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LEAF FLAVONOID PATTERNS IN THE WINTERACEAE

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Abstract—In a leaf flavonoid survey of 59 specimens of the Winteraceae and related families, representing nine genera, luteolin 7,3′-dimethyl ether (in 77%) and flavonols (in 81%) were found to be major constituents. Indeed the high incidence of luteolin 7,3′-dimethyl ether chemically isolates the family from all other angiosperm groups, including families and genera that have been taxonomically associated with the Winteraceae in the past. Simple flavones (in 16%), on the other hand, were found only in some *Drimys s. str.*, *Tasmannia* and *Pseudowintera* species. Similarly, the distribution of flavone *C*-glycosides was restricted to specimens of *T. piperita* and one specimen of *D. winteri*. The frequent occurrence of procyanidin (in 60%) and dihydroquercetin (in 44%) reflects the primitive and woody nature of the family. The combined flavonoid data clearly support previous cytological, morphological and phylogenetic studies in the division of the Winteraceae into three groups of genera: (1) *Bubbia*, *Belliolum*, *Exospermum* and *Zygogynum*; (2) *Drimys s. str.* and *Pseudowintera* and (3) *Tasmannia*. Some generic variations were found within the *Bubbia*, *Belliolum*, *Expospermum* and *Zygogynum* group but apart from minor geographic variations within *Belliolum* the flavonoid results do not appear to provide suitable evidence for subgeneric taxonomy.

INTRODUCTION

The Winteraceae is a family of trees and shrubs that occupies forest habitats on either side of the southern Pacific Ocean. It consists of about 100 species in six or seven genera. Exceptional morphological features have caused the family to be the subject of much comment and discussion by phylogenists. Thus the secondary wood is always without vessels, a character shared only with a few small angiosperm families such as the Trochodendraceae and Tetracentraceae. The flowers mostly have more than one free carpel with an accentuated and sessile stigma. The stamens are spirally arranged and sometimes flattened with laterally placed pairs of sporogenous locules. Recent collections show a considerable diversity in floral features, inflorescence type and position which sometimes present difficulties in ascribing material to the presently accepted genera. However, revisional studies on the family are continuing [1–3].

The most recent taxonomic review of the whole family was conducted by Smith [4, 5] but was hindered by the unavailability of collections held in European herbaria. Accordingly, very few new taxonomic combinations were made and species were described within the generic framework proposed by van Tieghem in 1900 [6]. Smith's revision recognized the following genera: *Drimys* section *Wintera* (Central and South America) 4 species; *Drimys* section *Tasmannia* (Australia, New Guinea, South-East Asia) 36 species; *Pseudowintera* (New Zealand) 2 species; *Bubbia* (Australia, New Guinea, New Caledonia) 30 species; *Belliolum* (Solomon Islands, New Caledonia) 8 species; *Exospermum* (New Caledonia) 2 species; *Zygogynum* (New Caledonia) 6 species.

From its earliest descriptions, *Drimys* had been considered as closely related to the Asian genus *Illicium*. Van Tieghem's classification excluded *Illicium* from the Winteraceae and, with the exception of Hutchinson [7], this separation has been retained. In a later work Hutchinson [8] also included the recently described Fijian genus *Degeneria* [9], but neither of these decisions have been supported by later workers. Nevertheless, the Illiciaceae and Degeneriaceae are accepted as near relatives to the Winteraceae [10].

In a later paper Smith [11] dissolved the sectional division of *Drimys* by elevating his section *Tasmannia* to the genus *Tasmannia* with an exclusively Old World distribution, and his section *Wintera* to the genus *Drimys* with a New World distribution. The change was largely because of substantial chromosomal differences between the two groups, originally recognized by Hotchkiss [12] and subsequently confirmed by Ehrendorfer *et al.* [13]. However, not all workers have accepted this change despite accumulating evidence from morphological studies [14, 15]. While *Pseudowintera*, based on van Tieghem's genus *Wintera*, is a well circumscribed genus, the remaining four genera are somewhat problematic in their delimitation, largely because van Tieghem relied to an unreasonable extent upon single character differences between genera. Thus, for example, *Bubbia* and *Belliolum* are distinguished by stamen shape and the location of anther locules, and *Exospermum* and *Zygogynum* by the extent to which the carpels are fused.

Perhaps because of a lack of economic importance and restricted distribution, the Winteraceae has not been extensively surveyed for chemical constituents. Thus, Hegnauer [16] records essential oils, cyanogenic compounds and saponins from only a small number

of species. Bate-Smith [16, 17] in a leaf flavonoid study examined six members of the family representing the genera *Drimys s. str.*, *Pseudowintera* and *Tasmannia*. He found quercetin in five, kaempferol in four, and procyanidin in five of these species, respectively. In the related genera *Degeneria* [16] and *Illicium* [17], Bate-Smith records quercetin and procyanidin and quercetin, kaempferol and procyanidin, respectively. More recently Young and Sterner [18] have characterized quercetin 3-glucoside, quercetin 3-rhamnoside and rutin in leaves of *Degeneria*. Kubitzki and Vink [19] in a flavonoid aglycone study of *Tasmannia* (as *Drimys* section *Tasmannia*) species from New Guinea found quercetin, kaempferol, dihydroquercetin, luteolin, apigenin and procyanidin as common leaf components and diosmetin as a trace constituent in a small number of species. These authors found that the flavonoid data enabled them to group the specimens into six chemical types, which showed sufficient correlation with geographical and morphological data to be employed in the taxonomic circumscription of the taxa.

The present leaf flavonoid survey forms part of a wider study by one of us (W.J.H.) of the generic delimitation and relationships of the Winteraceae which occur in north-eastern Australia, New Guinea, the Solomon Islands and New Caledonia in the south-west Pacific.

RESULTS

The results of the leaf flavonoid survey of 59 herbarium specimens of the Winteraceae and related families are shown in Table 1. The specimens were selected as representing the geographical and morphological range of the Winteraceae, with an emphasis upon those genera not previously surveyed. The data refer to flavonoid aglycones, which were detected in acid hydrolysed leaf tissue. The aglycones were identified by comparison of R_f values and colour reactions in UV light with standard markers. These results were confirmed by two dimensional paper chromatography (2D-PC) of direct leaf extracts and by more detailed investigation of some specimens.

During the course of the survey several unusual methylated flavones were found free in significant amounts in direct leaf extracts. Thus, luteolin 7,3′-dimethyl ether, first isolated from *Ceanothus velutinus* (Rhamnaceae) [20], was found to be a characteristic leaf constituent of the Winteraceae (present in 77% of the sample). This compound is especially typical of the genera *Bubbia* (in 91% of specimens), *Belliolum* (in 82%), *Exospermum* (in both specimens) and *Drimys s. str.* (three of the four specimens). It was, however, not detected in *Degeneria* (Degeneriaceae) or *Illicium* (Illiciaceae) and was found in only 46% of the *Tasmannia* material surveyed. The related flavone, luteolin 7,4′-dimethyl ether, previously isolated from *Ovidia pillo-pillo* (Thymelaeaceae) and *Alnus japonica* (Betulaceae) [20], was identified in only three plants: *Tasmannia piperita* (Elmer 9912), *Belliolum gracile* and *Zygogynum* sp. (McPherson 2603). Apigenin 7,4′-dimethyl ether, also originally isolated from *Alnus japonica* [20], was detected in two other specimens of *Tasmannia piperita* (Thien 10 and Thien and Surry 17), *Tasmannia* sp. (Womersley s.n., NGF 11347) and in *Zygogynum* sp. (McPherson 2603). In these four specimens apigenin 4′-monomethyl ether was also tentatively identified.

The flavonols quercetin (in 81% of the sample) and kaempferol (in 53%) were also predominant leaf constituents of the Winteraceae and were found in all the genera. Isorhamnetin was not detected in any member of the Winteraceae or in *Degeneria*, but was found in *Illicium*. The simple flavones luteolin (in 16% of the sample) and apigenin (in 14%) were much restricted in their distribution. These compounds were found only in *Drimys winteri* (two of four specimens), *Pseudowintera colorata* (one specimen examined) and in 46% of the *Tasmannia* material. Flavone *C*-glycosides, although rare in the Winteraceae (in 14% of the sample), were found to be characteristic leaf components of *Tasmannia piperita* (in seven of nine specimens) and were detected in one of four specimens of *Drimys winteri*.

Dihydroquercetin, a frequent constituent of the Winteraceae (in 43% of the sample) was found in all genera, including *Illicium*, but was absent from *Degeneria*. It was also notably less frequent in the genus *Bubbia* where it occurred in only 14% of the specimens included in the survey. Procyanidin was also a common leaf component (in 60% of the Winteraceae sample) and was detected in all the genera except *Exospermum* and *Degeneria*.

DISCUSSION

A summary of the present results (Table 2) clearly shows that the Winteraceae has a particularly distinctive leaf flavonoid profile in which luteolin 7,3′-dimethyl ether and flavonols together form the most characteristic components. Indeed this is the first report of luteolin 7,3′-dimethyl ether as a major leaf constituent in such a large number of related species and is therefore of considerable diagnostic value in delimiting the Winteraceae. Previously, luteolin 7,3′-dimethyl ether and the related luteolin and apigenin 7,4′-dimethyl ethers have been reported only from a small number of isolated plant sources [21]. However, these substances could easily have been missed in surveys based solely on hydrolysed plant tissue since they are most easily detected by 2D-PC of direct methanolic extracts. It is of note that methylated flavones were not found in the two specimens of *Degeneria* and *Illicium* examined in the present survey and have not been reported from the Magnoliaceae [22].

The high incidence of dihydroquercetin and procyanidin as leaf constituents in members of the Winteraceae (Tables 1 and 2) reflects the 'primitive' and woody nature of the family [17]. Flavones and flavone *C*-glycosides on the other hand have a restricted distribution within the family. Thus simple flavones are seen to characterize the genera *Drimys s. str.*, *Pseudowintera* and *Tasmannia*. It is also of interest that the *Tasmannia* taxa which produce simple flavones do not generally synthesize luteolin 7,3′-dimethyl ether or the other methylated flavones. This could indicate a form of chemical advancement by reduction in these plants.

Flavone *C*-glycosides were found in only one specimen of *Drimys winteri* and in seven of the eleven specimens of *Tasmannia piperita* examined, a species which shows remarkable variation in its flavonoid

Table 1. The distribution of flavonoids in leaves of the Winteraceae and related families

Family, genus, species	Flavonols	Flavones	Lu7,3'-DIME	Lu7,4'-DIME	Ap7,4'-DIME	Flavone C-glycosides	DiHQu	Procy	Plant source*	Collector's name and number or accession number, collection data, location† and altitude
Winteraceae‡										
Drimys winteri J. R. and G. Forster	Qu, Km	Lu, Ap	+	–	–	–	+	+	RNG	599241, ix.1979, cultivated
	Qu, Km	—	+	–	–	–	+	+	RNG	599245, ix.1979, cultivated
	Qu	Lu, Ap	+	–	–	+	+	+	RNG	599774, ix.1979, cultivated
	Qu, Km	—	–	–	–	–	+	+	RNG	670643, ix.1979, cultivated
Tasmannia lanceolata (Poir.) A. C. Smith	Qu	Lu, Ap	–	–	–	–	–	+	E	E. Adams 1956, 11.x.1967 NSW, 1200 m
	Qu, Km	Ap	–	–	–	–	–	+	RNG	599244, ix.1979, cultivated
T. piperita entity *cordata* Vink	Qu, Km	—	+	–	+§	+	+	+	NO	Thien, 10, vii.1976, PNG, 2500 m
T. piperita entity *heteromera* Vink	Qu	—	+	–	–	+	–	–	NO	Thien, 4, 15.viii.1976, PNG, 3500 m
T. piperita entity *montis-wilhelmii* Vink	Qu	Ap	–	–	–	+	–	+	E	Stevens and Foreman s.n. (LAE 55812), 9.viii.1972, PNG, 3415 m
T. piperita entity polymera Vink	Qu	—	+	–	+§	–	–	+	NO	Thien and Surry 17, 20.vii.1976
T. piperita entity *subalpina* Vink	Qu, Km	—	(+)	–	–	+	+	+	NO	Thien 9, vii.1976, PNG
T. piperita (Hook. f.) Miers	Qu	Lu	–	–	–	+	+	+	E	Vinas and Wiakabu s.n. (LAE 67066) 11.iv.1975, PNG, 3000 m
Tasmannia sp.	—	Lu, Ap	+	–	+§‖	–	–	–	BRI	Womersley s.n. (NGF 11347), vi.1959, PNG, 2000 m
Tasmannia sp.	—	—	–	–	–	–	–	–	BRI	Womersley and Thorne s.n. (NGF 12840) vi.1960, PNG, 2000 m
T. piperita (Hook. f.) Miers	Qu	Lu	+	–	–	+	+	+	E	Bogle, Bogle and Meijer 429, 11.ii.1962, Borneo, 3500 m
	Qu	Lu, Ap	–	–	–	–	+	(+)	E	Burtt and Martin B 5348, 10.ix.1967, Sarawak, 2000 m

Table 1—*continued.*

Family, genus, species	Flavonols	Flavones	Lu7,3'-DiME	Lu7,4'-DiME	Ap7,4'-DiME	Flavone *C*-glycosides	DiHQu	Procy	Plant source*	Collector's name and number or accession number, collection data, location† and altitude
	Qu	Lu	−	+	−	+	+	+	E	Elmer 9912, iv.1908, Philippines
Pseudowintera colorata (Raoul) Dandy	Qu	Lu, Ap	+	−	−	−	+	(+)	RNG	709240 ix.1979, cultivated
Bubbia argentea A. C. Smith	(Qu), (Km)	—	+	−	−	−	−	−	BRI	Ridsdale s.n. (NGF 36813), 7.viii.1968, PNG, 3000 m
B. howeana (F. Muell.) van Tieghem	Qu, Km	—	+	−	−	−	−	−	NSW	Rodd 1858, 23.viii.1971, LH, 450 m
B. semecarpoides (F. Muell.) B. L. Burtt	Qu, Km	—	+	−	−	−	−	−	NSW	Rodd 286, 9.i.1966, Q, 1000 m
B. sororia (Diels) A. C. Smith	Qu, Km	—	+	−	−	−	−	(+)	BRI	Sayers s.n. (NGF 21357), 6.vi.1964, PNG, 2500 m
B. whiteana A. C. Smith	Qu, Km	—	+	−	−	−	−	−	QRS	Irvine 1152, 12.ii.1975, Q, 1000 m
Bubbia sp.	Qu, Km	—	+	−	−	−	−	+	BRI	Craven and Schodde 1091, 30.iii.1966, PNG, 1700 m
Bubbia sp.	Qu	—	+	−	−	−	−	+	BRI	Frodin s.n. (NGF 26281), 17.xi.1965, PNG 1000 m
Bubbia sp.	Qu, Km	—	+	−	−	−	−	+	BRI	Hartley 11829, 8.v.1963, PNG, 2600 m
Bubbia sp.	(Qu)	—	(+)	−	−	−	−	−	BRI	Henty s.n. (NGF 27070), 6.xi.1965, PNG, 600 m
Bubbia sp.	—	—	+	−	−	−	−	−	BRI	Hoogland 3689, 20.viii.1953, PNG, 25 m
Bubbia sp.	Qu	—	+	−	−	−	−	+	BRI	Hoogland and Schodde 7323, 4.viii.1960, PNG, 2800 m
Bubbia sp.	Qu, Km	—	+‖	−	−	−	+	(+)	RNG	McPherson 2189, 6.xii.1979, NC
Bubbia sp.	Qu, (Km)	—	−	−	−	−	−	+	RNG	McPherson 3414, 10.xii.1980 NC, 900 m
Bubbia sp.	Qu, Km	—	(+)	−	−	−	−	−	BRI	Pullen 448, 3.viii.1957, PNG, 2500 m
Bubbia sp.	Qu	—	+	−	−	−	−	+	BRI	Van Royen s.n. (NGF 20390), 14.i.1965, PNG, 3100 m

Table 1—*continued.*

Family, genus, species	Flavonols	Flavones	Lu7,3'-DIME	Lu7,4'-DIME	Ap7,4'-DIME	Flavone *C*-glycosides	DiHQu	Procy	Plant source*	Collector's name and number or accession number, collection data, location† and altitude
Bubbia sp.	—	—	–	–	–	–	–	–	BRI	Van Royen and Sleumer 6328, 31.vii.1961, IJ, 450 m
Bubbia sp.	Qu, Km	—	(+)	–	–	–	–	(+)	BRI	Schodde and Craven 4752, 31.iii.1966, PNG, 1800 m
Bubbia sp.	Qu, Km	—	+	–	–	–	+	–	BRI	Schodde and Craven 5082, 29.iv.1966, PNG, 1200 m
Bubbia sp.	Qu, Km	—	(+)	–	–	–	–	–	NO	Thien 346, 23.xi.1976, NC, 950 m
Bubbia sp.	Qu, Km	—	+	–	–	–	–	–	BRI	Vandenburg s.n. (NGF 39626) 20.vi.1968, PNG, 3500 m
Bubbia sp.	Qu	—	+	–	–	–	+	–	MEL	Veillon 1337, 6.ix.1969, NC, 200 m
Bubbia sp.	—	—	+	–	–	–	–	–	BRI	Womersley s.n. (NGF 11238), 12.vii.1959, PNG, 2500 m
Belliolum gracile A. C. Smith	—	—	+	+	–	–	–	+	BRI	Kajewski 2630, 4.v.1931, S, 1700 m
B. haplopus (B. L. Burtt) A. C. Smith	—	—	+	–	–	–	+	–	BRI	Kajewski 1994, 1.viii.1930, S, 800 m
	—	—	+	–	–	–	–	–	BRI	Kajewski 2007, 4.viii.1930, S, 800 m
	—	—	+	–	–	–	–	–	BRI	Walker and White s.n. (BSIP 154) 24.ix.1945, S
B. pancheri (Baillon) van Tieghem	Qu, Km	—	+	–	–	–	+	+	NO	Thien s.n. ix.1976, NC
B. vieillardi van Tieghem	Qu, Km	—	+	–	–	–	+	+	NO	Thien 278, 29.x.1976, NC
Belliolum sp.	Qu, Km	—	–	–	–	–	+	–	RNG	McPherson 2186, 6.xii.1979, NC
Belliolum sp.	Qu	—	+	–	–	–	–	+	RNG	McPherson 2327, 18.i.1980, NC
Belliolum sp.	Qu	—	–	–	–	–	(+)	+	RNG	McPherson 2947, 8.viii.1980, NC
Belliolum sp.	Qu, Km	—	+	–	–	–	+	–	RNG	McPherson 3126, 20.ix.1980, NC
Belliolum sp.	Qu, Km	—	+	–	–	–	+	(+)	NO	Thien 395 ix.1976, NC

Table 1—*continued.*

Family, genus, species	Flavonols	Flavones	Lu7,3'-DIME	Lu7,4'-DIME	Ap7,4'-DIME	Flavone *C*-glycosides	DiHQu	Procy	Plant source*	Collector's name and number or accession number, collection data, location† and altitude
Exospermum stipitatum	(Km)	—	+	–	–	–	+	–	RNG	McPherson 2184, 6.xii.1979, NC
(Baillon) van Tieghem	(Km)	—	+	–	–	–	–	–	RNG	McPherson 2606, 17.iv.1980, NC
Zygogynum baillonii van Tieghem	Qu	—	+	–	–	–	–	+	NO	Thien 176, 14.x.1976, NC
Zygogynum sp.	Qu, (Km)	—	+	–	–	–	+	+	RNG	McPherson 2358, 24.i.1980, NC, 900 m
Zygogynum sp.	Qu, Km	—	–	+	+§‖	–	–	+	RNG	McPherson 2603, 17.iv.1980, NC, 300 m
Zygogynum sp.	Qu, Km	—	+	–	–	–	+	–	NO	Thien 399, 23.xi.1976, NC, 750 m
Degeneriaceae										
Degeneria vitiensis I. W. Bailey and A. C. Smith	Qu	—	–	–	–	–	–	–	RNG	Sundaresan and Naidu s.n., iv.1981, Fiji
Illiciaceae										
Illicium munipurense Watt ex King	Qu, Km Isorh	—	–	–	–	–	(+)	(+)	ABD	Kingdon Ward 8050, 11.iv.1928, Assam, 2500 m

*Plant sources: ABD = Department of Botany, University of Aberdeen; BRI = Queensland Herbarium, Indooroopilly, Queensland; E = Royal Botanic Gardens, Edinburgh; MEL = National Herbarium of Victoria, Melbourne; NO = Tulane University Herbarium, New Orleans; NSW = National Herbarium of New South Wales, Sydney; QRS = Queensland Research Station, Forest Research Institute, Atherton, Queensland; RNG = Plant Science Laboratories, University of Reading.

†Location abbreviations: IJ = Irian Jaya; LH = Lord Howe Island; NC = New Caledonia; NSW = New South Wales; PNG = Papua New Guinea; Q = Queensland; S = Solomon Islands including Bougainville.

‡Generic classification according to Smith [4, 5, 11]; species are according to herbarium determinations.

§Apigenin 4'-methyl ether may also be present in this plant.

‖Apigenin 7-methyl ether may also be present in this plant.

Key: Qu = quercetin, Km = kaempferol, Isorh = isorhamnetin, Lu = luteolin, Ap = apigenin, DIME = dimethyl ether, DiHQu = dihydroquercetin, Procy = procyanidin. Figures in parentheses = present in trace amount.

Table 2. The distribution of leaf flavonoids at generic level in the Winteraceae and related families

Family, genus	Flavonols	Luteolin 7,3'-DIME	Other methylated flavones	Simple flavones	Flavone *C*-glycosides	DiHQu	Procy
Winteraceae*							
Drimys (4)	+ + + +	+ + +	−	+ + +	+	+ + + +	+ + + +
Tasmannia (12)	+ + + +	+ +	+	+ +	+ + +	+ +	+ + + +
Pseudowintera (1)	+ + + +	+ + + +	−	+ + + +	−	+ + + +	+ + + +
Bubbia (22)	+ + + +	+ + + +	−	−	−	+	+ +
Belliolum (11)	+ + +	+ + + +	(+)	−	−	+ + +	+ + +
Expospermum (2)	+ + + +	+ + + +	−	−	−	+ +	−
Zygogynum (4)	+ + + +	+ + +	+	−	−	+ +	+ + +
Degeneriaceae							
Degeneria (1)	+ + + +	−	−	−	−	−	−
Illiciaceae							
Illicium (1)	+ + + +	−	−	−	−	+ + + +	+ + + +

*Classification according to Smith [4, 5, 11]. Number of specimens surveyed in parentheses. (+) in less than 10% of specimens; + in 10–25% of specimens; + + in 26–50% of specimens; + + + in 51–75% of specimens; + + + + in more than 75% of specimens.

profile (Table 1). Other workers [19] have already remarked on the chemical variability of this species.

On the basis of the present small sample, *Degeneria* and *Illicium* are clearly distinguished from members of the Winteraceae by the apparent absence of flavone derivatives but a more comprehensive survey of these genera is needed in order to establish this difference definitely.

Within the Winteraceae there occurs sufficient flavonoid variability to permit the grouping of genera into chemically similar classes. Thus, *Tasmannia* is seen to be the most diverse group in the present survey. Our findings are largely in agreement with those of Kubitzki and Vink [19] except that we record a lower incidence of kaempferol and were unable to detect the methylated flavone, diosmetin (luteolin 4'-methyl ether). The genus is widely acknowledged to be polymorphic [4, 14, 15] and Vink, in a recent taxonomic revision [2], resorted to the clustering of a large number of previously recognized species under one epithet, namely *T. piperita*, subdivided into 39 morphologic 'entities' which he described as "focal points in the more or less continuous variation". However, many more specimens of *Tasmannia* need to be examined before any meaningful comments may be made on the possible significance of leaf flavonoids as taxonomic markers in subgeneric classifications.

Drimys s. str. and *Pseudowintera* are chemically more uniform than *Tasmannia*. These genera have very similar leaf flavonoid profiles in which flavonols, dihydroquercetin and procyanidin are universally present and methylated and simple flavones are common constituents.

However, the highest level of chemical uniformity is reached in the remaining four genera, *Bubbia*, *Belliolum*, *Exospermum* and *Zygogynum*. In this group methylated flavones, especially luteolin 7,3'-dimethyl ether, show the highest incidence in the family. Flavonols are also common leaf constituents but simple flavones and flavone *C*-glycosides are notably absent. The genera *Bubbia* and *Belliolum* are largely indistinguishable from one another on the results of the flavonoid aglycone survey. *Exospermum* differs from the other three genera only in the absence of procyanidin and *Zygogynum* differs in the variety of methylated flavones present in one of the four specimens examined. Within the genus *Belliolum* there is a marked differentiation between New Caledonian and Solomon Island material in that flavonols are absent from the latter. However, no such simple geographic distinctions were obvious amongst specimens of *Bubbia* or other genera that were surveyed.

The present flavonoid evidence for the division of the Winteraceae into three groups of genera supports recent cytological studies [23]. These findings are also in agreement with the proposed evolutionary scheme for the family [24, 25] with an origin in the south-west Pacific and subsequent spread via southern Australia, New Zealand and Antarctica to South America. In this scheme, *Tasmannia* is recognized as an actively evolving and heterogenous product of the recent expansion of the family from Australia towards south-east Asia via New Guinea. The genus has a distinctive karyotype and is considered phylogenetically removed from the remainder of the family and most particularly from *Drimys s. str.* This has led to the suggested elevation of the genus to familial status [23] but the incidence, albeit reduced, of luteolin 7,3'-dimethyl ether, along with other characters such as features of the carpel, pollen and wood, confirms its association with the other genera. Subfamilial status may be more acceptable and must wait the re-examination of the other south-west Pacific genera.

Zygogynum is considered by some workers to be relatively advanced within the family because of an unusually high polyploid level in at least one species [13] and the release of pollen as monads in a further

two species rather than as tetrads as in the rest of the family [26]. Degrees of advancement within this genus may be reflected by variations in the methylated flavones observed in the few specimens included in this survey. However, the data presented here confirm its chemical association with *Bubbia*, *Belliolum* and *Exospermum*.

In the light of this phylogenetic interpretation, we may suppose the overall chemical profile of the *Bubbia*, *Belliolum*, *Exospermum* and *Zygogynum* class to be primitive within the family, with that of *Drimys s. str.* and *Pseudowintera* as further advanced and that of *Tasmannia* as most advanced. Thus chemical advancement within the family seems to be indicated by a loss of the ability to synthesize methylated flavones with an associated increase in the production of simple flavones and flavone *C*-glycosides.

EXPERIMENTAL

Plant material. Herbarium material was received from various sources, details of which are given in Table 1.

Identification of leaf flavonoids. Simple flavonoid aglycones were identified in acid hydrolysed leaf extracts using standard procedures and in comparison with authentic markers. Dihydroquercetin was detected on paper chromatograms of hydrolysed leaf extracts run in BAW by the red colour formed with Zn dust/6N HCl. Direct 80% methanolic leaf extracts were run by 2D-PC in BAW and 15% HOAc. Methylated flavones were also isolated from 80% methanolic leaf extracts by over-running the concentrated extracts in 15% HOAc on Whatman 3MM paper for 15 hr. The relevant bands were eluted and the concentrated eluates run on cellulose TLC plates in 50% HOAc, CAW, BAW and Forestal against authentic markers (for R_f values see Table 3). For detailed characterization of methylated flavones see below. Flavone *C*-glycosides were confirmed by 4 hr acid hydrolysis, extraction into amyl alcohol and TLC on cellulose against standard markers in CAW, BAW and H_2O.

Identification of luteolin 7,3'-dimethyl ether (**1**). **1** was isolated from the combined 80% methanolic leaf extracts of a number of Winteraceae specimens as described above and was purified by re-running on 3MM paper in 15% HOAc for 24 hr. Chromatographic data are given in Table 3. Demethylation of **1** for 6 hr gave luteolin 7-methyl ether and unchanged **1**. Further demethylation for 5 hr gave a mixture of luteolin, luteolin 7-methyl ether and a trace of **1**. λ_{max} values for **1** are: MeOH 269, 346; +NaOAc 269, 357, 400 sh; $+H_3BO_3$ 269, 346 nm. The absence of a NaOAc shift indicates that the 7-position is blocked and the negative borate shift indicates the absence of two free adjacent hydroxyl groups on the B-ring. The MS fragmentation pattern for **1**, which is presented in Table 4, confirms the presence of one methoxyl on each of the A- and B-rings. The dark to yellow colour reaction of **1** in UV + NH_3 suggests that there is a methoxyl at the 3'-position. In all its properties **1** agrees with

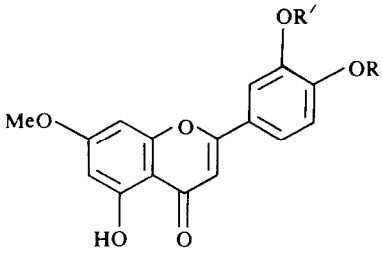
Table 3. Chromatographic properties of methylated flavones from Winteraceae species

Flavones	R_f values (× 100)* 50% HOAc	Forestal	CAW	BAW	Colour† in UV light + NH_3
Luteolin	45	58	41	83	
7,3'-dimethyl ether (**1**)	61	88	100	94	Dk/Y
7,4'-dimethyl ether (**2**)	61	89	100	94	Dk/Dk
7-methyl ether	55	75	91	85	Dk/Y
Apigenin	61	79	81	93	
7,4'-dimethyl ether (**3**)	79	90	100	96	Dk/Dk
7-methyl ether	68	90	100	96	Dk/Y
4'-methyl ether	63	90	100	94	Dk/Dk

*Solvent key: Forestal = concentrated HCl–HOAc–H_2O (3 : 30 : 10); BAW = *n*-BuOH–HOAc–H_2O (4 : 1 : 5, top layer); CAW = $CHCl_3$–HOAc–H_2O (2 : 1 : 1, bottom layer). Support cellulose TLC.
†Dk = dark, Y = yellow.

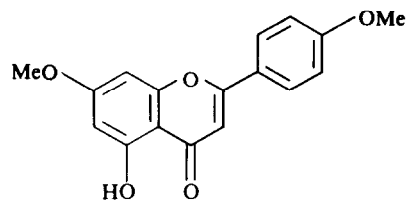
Table 4. Mass spectral properties of methylated flavones from Winteraceae species

Methylated flavone	M	MS (*m/z*) M − 43	A-ring	B-ring	B-ring − CH_2
Luteolin 7,3'-dimethyl ether (**1**)	314 (100)	271 (7.5)	167 (14.1)	147 (2.7)	133 (9.4)
Luteolin 7,4'-dimethyl ether (**2**)	314 (100)	271 (11.9)	167 (14.5)	147 (5.8)	133 (12.3)
Apigenin 7,4'-dimethyl ether (**3**)	298 (67.0)	255 (10.4)	167 (1.6)	131 (4.0)	117 (7.9)



1 R′ = Me, R = H

2 R′ = H, R = Me



3

the literature data and is thus identified as luteolin 7,3′-dimethyl ether. **1** was used as a marker in the general survey of the Winteraceae for methylated flavones.

Identification of luteolin 7,4′-dimethyl ether (**2**). The same purification procedure as for **1** was employed. Chromatographic data are given in Table 3. λ_{max} values for **2** are: MeOH 269, 335; +NaOAc 269, 335; +H_3BO_3 269, 335; +alk. 364 nm. The absence of a NaOAc shift indicates that the 7-position is blocked and the negative borate shift the absence of an *ortho*-dihydroxyl on the B-ring. MS fragmentation data are presented in Table 4. **2** gave the same parent ion (314) and similar A-ring, B-ring and B-ring minus CH_2 fragments to **1** suggesting the presence of one methoxyl on both the A- and B-rings. **2** also gave a dark to dark colour reaction in UV + NH_3 which suggests that there is a methoxyl at the 4′-position, otherwise it has similar chromatographic properties to **1** (Table 3). Thus **2** is characterized as luteolin 7,4′-dimethyl ether.

Identification of apigenin 7,4′-dimethyl ether (**3**). The same purification procedure as for **1** was employed. Chromatographic data are given in Table 3. The identity of **3** was established as apigenin 7,4′-dimethyl ether by co-chromatography with an authentic marker. This was confirmed by the MS fragmentation pattern (Table 4), which gave the correct parent ion and the corresponding A-ring, B-ring and B-ring minus CH_2 fragments for apigenin 7,4′-dimethyl ether.

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