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# THE LEAF FLAVONOIDS OF THE ORCHIDACEAE

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**Key Word Index**—Orchidaceae; flavonols; flavone *C*-glycosides; 6-hydroxyflavones; luteolin 3′,4′-diglucoside; scutellarein 6-methyl ether 7-rutinoside; pectolinarigenin 7-glucoside; mangiferin sulphate; dihydroquercetin 3-glucoside; biochemical systematics.

**Abstract**—In a leaf survey of 142 species from 75 genera of the Orchidaceae, flavone *C*-glycosides (in 53%) and flavonols (in 37%) were found to be the most common constituents. However, since these compounds are not found uniformly and their distribution shows a strong correlation with plant geography, it is not possible to represent the Orchidaceae by a single flavonoid profile. Thus, flavone *C*-glycosides are most common in tropical and subtropical species of the Epidendroid and Vandoid tribes (in 63%) and flavonol glycosides are more characteristic of temperate species of the Neottioid tribes (in 78%). By contrast 6-hydroxyflavones (in 6 species), luteolin (in 2 species) and tricin as the 5-glucoside (in 1 species) are all rare. Three new glycosides were characterised: scutellarein 6-methyl ether 7-rutinoside from *Oncidium excavatum* and *O. sphacelatum*, pectolinarigenin 7-glucoside from *O. excavatum* and *Eria javanica*, and luteolin 3′,4′-diglucoside from *Listera ovata*. The xanthones, mangiferin and isomangiferin were found in *Mormolyca ringens*, *Maxillaria* aff. *luteo-alba* and 5 *Polystachya* species and a mangiferin sulphate tentatively identified in *P. nyanzensis*. Other unusual phenolic constituents include 6,7-methylenedioxy- and 6,7-dimethoxycoumarins from *Dendrobium densiflorum* and *D. farmeri*, formed by the rearrangement during the extraction process from the corresponding *O*-glucosyloxycinnamic acids. The origin and relationship of the Orchidaceae to other monocot groups are discussed in the light of the flavonoid evidence.

## INTRODUCTION

The Orchidaceae is one of the largest of the angiosperm families. Hunt [1, 2], from herbarium material at Kew, recognised approximately 17000 species and some 750 genera, while Garay [3], counting type descriptions gave a figure of nearer 30000, which he suggested might be reduced to *ca* 12000 if major taxonomic revision were ever carried out. Although the family is distributed throughout the world the majority of orchid species are found in the tropics. Its size and the morphological complexity and inaccessibility of many species make classification difficult, especially at tribal and subtribal levels. There are numerous treatments of the family but the present results are arranged according to the system of Dressler [4], because it is one of the most recent comprehensive classifications and aims to be phylogenetic rather than phenetic as is the case of Schlechter's [5] classic system.

Previous chemical investigations of the orchid family have concentrated largely on alkaloid constituents [6] and the identification and inheritance of flower pigments in species of ornamental value [7]. Apart from chlorophyll in green flowered forms and carotenoids in some yellow flowers, anthocyanins play a major role in orchid flower pigmentation. Limited surveys indicate that three anthocyanidins predominate: cyanidin, pelargonidin and petunidin, and that complex mixtures of their glycosides and acylated derivatives are often present in a single flower. Few detailed studies of glycosidic patterns have been undertaken [7–12]. Leaf flavonoids have been identified in only two species: quercetin 3-glucoside, 7-glucoside and 3,7-diglucoside in *Orchis sambucina* and quercetin 3-glucoside in *O. morio* [13] and Bate-Smith [14] reports quercetin from *Aceras anthropophorum*.

A number of unusual coumarins and cinnamic acid derivatives have been reported from orchid species (see Hegnauer [15]). More recently Dahmén *et al.* [16] have characterised 2-(β-D-glucopyranosyloxy)-4,5-dimethoxy-*trans*-cinnamic acid, densifloroside, and the corresponding *cis* isomer from *Dendrobium densiflorum*.

The present study represents the culmination of a larger survey of the leaf flavonoids in the families of the monocotyledons [17–28]. Because of the enormous size of the Orchidaceae and scarcity of material, a detailed flavonoid study of the whole family was not feasible. The aim of the present work was therefore a broad survey of as many genera as possible with a more thorough investigation of some species, in order to determine a flavonoid profile of the family for comparison with other monocot groups.

## RESULTS

The results of the leaf flavonoid survey are given in Table 1. Herbarium material was used from the Botany Department, University of Reading and both fresh and herbarium leaf tissue was received from the Royal Botanic Gardens, Kew. Some dried leaf material was also received from the University of California. The data in Table 1 refer to flavonoid aglycones detected in leaf

Table 1. The distribution of flavonoids in leaves of the Orchidaceae.

| Subfamily, tribe, subtribe, genus, species* | Flavonols | | Flavones | | | Plant source† | Collector's name, number and locality |
|---|---|---|---|---|---|---|---|
| | Qu | Km | Lu | 6-Hydroxy- | C-glycosides | | |
| Cypripedioideae Lindley | | | | | | | |
| *Paphiopedilum venustrum* (Wall.) Pfitz | — | | — | — | | K* | 190-67 |
| *Phragmipedium sargentianum* Rolfe | — | — | — | — | + | K* | 366 05 |
| Orchidoideae | | | | | | | |
| I—Neottioid tribes | | | | | | | |
| 1. Neottieae Lindley | | | | | | | |
| Limodorinae Bentham | | | | | | | |
| *Cephalanthera damasonium* (Miller) Druce | + | — | — | — | — | RNG | L. J. Foster, 76 London Cat. No. 1794, Leatherhead, England |
| *C. longifolia* (L.) Fritsch | + | + | | — | — | RNG | J. Percival, 1886, Forge Valley, Scarborough |
| *C. rubra* (L.) L. C. Richard | + | | — | — | — | RNG | Guadaljana, Spain, 9-VII-37 |
| *Epipactis atrorubens* (Hoffman) Schultes | + | + | — | — | — | RNG | G. Halliday, 218/72 Mali Grad S.E. of Bohinjsko, Jezero, Slovenija, Yugoslavia |
| | + | + | — | — | — | RNG | P. W. Mueller, 133-3-9, Inchnadamph, Scotland |
| *E. helleborine* (L.) Crantz | + | — | — | — | | RNG | J. Percival, 1883, Bolton Gill, Wensleydale, N. Yorks |
| *E. leptochila* (Godfery) Godfery | + | — | — | — | — | RNG | D. P. Young, 28 July, 1951 Maidensgrove, Scrubs |
| *E. palustris* (L.) Crantz | — | — | — | — | — | RNG | C. Grant 7.7.66 Harlech, Merionethshire |
| | + | — | — | — | — | RNG | F. Guinea 2728, Los Aricares, Galicia, Vizcaya, Spain |
| | + | — | — | — | | | |
| *E. veratifolia* Boiss. | | | | | — | RNG | E. Gengiddon, 87, Brissi, Toödos Mts., Cyprus |
| Listerinae Schlechter | | | | | | | |
| *Listera ovata* (L.) R.Br. | — | — | — | — | — | RNG | J. Symons, TQ 911621, Nr. Sittingbourne, Kent |
| | — | — | + | — | — | RNG | Diana Friar, 32, Harlech, Merionethshire |
| | — | — | + | — | — | RNG | J. E. Lousley, 1786, Devils-den Wood, Coulsdon, Surrey |
| *Neottia nidus-avis* (L.) L. C. Richard | — | — | — | — | — | RNG | J. E. Lousley, 1784, Crasham Woods, East Gloucester |
| 2. Diurideae Endlicher | | | | | | | |
| 3. Cranichideae Endlicher | | | | | | | |
| Spiranthinae | | | | | | | |
| *Spiranthes aestivalis* L. C. Richard | + | + | — | — | — | RNG | E. Guinea, 2727, Los Ancestes, Galicia, Spain |
| *S. cernua* L. C. Richard var. *odorata* | | | | | | RNG | *Sine loc.* |
| Goodyerinae Klotzsch | | | | | | | |
| *Goodyera repens* R.Br. | | | | — | — | RNG | A. Somerville, 1436, Bervine, Kincardineshire |
| 4. Epipogeae | | | | | | | |
| 5. Orchideae | | | | | | | |
| Orchidinae | | | | | | | |
| *Aceras anthropophora* (L.) R.Br. | + | + | | — | — | RNG | Walokoch 24-5-31 Kanton Wallis, Les Follaterres bei Brançon, Waldlichturgen, Switzerland |
| *Coeloglossum viride* Hartm. | + | + | | | | RNG | R. W. Rutherford, 00142, Husinish, N. Harris |
| *Cyclopogon congestus* (Vell.) Hoehne | + | + | — | — | — | K* | 032-74.00440 |

Table 1.—*Continued*

| Subfamily, tribe, subtribe, genus, species* | Flavonols | | Flavones | | | Plant source† | Collector's name, number and locality |
|---|---|---|---|---|---|---|---|
| | Qu | Km | Lu | 6-Hydroxy- | *C*-glycosides | | |
| *C. elatus* Schlechter | — | — | — | — | — | K* | 032-74.00445 |
| *Dactylorhiza fuchsii* (Druce) Soó | + | + | | — | — | RNG | 17th July, 1947, Nr. Brynrhug, Dolgelley, Merionethshire |
| *D. incarnata* (L.) Soó | — | — | — | — | + | RNG | C. Grant, 7.7.66, Dune Slacks, Harlech, Merionethshire |
| *D. praetermissa* (Druce) Soó | — | — | — | — | +? | RNG | H. Owen, 133.20., 11 Widemouth Bay, Bude, Cornwall |
| *Gymnadenia conopsea*‡ (L.) R.Br. | + | + | — | — | — | RNG | D. P. Holmes, Bix Bottom, Oxfordshire |
| *Leucorchis albida* (L.) E. Meyer | + | + | — | — | — | RNG | Gravesen and Hansen, 66-2241 |
| *Ophrys apifera* Hudson | + | + | — | — | — | RNG | Kjellquvist, J. Löve N., 187 Sierra de Cazorla, Prov. Jaén, Spain |
| | + | + | — | — | — | RNG | L. J. Foster, 128 S. Downs, Sussex |
| *O. bombyliflora* Link | + | + | — | — | — | RNG | Heywood *et al.*, 1972, 90 Arenillas, Nr. Soto-grande, Prov. de. Cádiz, Spain |
| | + | + | — | — | — | RNG | Heywood *et al.* 1969, 160 Tairfa, Prov. de Cádiz, Spain |
| *O. tenthredinifera* Willd. | + | + | — | — | — | RNG | P. E. Gibbs, 09.115, Sierra Morena, Prov. Sevilla, Spain |
| *Orchis italica* Poiret | + | — | — | — | — | RNG | Heywood *et al.*, 431, 3 km S.E. of Montellano, Prov. Sevilla, Spain |
| *O. mascula* (L.) L. | + | + | — | — | — | RNG | D. M. Moore and D. Bramwell, April 1973, Sierra de Somosierra, Prov. Madrid, Spain |
| *O. morio* L. | + | — | — | — | — | RNG | Heywood *et al.*, 215, Road to Chiclan Prov. Cádiz, Spain |
| | + | | — | — | — | RNG | P. E. Gibbs, 99.115, Sierra Morena, Prov. Sevilla, Spain |
| *O. papilionacea* L. | + | — | — | — | | RNG | C. J. Humphries, I.B.K., Richardson, 17, N. coast of Sardinia |
| *O. ustulata* L. | + | — | — | — | — | RNG | J. Percival, 1883, Carperby, Wensleydale, N. Yorks |
| *Plantanthera bifolia* L. C. Richard | + | + | — | — | + | RNG | Russia, 11.VI. 1956 |
| *P. hyperborea* (L.) Lindley | + | — | — | — | + | RNG | Jørgensen & Larson, 66-069, Botaniske, Undersøgelse, Greenland |
| *Serapias cordigera* L. | + | + | — | — | — | RNG | Heywood *et al.*, 209, Road to Chiclar, N.W. of Concil, Prov. Cádiz, Spain |
| *S. lingua* L. | + | — | — | — | — | RNG | M. Greenway, 5–11 Prov. Alp Maritime Biot. |
| *S. vomeracca* (Burm. f.) Briquet | + | + | — | — | — | RNG | F. Guinca, 29. IV–1941, Audiijar, Prov. Jaén, Spain |
| Disinac Bentham | | | | | | | |
| *Disa uniflora* Berg. | — | — | | — | + | K* | 224-75.02100 |

Table 1.—*Continued*

| Subfamily, tribe, subtribe, genus, species* | Flavonols | | Flavones | | | Plant source† | Collector's name, number and locality |
|---|---|---|---|---|---|---|---|
| | Qu | Km | Lu | 6-Hydroxy- | *C*-glycosides | | |
| II—Epidendroid tribes | | | | | | | |
| 6. Gastrodieae Lindley | | | | | | | |
| Vanillinae | | | | | | | |
| *Vanilla imperialis* Kränzlin | — | — | — | — | + | K* | 000-57.624 |
| | — | — | — | — | + | K* | 000-73.13709 |
| 7. Arethuseae Lindley | | | | | | | |
| Arethusinae Bentham | | | | | | | |
| Bletiinae Bentham | | | | | | | |
| *Bletia purpurea* DC. | — | — | — | — | + | K* | 476-50-47601 |
| *Calanthe vestita* Lindley | | — | — | — | + | K* | 000-69.51753 |
| Thuniinae Schlechter | | | | | | | |
| *Thunia pulchra* Reichb. fil. | | — | — | — | + | K* | 000-62.012 |
| Sobraliinae Schlechter | | | | | | | |
| *Arpophyllum giganteum* Hartw. ex Lindley | — | — | — | — | + | K* | 000-24.223 |
| *Meiracyllium trinasutum*§ Reichb. fil. | — | — | — | — | + | K* | 032-75.00380 |
| 8. Epidendreae H.B.K. | | | | | | | |
| Laeliinae Bentham | | | | | | | |
| *Brassavola nodosa* Lindley ssp. *grandiflora* | — | — | — | — | + | K* | 726-32.72601 |
| *Cattleya bowringiana* Veitch | — | — | — | — | + | K* | 000-58.081 |
| | — | — | — | — | + | C | |
| *Encyclia bractescens* (Lindley) Hoehne | | | — | — | + | K* | 775-28.77501 |
| *Epidendrum floribundum* H.B.K. | — | — | — | — | + | K* | 000-63.266 |
| *E. fragrans*? Sw. | — | — | | — | + | K* | 281-73.02768 |
| *E. huebneri* Schlechter§ | — | — | — | — | + | K* | 059-74.00775 |
| *Laelia anceps* Lindley | — | — | — | — | + | K* | 452-59.45227 |
| Eriinae Bentham | | | | | | | |
| *Eria javanica* Blume | — | — | — | + | — | K* | 00-67.190 |
| *Trichotosia elongata* (Blume)§ Kränzlin | — | — | | +? | + | K* | 175-24 |
| *Ceratostylis subulata* Blume | — | — | — | — | + | K* | 321-74.02716 |
| *Glomera* sp. | — | — | — | — | — | K* | 351-75.03298 |
| Pleurothallidinae Lindley | | | | | | | |
| *Masdevallia triangularis* Lindley | — | — | — | | + | K* | 336-73.04453 |
| *Pleurothallis cardiostola* Reichb. fil. | + | — | — | — | — | K* | Brazil |
| *P. dolichopos* Schlechter | + | — | — | — | — | K* | 361-34.36101 |
| *P. gelida* Lindley | + | | — | — | — | K* | 000-73.13668 |
| *P. lonchophylla* Reichb. fil. | — | — | — | — | + | K* | 000-63.577 |
| *P. revoluta* (Ruiz & Pavon) Garay | + | — | | — | — | K* | 782-29.78202 |
| *P. semipellucida* Reichb. fil. | + | — | — | — | | K* | 782-29 |
| *Restrepia elegans* Karsten‡§ | — | | — | — | + | K* | 091-42.09132 |
| *Stelis lutea* Lindley (uncertain position) | + | — | — | — | — | K* | 229-91.22901 |
| *Stolzia repens* | — | — | — | — | + | K* | 052.77.00301 |
| 9. Dendrobieae Endlicher | | | | | | | |
| Dendrobiinae | | | | | | | |
| *Dendrobium chrysanthum* Wall. | — | + | — | — | — | K* | 000-67.190 |
| *D. chrysotoxum* Lindley | — | — | — | — | + | K* | 032-75.00501 |
| *D. densiflorum* Wall.‖ | + | + | — | — | — | K* | 000-62.642 |
| ‖ | + | + | — | — | — | K* | 032-75.00504 |
| *D. farmeri* Paxt.‖ | + | + | — | — | — | K* | 000-67.190 |
| *D. fimbriatum* Hooker var. *oculatum* | — | + | — | — | — | K* | 000-68.366 |
| *D. gracilicaule* F. von Mueller var. *howeanum* | — | (+) | — | — | — | K* | 320-71.02765 |
| *D. kingianum* Bidw. | — | + | | — | — | K* | 000-42.091 |
| *D. moschatum* Swartz. | — | — | — | — | (+) | K* | 000-37.673 |
| *D.* × *superbiens* Reichb. fil. | — | — | — | — | — | K* | 000-62.751 |

Table 1. —*Continued*

| Subfamily, tribe, subtribe, genus, species* | Flavonols | | Flavones | | | Plant source† | Collector's name, number and locality |
|---|---|---|---|---|---|---|---|
| | Qu | Km | Lu | 6-Hydroxy- | *C*-glycosides | | |
| *Diplocaulobium* aff. (Reichb. f.) *tipuliferum* aff. Kränzlin | — | — | — | — | + | K* | 405-71.03844 |
| *Ephemerantha fimbriata* (Blume) P. F. Hunt and Summerhayes | — | — | — | — | + | K* | 651-59.65113 |
| *Epigeneium triflorum*§ (Blume) Summerhayes | — | — | — | — | + | K* | 484-55.48403 |
| Bulbophyllinae Schlechter | | | | | | | |
| *Bulbophyllum bequaertii* De Wild var. *brachyanthum* Summerhayes | — | + | — | — | — | K* | 052-77.00290 |
| *B. gibbosum* Lindley | — | — | — | — | + | K* | 247-22.24702 |
| 10. Maxideae Lindley | | | | | | | |
| *Liparis taiwaniana* Hayata | + | + | — | — | + | K* | 270-75.02572 |
| III—Vandoid tribes | | | | | | | |
| 11. Maxillarieae Pfitzer | | | | | | | |
| Zygopetalinae | | | | | | | |
| *Koellensteinia graminea* Reichb. fil. | — | — | — | — | + | K* | 078-77.02449 |
| *Zygopetalum* × 'John Banks' | — | — | — | — | + | K* | 078-77.00756 |
| *Cochleanthes flabelliformis* (Sw.) R. E. Schult. | — | — | — | — | + | K* | 321-77.02449 |
| *Xylobium palmifolium* Fawcett | — | — | — | — | + | K* | 433-75.04415 |
| *Anguloa brevilabris* Rolfe | — | — | — | — | +? | K* | 300-28.3001 |
| *Lycaste consorbrina*?§ Reichb. fil. | — | — | — | — | + | K* | 000-59.201 |
| *Neomoorea wallisii*¶ Schlechter | — | — | + | — | + | K* | 567-58.56712 |
| Maxillariinae Lindley | | | | | | | |
| *Maxillaria* aff.** *luteo-alba* Lindley | — | — | — | — | — | M | 73/138 Orige Misiones, Agentina |
| *M. meleagris* Lindley | — | — | — | — | + | K* | 116-28.11601 |
| *Mormolyca ringens*** (Lindley) Schlechter | — | — | — | — | + | K* | 170-14.17001 |
| Dichaeinae Schlechter | | | | | | | |
| *Dichaea graminoides* (Swartz) Lindley | — | — | — | — | + | K* | 059-74-00803 |
| 12. Vandeae Lindley | | | | | | | |
| Sarcanthinae Bentham | | | | | | | |
| *Aerides fieldingii* Lodd¶ ex E. Morr. | + | + | — | — | — | K* | 000-67.190 |
| *A. odorata* Lour.¶ | + | + | — | — | — | K* | 032-75.00468 |
| *Angraecum eburneum* Bory | — | — | — | — | + | K* | 461-57.46122 |
| *A. eichlerianum* Kränzlin | — | — | — | — | +? | K* | 000-48.302 |
| *Chamaeangis odoratissima* Schlechter | — | — | — | — | + | K* | 000-42.091 |
| *Doritis pulcherrima* Lindley | — | — | — | — | + | K* | 582-63.58204 |
| *Phalaenopsis cornu-cervi* Blume & Reichb. fil. | — | — | — | — | + | K* | 000-65.444 |
| *Renanthera inschootiana* Schlechter | — | — | — | — | + | K* | 193-73.01810 |
| *Sarcanthus racemifer* Reichb. fil. | — | — | — | — | + | K* | 737-62 |
| *Vanda merrillii* Ames & Quisumb. | — | — | — | — | + | K* | 529-69-04476 |
| *Vandopsis lissochiloides* Pfitz. | — | — | — | — | + | K* | |
| 13. Polystachyeae Pfitzer | | | | | | | |
| *Neobenthamia gracilis* Rolfe | + | + | — | — | — | K* | 000-23.177 |
| *Polystachya anceps* Ridley | — | — | — | — | + | K | Mason 151, Malagasy Rep. |
| *P. angustifolia* Summerhayes | — | — | — | — | + | K | C. D. Smith, 4593 |
| *P. ashantensis* Kränzlin | + | — | — | — | — | K | W. J. Eggeling, 3037, Uganda |
| *Polystachya bella* Summerhayes | + | — | — | — | — | K* | 014-71.00143 |

Table 1.—*Continued.*

| Subfamily, tribe, subtribe, genus, species* | Flavonols | | Flavones | | | Plant source† | Collector's name, number and locality |
|---|---|---|---|---|---|---|---|
| | Qu | Km | Lu | 6-Hydroxy- | C-glycosides | | |
| | + | — | — | — | — | K* | Mason, 148, Ethiopia 433–75 |
| *P. bicarinata* Rendle | + | + | — | — | — | K | W. J. Eggeling, 3246 |
| *P. caespitifica* Kränzlin ex Engler | — | — | — | — | + | K | W. J. Eggeling, 4142 |
| *P. cultriformis* (Thou.) Sprenger | — | — | — | — | + | K* | 433.75.04375 |
| | — | — | — | — | — | K | W. M. Moreau, 696, Kenya |
| | + | + | — | — | — | K | W. J. Eggeling, 3307, Uganda |
| | — | — | | | + | K | Drummond & Hemsley, 2097, Tanzania |
| ** | — | | | | — | K | H. Perrier de la Bathie (1824), Malagasy Rep. |
| *P. dolichophylla* Schlechter | — | — | — | — | + | K* | 796-58 |
| *P. fallax* Kränzlin | — | — | — | — | + | K* | 44-70.04172 |
| *P. fulvilabia* Schlechter** | — | | — | — | — | K* | 44-70.04172 |
| *P. galeata* Reichb. fil.** | | | | | | K* | *Sine loc.* |
| ** | — | — | — | — | — | K* | 117-72.01094 |
| *P. gerrardii* Harvey (= *P. cultriformis* Thou. Sprenger) | — | — | — | — | +? | K | H. Wild, 49363, Rhodesia |
| *P. golungensis* Reichb. fil. | | | | — | + | K | Mason, 2900 |
| *P. hislopii* Rolfe | + | — | — | — | — | K* | 347.62 |
| *P. inconspicua* Rendle | + | — | — | | | K | Olov Hedberg, 303, Kenya |
| *P. latilabris* Summerhayes | + | — | — | — | — | K | Greenway & Kanuri, 12456, Tanzania |
| *P. laxiflora* Lindley | + | — | — | — | — | — | 095-72.00751 |
| *P. megalogenys* Summerhayes | + | | | | | K | A. Leonard, 4788, Congolana |
| *P. nyanzensis* Rendle** | — | — | — | — | — | K | Stewart, 1243 |
| ** | — | — | — | — | — | K | W. J. Eggling, Uganda |
| *P. pachychila*? Summerhayes** | — | +? | — | — | — | K | Colin Leakey, Uganda |
| *P. paniculata* Rolfe | | | | | + | K | Moult, 72 |
| | — | — | — | — | + | K* | 373-72.02932 |
| *P. pubescens* Reichb. fil. | + | + | — | — | | K* | 000-24.223 |
| *P. stauroglossa* Kränzlin | — | — | — | — | — | K* | 031-69.00300 |
| *P. supfiana* Schlechter | — | — | — | — | + | K | Talbot, 440, S. Nigeria |
| *P. tessellata* Lindley | | | — | — | + | K | Stewart, 1282 |
| 14. Cymbidieae Pfitzer | | | | | | | |
| Cyrtopodiinae Bentham | | | | | | | |
| *Eulophia andamanensis* Reichb. fil. | — | — | — | — | + | K* | 000-67.190 |
| *E. paivaeana* (Reichb. fil) Summerhayes ssp. *borealis* | | | — | — | + | K* | 430-74.03771 |
| *Graphorkis scripta* (Sw.) Kuntze | | | | — | + | K* | 112-72.01132 |
| *Oeceoclades saundersianum* (Reichb. fil.) Garay & Taylor | — | — | — | — | + | K* | 132-69.01046 |
| Cymbidiinae Bentham | | | | | | | |
| *Cymbidium atropurpureum* Rolfe | — | — | — | — | + | K* | 000-63.417 |
| *C. finlaysonianum* Lindley | | | — | — | + | C | |
| *C. madidum* Lindley | — | — | — | — | + | C | |
| 15. Pachyphylleae Dressler | | | | | | | |
| Lockhartiinae Schlechter | | | | | | | |
| *Lockhartia acuta* Reichb. fil. | + | — | — | — | — | K* | 000-73.13628 |
| 16. Gongoreae Pfitzer | | | | | | | |
| *Gongora quinquenervis* Ruiz & Pavon | — | — | — | — | + | K* | 310-73.04339 |
| *Stanhopea devoniensis* Lindley | — | — | — | — | + | K* | 705-63.70514 |

Table 1. —*Continued*

| Subfamily, tribe, subtribe, genus, species* | Flavonols | | | Flavones | | Plant source† | Collector's name, number and locality |
|---|---|---|---|---|---|---|---|
| | Qu | Km | Lu | 6-Hydroxy- | C-glycosides | | |
| 17. Oncidieae Pfitzer | | | | | | | |
| *Odontoglossum pendulum* (La Llave & Lex.) Batem. | — | — | — | +? | — | K* | 000-59.452 |
| *Oncidium baueri* Lindley | | | | + | — | K* | *Sine loc.* |
| *O. excavatum* Lindley | — | — | — | + | — | K* | 270-75.02469 |
| *O. pumilum* Lindley | — | — | — | + | — | K* | 033-72.00326 |
| *O. sphacelatum* Lindley | — | — | — | + | | C | |
| | — | — | — | + | — | K* | 613-08.61301 |

Key: Qu = quercetin; Km = kaempferol; Lu = luteolin; + = present; — = absent; (+) = trace constituents.
* Classification according to Dressler [4].
† Plant sources: K = The Herbarium, The Royal Botanic Gardens, Kew; K* = plants growing at the Royal Botanic Gardens,
‡ Negatively charged flavonoid present.
Kew with accession number; RNG = Reading University Herbarium; C = plants from Professor J. Arditti, growing at the Dept of Organismic Biology, The University of California at Irvine; M = plants grown by Prof. D. M. Moore at The Botany Dept, University of Reading.
§ Procyanidin also present.
‖ These plants contain 2-glucosyloxy-4,5-dimethoxy *cis* and *trans*-cinnamic acid and 2-glucosyloxyl-4,5-methylenedioxy-cinnamic acid, which break down on extraction to give the corresponding coumarins.
¶ Dihydroquercetin also present.
** Mangiferin and isomangiferin present in these plants.

tissue after acid hydrolysis. The identity of the aglycones was established by comparison of $R_f$ values and colour reactions in UV light with standard markers. The results of the aglycone survey were confirmed by 2D-PC of direct leaf extracts and by the characterisation of the flavonoid glycosides in 19 species (Table 2). Flavone *C*-glycosides were confirmed by their resistance to acid hydrolysis and the presence of flavonoid and xanthone sulphates by electrophoresis of direct leaf extracts.

Two new 6-hydroxyflavone glycosides were characterised during the course of the survey: scutellarein 6-methyl ether 7-rutinoside from *Oncidium excavatum* and *O. sphacelatum*, and pectolinarigenin 7-glucoside from *O. excavatum* and *Eria javanica.* Pectolinarigenin 7-rutinoside, first isolated from *Linaria vulgaris* (Scrophulariaceae) [29], was also found in both the above *Oncidium* species and free pectolinarigenin in *E. javanica*. However, 6-hydroxyflavones are rare leaf constituents of the Orchidaceae being detected in only six of the 142 species surveyed, including the two other *Oncidium* species: *O. baueri* and *pumilum*. Dressler [4] places *Oncidium* and *Eria* in separate tribes: the Oncidieae and the Epidendreae (subtribe Eriinae), respectively, whereas in a previous treatment by Dressler and Dodson [31] these genera were in the same tribe, the Epidendreae, but in different subtribes. One other member of the Oncidieae, *Odontoglossum pendulum* was surveyed. In this plant some dark to dark glycosides (in UV + $NH_3$) were detected, which could possibly be 6-hydroxyflavone derivatives but there was insufficient material for identification. The presence of quercetin and the absence of 6-hydroxyflavones in *Lockhartia acuta* supports Dressler's [4] separation of this genus from the Oncidieae. The leaves of the other species examined in the subtribe Eriinae, *Trichotosia elongata*, accumulate an unusual dark to dark (UV + $NH_3$) glucoside, whose aglycone has similar $R_f$ and spectral properties to wogonin, 5,7-dihydroxy-8-methoxyflavone (see Experimental). However, all attempts to characterise this substance have so far failed.

The common flavones are also rarely present as orchid leaf constituents. Luteolin was found in only two of the 142 species studied: *Listera ovata* and *Neomoorea wallisii*. In all three samples of *Listera ovata* examined, the 3′,4′-diglucoside and a similar diglucoside with a possibly different sugar linkage were identified. This represents the first report of luteolin 3′,4′-diglucoside in nature. Another unusual flavone glycoside, tricin 5-glucoside, a frequent leaf constituent of the grasses, was detected in small amount in one orchid species, *Restrepia elegans*.

The most characteristic flavonoid constituents of the Orchidaceae are flavone *C*-glycosides (in 53% of the sample) and flavonols (in 37% of species). However, these compounds are not distributed uniformly through the family. Thus, flavone *C*-glycosides are found largely in the tropical and subtropical species of the Epidendroid and Vandoid tribes (in 63% of species compared with flavonols in 23% of the sample), whereas flavonols are most frequent in temperate species of the Neottioid tribes, where they occur in 78% (flavone *C*-glycosides in 19%) of the sample.

Several flavone *C*-glycosides and flavonol glycosides were characterised during the course of the survey (see Table 2). Vitexin 7-glucoside and isovitexin 7-glucoside were identified in leaves of *Cymbidium finlaysonianum* and *C. madidum*, vitexin was also found in *C. madidum* and both vitexin and isovitexin in *Maxillaria* aff. *luteo-alba*. An unidentified flavone *C*-glycoside sulphate was present in *Restrepia elegans* together with one luteolin *C*-glycoside and two apigenin di-*C*-glycosides. The flavonol glycosides identified include: rutin in *Pleurothallis gelida*, quercetin and isorhamnetin 3-glucosides and quercetin 3-(6-acetylglucoside) in *Neobenthamia gracilis*, and quercetin and kaempferol 3-glucosides in *Aerides fieldingii*. Leaves of the latter plant also contained an unusually large amount of the flavanol, dihydroquercetin 3-glucoside. Dihydroquercetin was also detected in two unrelated species: *Neomoorea wallisii* and *Gongora quinquenervis*.

Table 2. Flavonoid glycosides identified in the leaves of some Orchidaceae species

| Species | Flavonoid glycosides identified |
|---|---|
| *Aerides fieldingii* | Quercetin and kaempferol 3-glucosides, dihydroquercetin 3-glucoside |
| *Anguloa brevilabris* | Vitexin |
| *Cattleya bowringiana* | 3 Apigenin di-*C*-glycosides |
| *Chamaeangus odoratissima* | Chrysoeriol *C*-glycoside-7-*O*-glucoside |
| *Cymbidium finlaysonianum* | Vitexin 7-glucoside, isovitexin 7-glucoside and an apigenin di-*C*-glycoside |
| *C. madidum* | Vitexin 7-glucoside, vitexin, isovitexin 7-glucoside and an apigenin di-*C*-glycoside |
| *Eria javanica* | Pectolinarigenin and pectolinarigenin 7-glucoside |
| *Listera ovata* | Luteolin 3,4′-diglucoside |
| *Maxillaria* aff. *luteo-alba* | Mangiferin, isomangiferin, mangiferin isomer, vitexin, isovitexin |
| *Mormolyca ringens* | Mangiferin, isomangiferin, two unidentified xanthones with no *C*-sugar, a flavone *C*-glycoside |
| *Neobenthamia gracilis* | Quercetin and isorhamnetin 3-glucosides, quercetin 3-(6-acetylglucoside) |
| *Oncidium excavatum* | Pectolinarigenin 7-glucoside and 7-rutinoside, scutellarein 6-methyl ether 7-rutinoside |
| *Oncidium sphacelatum* | Scutellarein 6-methyl ether 7-rutinoside, pectolinarigenin 7-rutinoside |
| *Pleurothallis gelida* | Quercetin 3-rutinoside |
| *Polystachya fulvilabia* } *P. galeata* } | Mangiferin and isomangiferin glucosides |
| *P. nyanzensis* | Mangiferin, isomangiferin and mangiferin sulphate |
| *P. pachychila* | Mangiferin, isomangiferin and mangiferin sulphate |
| *Restrepia elegans* | Tricin 5-glucoside, flavone *C*-glycoside sulphate, luteolin *C*-glycoside, two apigenin di-*C*-glycosides |
| *Trichotosia elongata* | Flavone *C*-glycoside, glucoside of unknown dark to dark (in UV $+ NH_3$) aglycone |

Bate-Smith [14] records procyanidin in two of the 31 species he surveyed: in *Eria rosea*, a member of the tribe Epidendreae, subtribe Eriinae and in *Dendrobium thyrsiflorum* of the tribe Dendrobieae, subtribe Dendrobiinae. In the present survey, procyanidin was found in two other members of these groups: in *Trichotosia elongata* (Epidendreae, Eriinae) and *Epigeneum triflorum* (Dendrobieae, Dendrobiinae), respectively, and in two other taxa in different subtribes of the tribe Epidendreae: *Epidendrum huebneri* (subtribe Laeliinae) and *Restrepia elegans* (subtribe Pleurothallidinae). Procyanidin was detected in only two other species: *Meiracyllium trinasutum* (Arethuseae, Arethusinae) and *Lycaste consobrina* (Maxillarieae, Zygopetalinae) and is thus a rare leaf constituent of the Orchidaceae.

Other phenolic constituents that were detected during the survey include xanthones and cinnamic acid derivatives. Mangiferin and isomangiferin were identified in *Mormolyca ringens*, *Maxillaria* aff. *luteo-alba* and in five of 25 species of *Polystachya* surveyed: *P. fulvilabia*, *P. galeata*, *P. nyanzensis*, *P. pachychila* and *P. cultriformis* (in one of five samples). *Mormolyca* and *Maxillaria* are both members of the tribe Maxillarieae, subtribe Maxillariinae, whereas *Polystachya* species are placed in a separate tribe, the Polystachyeae [4]. In *P. nyanzensis* a negatively charged xanthone with the same orange to bright yellow colour reaction (in UV + $NH_3$) as mangiferin was detected. This compound gave mangiferin on acid hydrolysis and is tentatively identified as mangiferin sulphate. Unfortunately, there was insufficient material to complete the characterisation. This is the first report of a xanthone sulphate in nature.

Some unusual cinnamic acid derivatives, 2-glucosyloxy-4,5-dimethoxy-*trans*-cinnamic acid and its *cis* isomer have recently been reported from *Dendrobium densiflorum* [16]. These compounds break down on extraction to give the corresponding coumarin, 6,7-dimethoxycoumarin. Ayapin (6,7-methylenedioxycoumarin) and 6,7-dimethoxycoumarin have previously been identified in leaves of *D. thyrsiflorum* [30], now considered a synonym of *D densiflorum* [36]. In the present survey both ayapin and 6,7-dimethoxycoumarin were identified in *D. densiflorum* and in another closely related species, *D. farmeri*. The presence of ayapin in the leaf extract suggests that the corresponding 2-glucosyloxy-4,5-methylenedioxycinnamic acid may also occur *in vivo* in these plants.

## DISCUSSION

While most flavonoid classes: flavone *C*-glycosides, flavonols, flavones, 6-hydroxyflavones, proanthocyanidins and xanthones are all represented in leaves of the Orchidaceae, perhaps the most interesting feature of the present survey is the lack of advanced flavonoid characters, e.g. highly methylated or glycosylated derivatives in a family where morphological complexity has reached a climax. However, the almost complete absence of proanthocyanidins does suggest a high degree of advancement for the family as a whole, and perhaps a survey of orchid flowers, morphologically the most derived tissue in these plants, could well provide more evidence for this.

Garay [32] has already pointed out the morphologically heterogeneous nature of the Orchidaceae in that both advanced and primitive characters are frequently found within a single species. This is to some extent reflected in the present flavonoid results, in that in contrast with most other monocot families a single familial pattern is not easily distinguished. However, when the results are considered at subfamily and tribal levels (see Table 3) an apparently clear correlation with plant geography may be seen. Thus, most temperate species of the Neottioid tribes accumulate flavonol glycosides, whereas the largely tropical species of the Cypripedioideae, the Epidendroid and Vandoid tribes produce mainly flavone *C*-glycosides. Since glycosylflavones are generally considered to be retained primitive characters, this distribution supports Garay's [32] suggestion that the orchids arose somewhere in the Asiatic tropics, where those species with the most primitive morphological features, e.g. species of the Malayan genus *Neuwiedia*, are still to

Table 3. A comparison of the leaf flavonoids of the Orchidaceae with those of some other monocot groups

| Order or family | Flavone C-glycosides | Flavonols | 6-Hydroxy flavones | Flavones (Lu or Ap) | Tricin | Mangi-ferin | Flavonoid sulphates | Reference |
|---|---|---|---|---|---|---|---|---|
| Orchidaceae | + + | + + | + | (+) | (+) | (+) | (+) | — |
| Bromeliaceae | + | + + | + | + | — | — | — | [28] |
| Commelinaceae | + + | + | (+) | + | (+) | (+) | — | [*] |
| Cyperaceae | + + | + | + | + | + | — | (+) | [26] |
| Fluviales | + + | (+) | — | + | - | — | + | [24] |
| Gramineae | + + + | (+) | — | + | + + + | — | + | [25] |
| Iridaceae | + + | + | — | nd | + | + | nd | [14, *] |
| Juncaceae | (+) | (+) | — | + + + | - | | + | [22] |
| Liliaceae | (+) | + | — | + | (+) | (+) | (+) | [23] |
| Palmae | + + + | + | — | + | + + | — | + + + | [21] |
| Zingiberales | + | + + | — | + | — | — | (+) | [27] |

Key: (+) in >10% of species; + in 10–25% of species, + + in 25–50% of species; in <50% of species; nd = not determined.
* Unpublished data, see text below.

be found. As flavonols are formed at a later stage in the biosynthetic pathway than flavone *C*-glycosides they could be considered as advanced characters in the Orchidaceae. There is, however, no morphological evidence to suggest that members of the Neottioid tribes could have arisen from present day or ancestral species of the tropical Epidendroid or Vandoid tribes. The present flavonoid data may therefore indicate that either the ancestors of the present day temperate and tropical species were separated very early in the course of evolution, or that the origin of the Orchidaceae was polyphyletic as suggested by Garay [32].

In the almost complete absence of any fossil record for the orchids, the relationship of the Orchidaceae with other families of the monocotyledons will always remain uncertain. Several taxonomists [33–35] have suggested that the Orchids arose from Liliaceous ancestors on morphological grounds. However, a comparison of the flavonoid data of the Orchidaceae with other monocot families previously surveyed (Table 3) apparently refutes this idea in that flavone *C*-glycosides, the characteristic flavonoid constituents of tropical orchids, are rarely found in leaves of the Liliaceae. Instead the Orchidaceae show most similarity to the Commelinaceae, a family in which flavone *C*-glycosides and flavonols are both common leaf constituents [Del Pero de Martinez, M. A., unpublished results], the Iridaceae [14; Harborne, J. B., unpublished data], where flavone *C*-glycosides, flavonols and mangiferin are characteristic leaf components and the isolated group, the Bromeliaceae [28], in which flavone *C*-glycosides, flavonols, flavones and 6-hydroxyflavones are all represented. Takhtajan [33] suggests a possible common origin for the Bromeliales and Commelinales and from the present evidence, the orchids could have arisen from a similar stock or at a much earlier stage in the evolution of the angiosperms (see Garay [32]).

Below tribal level the present sampling is hardly sufficient to be significant for taxonomic purposes. However, a few groups are worthy of further discussion. Thus, the Pleurothallidineae are unique in the Epidendreae, where flavone *C*-glycosides are the characteristic leaf constituents, in the high proportion of species producing flavonols. Similarly, in the tribe Vandeae, *Aerides* species differ from the taxa of eight other genera of the Sarcanthinae in accumulating flavonol glycosides rather than glycosylflavones. A number of genera have uniform leaf flavonoid profiles, e.g. *Epipactis*, *Ophrys* and *Orchis* amongst the temperate orchids and the tropical genera, *Pleurothallis* and *Oncidium*.

It is generally agreed that species of *Oncidium* are morphologically highly evolved and this is reflected in the presence of the advanced leaf flavonoid constituents, 6-hydroxyflavones. In some larger genera, e.g. *Dendrobium* and *Polystachya*, the flavonoid results clearly indicate the heterogeneous nature of these groups.

The genus *Polystachya* is unusual in that among the 26 species examined three different leaf flavonoid patterns could be distinguished: (1) flavonol, (2) flavone *C*-glycoside and (3) xanthone (mangiferin and isomangiferin). *P. cultiformis* proved to be chemically the most heterogeneous species in that all three flavonoid patterns were found amongst the five accessions surveyed.

## EXPERIMENTAL

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

*Identification of flavonoids.* Flavonoid aglycones were characterised from acid hydrolysed leaf extracts using standard procedures and by comparison with authentic markers. The results of the aglycone survey were confirmed by 2D-PC of direct 80% methanolic leaf extracts using the solvents BAW and 15% HOAc. Known glycosides, isolated and purified by standard procedures, were identified on the basis of $R_f$, UV spectral analysis, acid hydrolysis to aglycone and sugar and by direct comparison with authentic markers. Flavone *C*-glycosides were confirmed by 4 hr acid treatment, extraction into amyl alcohol and PC against authentic markers in BAW and $H_2O$. The presence of phenolic sulphates was confirmed by electrophoresis on Whatman 3MM paper at pH 2.2 ($HOAc/HCO_2H$ buffer) for 2 hr at 400 V.

*Identification of scutellarein 6-methyl ether-7-rutinoside from* Oncidium sphacelatum. The glycoside was isolated from an 80% methanolic leaf extract by PPC in 15% HOAc, BAW and CAW. $R_f$ data are given in Table 4. Acid hydrolysis with 2 N HCl for 40 min gave scutellarein 6-methyl ether (UV, MS, $R_f$ compared with scutellarein 4'-methyl ether), glucose and rhamnose. $\lambda_{max}$ for the glycoside: MeOH 275, 337; +NaOAc 275, 337; $+H_3BO_3$ 275, 337; + alk 277, 386 nm. The absence of a NaOAc shift and the dark to yellow colour (in UV + $NH_3$) of the glycoside indicates that both sugars are attached at the 7-position.

Table 4. $R_f$ ( × 100) data for new and unusual flavonoid glycosides found in the Orchidaceae*

| Flavonoids | Solvents BAW | Forestal | 50% HOAc | CAW | Phenol | 15% HOAc | $H_2O$ | BEW |
|---|---|---|---|---|---|---|---|---|
| Scutellarein | 66 | 57 | 44 | 30 | | — | | — |
| Scutellarein 6-methyl ether | 59 | 88 | 77 | 67 | — | — | — | — |
| -7-rutinoside | 90 | 77 | 63 | 72 | 94 | 09 | — | — |
| Scutellarein 4′- methyl ether | 77 | 69 | 50 | 74 | 96 | 02 | — | |
| Pectolinarigenin | 95 | 84 | 74 | 93 | 98 | 11 | — | — |
| 7-glucoside | 51 | — | — | — | 87 | 23 | 02 | — |
| 7-rutinoside | 50 | — | — | 55 | 88 | 58 | 14 | — |
| Luteolin 3′,4′-diglucosides | | | | | | | | |
| (1) | 15 | — | — | 03 | 51 | 32 | 10 | |
| (2) | 17 | — | — | 05 | 75 | 32 | 13 | — |
| Methylated product of (1) and (2) | 66 | 76, (84) | 55 (63) | 67 | | — | — | — |
| Luteolin 5,4′-dimethyl ether | 70 | 78 | 56 | 69 | — | — | — | — |
| Dihydroquercetin 3-glucoside | 51 | | — | — | 51 | 66 | 52 | 65 |
| *Neobenthamia gracilis* | | | | | | | | |
| Quercetin 3-glucoside | 49 | — | — | — | 44 | 31 | 18 | 48 |
| 3-acetylglucoside | 69 | — | — | — | 69 | 42 | 28 | 65 |
| *Trichotosia elongata* | | | | | | | | |
| DK/DK glycoside | 57 | — | — | — | 92 | 60 | 29 | 54 |
| DK/DK aglycone | 74 | 87 | 81 | 78 | 88 | | — | — |
| Demethylated aglycone | | | | | | | | |
| B/Y | 84 | 77 | 62 | 84 | 76 | — | | — |
| DK/DK | 76 | 82 | 73 | 94 | 85 | — | — | — |
| DK/Y | 92 | 82 | 82 | 94 | 85 | — | | — |
| Methylated aglycone | 74, 83 | — | 88 | 81 | — | 53 | — | — |
| 5,7-dihydroxy-8-methoxyflavone (wogonin) | 84 | 81 | 70 | 82 | 76, 85 | — | — | — |
| 5,7,8-trihydroxyflavone | 91 | 87 | 81 | 93 | 91 | — | — | |
| *Restrepia elegans* | | | | | | | | |
| Flavone *C*-glycoside sulphate | 05 | — | — | — | — | 25 | — | 03 |
| Vitexin | 37 | — | — | | 63 | 25 | 09 | 41 |

* Some markers are given for comparison.
Key: B/Y = blue to yellow, DK/DK = dark to dark and DK/Y = dark to yellow in UV light + $NH_3$, respectively.

*Identification of pectolinarigenin 7-glucoside from* Eria javanica. The glycoside was isolated as above and purified by PPC in BAW, 15% HOAc and $H_2O$. $R_f$ data are given in Table 4. Acid hydrolysis gave pectolinarigenin (co-PC and UV) and glucose only. $\lambda_{max}$ for glycoside: MeOH 251, 276, 340, + NaOAc 251, 276, 340; + $H_3BO_3$ 251, 276, 340; + alk 291, 308 nm. The absence of a NaOAc shift and the colour of the glycoside (dark to dark in UV + $NH_3$) suggests that the glucose is attached to the 7-hydroxyl.

*Identification of luteolin 3′,4′-diglucoside from* Listera ovata. The glycoside was isolated from herbarium leaf material as above and purified by PPC in 15% HOAc, phenol and $H_2O$. $R_f$ data are given in Table 4. Acid hydrolysis gave luteolin and glucose only. $\lambda_{max}$ for the glycoside: MeOH 273, 328; + NaOAc 278, 328; + $H_3BO_3$ 273, 328; and + alk 276, 385 nm. The positive NaOAc shift indicates that the 7-position is free. Partial acid hydrolysis gave luteolin 4′-glucoside (co-PC) and another dark to bright yellow (in UV + $NH_3$) glycoside (not Lu 7-G) with a similar $R_f$ to luteolin 4′-glucoside, which could be luteolin 3′-glucoside (no marker was available). Both intermediate compounds broke down completely to give luteolin in 5 min. Methylation gave a blue to yellow (in UV + $NH_3$) product with a similar $R_f$ to luteolin 5,4′-dimethyl ether in four solvents and a trace amount of a 2nd blue to yellow compound with higher $R_f$. $R_f$ data are given in Table 4. Spectral data for the methylated glycoside after acid hydrolysis: $\lambda_{max}$ MeOH 253, 265, 338; + NaOAc 253, 265, 354; + $H_3BO_3$ 253, 265, 360 nm. The blue colour in UV and no NaOAc shift suggests that the 5 and 7 positions are blocked, and the positive borate shift that the 3′ and 4′-hydroxyls are free in this product. The original glycoside is therefore identified as luteolin 3′,4′-diglucoside. A second glycoside was also found which was identical in all respects to the first except for a lower $R_f$ in phenol (see Table 4) and it is suggested that this could be due to a difference in the sugar linkages between the two glycosides.

*Data for unknown dark to dark (in UV + $NH_3$) glycoside from* Trichotosia elongata. The glycoside was extracted as above and purified by PPC in 15% HOAc, BAW and $H_2O$. $R_f$ data are given in Table 4. UV data for glycoside: $\lambda_{max}$ MeOH 248′, 273, 315; + NaOAc 248′, 273, 340; + $H_3BO_3$ 248′, 273, 315; + alk 280, 375 nm. Acid hydrolysis gave glucose and an unknown dark to dark (in UV + $NH_3$) aglycone, for which $R_f$ data are given in Table 4. UV data for aglycone: $\lambda_{max}$ MeOH 248′, 274, 320; + NaOAc 243′, 279, 370; + $H_3BO_3$ 248′, 274, 320; + alk 267, 280, 270; + $AlCl_3$ 285, 333, 388; + $AlCl_3$ + HCl 285, 333, 388 mn. The absence of a NaOAc shift in the glycoside suggests that the glucose is attached to the 7-hydroxyl. Demethylation of the aglycone gave 3 products: dark to dark, blue to yellow and dark to yellow (in UV + $NH_3$), respectively. $R_f$ data are given in Table 4. Methylation gave two blue to yellow products; $R_f$ data are given in Table 4. Both products were fully methylated, i.e. blue to yellow colour in UV and absence of NaOAc and $H_3BO_3$ shifts. $\lambda_{max}$ MeOH product (1) 261, 307 and product (2) 260, 305 nm. All attempts to obtain a molecular ion for the aglycone and the methylated products by MS failed.

*Data for acylated quercetin 3-glucoside from* Neobenthamia gracilis. The glycoside was isolated as above and purified by PPC in BAW, 15% HOAc and $H_2O$. $R_f$ data compared with quercetin 3-glucoside, are given in Table 4. $\lambda_{max}$ MeOH 258, 267', 364; +NaOAc 271, 382; +$H_3BO_3$ 264, 386 nm. Acid hydrolysis gave quercetin and glucose. Alkaline hydrolysis gave quercetin 3-glucoside. The acyl group was identified as acetic acid and the compound was characterised as quercetin 3-(6-acetylglucoside) by Dr. V. M. Chari of the University of Munich, using $^{13}C$ NMR.

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