_2O, 5:3:1:1) revealed a 1:1 mixture of amentoflavone **3** (R_f 0.74) and robustaflavone (R_f 0.67) when compared with authentic samples (ex H. Geiger).

7-O-*Methyl*-2,3-*dihydroamentoflavone* (**6**). FABMS: *m/z* 555 [M+H]; EIMS, *m/z* (rel. intensities): 554 (100), 539 (9), 537 (25), 536 (31), 525 (19), 389 (24), 388 (27), 375 (38), 371 (24), 362, 345 (12), 270 (26), 252 (16), 226 (19), 213 (13), 193 (23), 167 (46), 153 (31), 140 (79), 121 (69), 111 (44), 110 (34).

Acid hydrolysis of flavonoid glycosides. 2 N TFA–MeOH (1:1), reflux, separation of aglycones from sugars by CC on RP-8. Sugar analyses: TLC on silica gel using *n*-BuOH–Me_2CO–1.6% aq. NaH_2PO_4 (5:4:1) and PC in *n*-BuOH–pyridine–HOAc–H_2O (5:1:3:3), and *p*-anisidine–HCl spray reagent. R_{glc} values (TLC/PC): galactose 0.74/0.87, arabinose 1.32/1.18, xylose 2.07/1.38, rhamnose 3.12/1.80. Aglycones were identified by co-chromatography with standards (TLC, cellulose, TBA and 50% HOAc).

8-*Hydroxyluteolin* 7-O-*xyloside* (**1**). Comparative R_f values on TLC (cellulose) in (i) TBA, (ii) 15% HOAc–run 10×, (iii) 50% HOAc, and on RP-18 in MeOH–HOAc–H_2O, 60:1:39 (run 2×) are: **1** 0.29, 0.20, 0.25, 0.45; 8-hydroxyluteolin 7-glucoside ex *Bryum* [16] 0.23, 0.40, 0.35, 0.48; luteolin 7-glucoside 0.35, 0.41, 0.51, 0.56; 8-hydroxyluteolin 0.35, 0.15, 0.22, 0.42; 6-hydroxyluteolin 0.27, 0.09, 0.17, 0.40; luteolin 0.90, 0.15, 0.45, 0.22. Compound **1** gave λ_{max}^{MeOH} 257 sh, 268 sh, 275, 302 sh, 344; (NaOMe) 266, 273 sh, 340 sh, 398 (dec.); ($AlCl_3$) 275, 318, 433; ($AlCl_3$ + HCl) 264 sh, 275, 285 sh, 310, 362, 420 sh; (NaOAc) 268, 274 sh, 302 sh, 345; (NaOAc + H_3BO_3) 267, 275 sh, 302 sh, 375 nm, and ^{1}H NMR (δ, Me_2CO-d_6): 7.59 (*d*; 2.1 Hz; H-2′), 7.52 (*dd*; 8.3 Hz, 2.1 Hz; H-6′), 6.99 (*d*; 8.3 Hz; H-5′), 6.62 (*s*; H-3), 6.58 (*s*; H-6), 5.06 (*d*; 7.5 Hz; H-1″), 3.3–3.9 (*m*; H-sugar).

Hydrolysis of **1** with 1 M HCl (100°), 15 min resulted in decomposition whereas 1 M TFA (100°), 1 hr yielded xylose and 6-hydroxyluteolin (TLC co-chrom. with standard). A mixture of β-glucosidase (Koch-Light) and pectinase (Koch-Light) did not cause hydrolysis at room temp., but after 2 hr at 30° and work-up by CC on RP-8, 8-hydroxyluteolin was identified (R_f values as above).

8-*Hydroxyapigenin*-7-O-*xyloside* (**2**). Comparative R_f values (solvents: TBA, 50% HOAc): **2**, 0.65, 0.50; 6-hydroxyapigenin, 0.26, 0.41; apigenin-7-glucoside, 0.76, 0.80; apigenin, 0.95, 0.63. Compound **2** gave λ_{max}^{MeOH} 279, 306, 326; (NaOMe) 272, 290 sh, 340 sh, 374 (dec); ($AlCl_3$) 274, 322, 348 sh, 412 sh; ($AlCl_3$ + HCl) 274, 320, 348 sh, 412 sh; (NaOAc) 280, 312, 386 sh; (NaOAc + H_3BO_3) 279, 308, 330 sh, nm. TFA hydrolysis (as for **1**) yielded xylose and 6-hydroxyapigenin (co-TLC with standards).

Chromatographic screening of non-N.Z. species. 2D-PC patterns were obtained for all species using TBA and 15% HOAc. The less polar components (biflavonoids and **7**) were analysed by silica gel TLC ($CHCl_3$–MeOH–HCO_2H, 18:2:1) of the 'aglycone' spots from the 2D-PCs, against a variety of authentic standards and the *L. bidwillii* biflavonoid fraction.

Acknowledgements—The authors are indebted to Professor Hans Geiger (Inst. Chemistry, U. of Hohenheim, Stuttgart) for the supply of various biflavone standards, to Drs K. Morgan and R. Meinhold (Chemistry Division, DSIR) for determining the NMR spectra, and to Miss L. Lang for technical assistance. A. F. wishes to acknowledge receipt of a Deutsche Forschungsgemeinschaft fellowship during the period July 1987–June 1988. We are also grateful for permission to collect samples from the sources listed in this paper. B. P. J. M. wishes to acknowledge field assistance provided by staff of the Forestry College, Bulolo, Papua, New Guinea; Tanguy Jaffre and Jean-Marie Veillon, ORSTOM, Noumea; and Jean-François Cherrier, CTFT, Noumea, New Caladonia.

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