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LEAF SURVEY OF FLAVONOIDS AND SIMPLE PHENOLS IN THE GENUS *RHODODENDRON*

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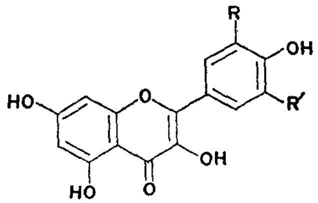
Abstract—A leaf survey of 206 *Rhododendron* species, subspecies and varieties showed that the genus possesses a relatively uniform flavonoid pattern. Three compounds which are generally rare in the angiosperms, gossypetin, azaleatin and caryatin, occur in 76, 34 and 10 per cent of the species respectively. Quercetin and leucoanthocyanidins are present in all taxa, while dihydroflavonols are present in 68 per cent, myricetin in 51 per cent and kaempferol in 23 per cent of the sample. Gossypetin is notably absent from the subgenus Pentanthera while caryatin characterises the single subgenus Hymenanthes. Of the three dihydroflavonols, dihydroquercetin and dihydromyricetin are reported in the genus for the first time, the former being isolated as the 3-arabinoside. The flavonols of *Rhododendron* leaf were found to be present as the 3-arabinosides, 3-rhamnosides, 3-galactosides and 3-glucosides. Simpler phenols were surveyed in leaves of 55 species with the following results: orcinol in 7 per cent, hydroquinone in 9 per cent, rhododendrol in 37 per cent, *o*-coumaric acid in 19 per cent, gentisic acid in 80 per cent and gallic acid in 84 per cent. Taxonomically, the results generally support the accepted sectional and subsectional classifications, although they suggest that on chemical grounds certain species might be misplaced. Phyletically, the data indicate that the genus still retains a wide range of primitive phenolic characters. Geographically, the separation of *R. lochae*, the only Australian species, from the rest of the genus in S.E. Asia is reflected in its chemistry.

INTRODUCTION

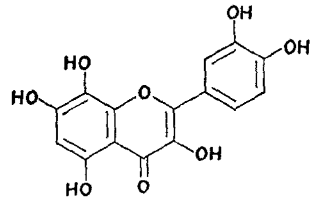
THE ORNAMENTALLY important genus *Rhododendron* (Ericaceae) is systematically difficult partly because of its size (5-600 spp.) and partly because certain species, when brought into cultivation, hybridize readily with each other. The taxonomy and leaf anatomy of these plants have received considerable attention,[1–3] one of the most recent systematic treatments being that of Sleumer.[4] It is a group, therefore, in which chemical data might provide additional characters of value for systematic purposes. Phytochemically, plants of this genus have been much studied, particularly for their phenolic constituents, toxic diterpenes and leaf triterpenes (for summary, see Hegnauer[5]). However, surveys for these compounds have been limited to relatively few taxa and the distribution patterns that have emerged have been relatively uniform ones.

There is a remarkably wide range of flower colour in the genus and our own interest in these plants stemmed first from a study of the flavonoid pigments responsible for most of these colours.[6] As a result of studying the phenolic constituents of the flowers, several unusual flavonols were found to be present, in addition to the three common types, kaempferol, quercetin and myricetin (for formulae, see Fig. 1). These included flavonols with an

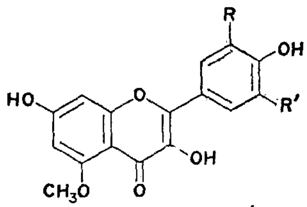
[1] C. G. BOWERS, *Rhododendrons and Azaleas.* Macmillan, New York (1936).
[2] J. M. COWAN, *The Rhododendron Leaf.* Oliver & Boyd, Edinburgh (1950).
[3] J. B. STEVENSON (ed.) *The Species of Rhododendron.* The Rhododendron Society, London (1947).
[4] H. SLEUMER, *Bot. Jahrb.*, **74,** 512 (1949).
[5] R. HEGNAUER, *Chemotaxonomie der Pflanzen* Vol. 4: 65–93. Birkhauser Verlag, Basel (1966).
[6] J. B. HARBORNE, *Arch. Biochem. Biophys.* **96,** 171 (1962).



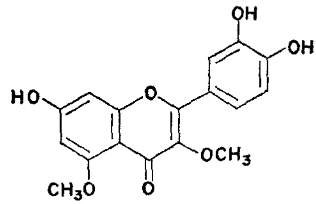
Kaempferol (R=R'=H)
Quercetin (R=OH, R'=H)
Myricetin (R=R'=OH)



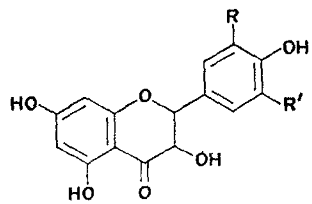
Gossypetin



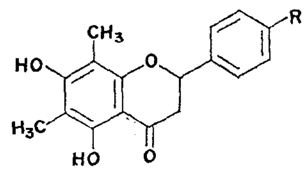
5-Methylkaempferol (R=R'=H)
Azaleatin (R=OH, R'=H)
5-Methylmyricetin (R=R'=OH)



Caryotin



Dihydrokaempferol (R=R'=H)
Dihydroquercetin (R=OH, R'=H)
Dihydromyricetin (R=R'=OH)



Farrerol (R=OH)
Matteucinol (R=OCH_3)

FIG. 1. FLAVONOID AGLYCONES OF *Rhododendron*.

extra 8-hydroxyl substitution, i.e. the yellow flower pigment gossypetin,[7,8] flavonols with 5-*O*-methylation, e.g. azaleatin[6,9,10] and flavonols with 3,5-di-*O*-methylation.[10] All these unusual flavonol types are of restricted distribution in the angiosperms and thus of general taxonomic and phylogenetic interest.[11,12]

It, therefore, seemed of interest to survey *Rhododendron* thoroughly for these flavonoids, in case they provided useful taxonomic markers within the genus. At the same time, plants have been surveyed for common flavonols and also for the related dihydroflavonols, since such compounds have been detected in a few species. The opportunity has also been taken to survey the plants for simpler phenols, since a number of rarer constituents of this class, such as hydroquinone, rhododendrol and *o*-protocatechuic acid[5] have been reported.

[7] J. B. HARBORNE, *Phytochem.* **4**, 647 (1965).
[8] J. B. HARBORNE, *Phytochem.* **8**, 177 (1969).
[9] K. EGGER, *Z. Naturforch.* **176**, 489 (1962).
[10] J. B. HARBORNE, *Phytochem.* **8**, 419 (1969).
[11] J. B. HARBORNE, *Comparative Biochemistry of the Flavonoids*, Academic Press, New York (1967).
[12] J. B. HARBORNE, Evolution of flavonoid pigments, in *Recent Advances in Phytochemistry*, Vol. 4, Appleton–Century–Crofts, New York (in press).

Attention was concentrated in the present work on leaf tissue, since flower pigments have now been adequately surveyed in the genus.[6,7,13,14] Leaf tissue, in any case, has certain advantages over flower tissue for chemotaxonomic surveys and was used, for example, by Bate-Smith in his now classic survey of the phenolics in the angiosperms[15,16] in preference to corolla material.

RESULTS

The results of surveying 206 *Rhododendron* taxa, approximately a third of the species in the genus, for leaf flavonoids are presented in Table 1. Fresh leaves were taken from plants growing in the species collections of the University of Liverpool and of Windsor Great Park. In many cases, at least two samples of a given species (i.e. from different plants) were examined. The data in Table 1 refer to flavonoid aglycones detected in leaf tissue after acid hydrolysis. The different aglycones of *Rhododendron* were very easily distinguished on the basis of R_f and colour reaction, as indicated in Table 2. The results of the aglycone survey were confirmed by two-dimensional chromatography (see Fig. 2) of alcoholic leaf extracts, when glycosides corresponding to the major aglycones were identified and then scored. For example, gossypetin is regularly present as the 3-galactoside, which appears as a dark spot with a particular R_f on the two-dimensional chromatogram (Fig. 2). Quercetin was found to be present in every taxon and was therefore omitted from the Table. The results of a more limited survey of the leaves for phenolic acids and phenols are shown in Table 3.

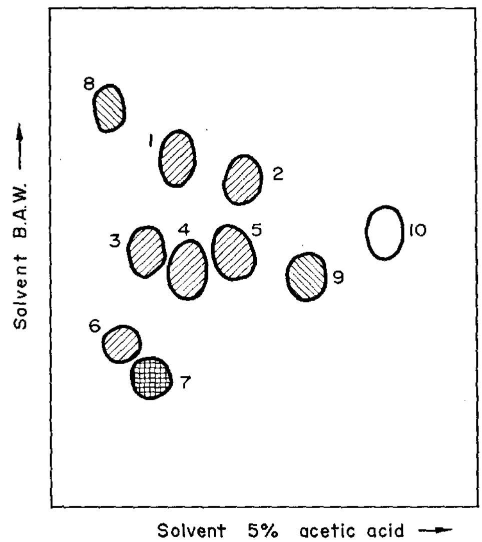


FIG. 2. TWO-DIMENSIONAL CHROMATOGRAM OF LEAF EXTRACT OF *Rhododendron* SHOWING MAJOR FLAVONOID CONSTITUENTS.

Key: 1, quercetin 3-arabinoside; 2, quercetin 3-rhamnoside and kaempferol 3-galactoside; 3, myricetin 3-arabinoside; 4, quercetin 3-galactoside; 5, myricetin 3-rhamnoside; 6, myricetin 3-galactoside; 7, gossypetin 3-galactoside; 8, caryatin; 9, azalein; 10, dihydroquercetin 3-arabinoside. Colours in UV light ▨ = dark brown changing to bright yellow-green or yellow-brown with ammonia, ▦ = black (yellow in visible light), □ = dark absorbing, ▧ = blue, changing to green with ammonia.

[13] R. DE LOOSE, *Phytochem.* **9**, 875 (1970).
[14] T. REYNOLDS, S. M. SMITH and P. A. THOMPSON, *Kew Bull.* **23**, 413 (1969).
[15] E. C. BATE-SMITH, *J. Linn. Soc.*, (*Bot.*), **58**, 95 (1962).
[16] E. C. BATE-SMITH, *J. Linn. Soc.*, (*Bot.*), **60**, 325 (1968).

TABLE 1. DISTRIBUTION OF FLAVONOLS, DIHYDROFLAVONOLS AND COUMARINS IN THE LEAVES OF *Rhododendron*

*Rhododendron** Subgenus, Section, Subsection and Species	Compound								
	Gossypetin	Kaempferol	Myricetin	Azaleatin	Caryatin	Dihydromyricetin	Dihydroquercetin	Dihydrokaempferol	Coumarins
RHODODENDRON									
Section—Rhododendron									
Subsection Glaucophylla									
R. glaucophyllum Rehd.	+	+	−	−	−	−	−	−	−
Subsection Boothii									
R. leucaspis Tagg.	+	−	+	−	−	+	+	−	−
R. megeratum Balf. & Forr.	+	+	−	+	−	−	+	+	−
R. tephropeplum Balf. & Forr.	+	−	+	−	−	−	−	−	−
Subsection Campylogyna									
R. campylogynum (L & S form) Franch.	+	−	−	−	−	−	−	−	−
Subsection Lepidota									
R. lepidotum Wall; G.Don.	−	−	+	−†	−	−	−	−	−
R. ludlowii Cowan	+	−	+	−	−	−	+	−	−
Section Baileya									
R. baileya Balf.	+	+	+	−	−	−	+	−	−
Subsection Uniflora									
R. pumilum Hook.	+	+	−	+	−	−	+	−	−
R. pemakoense Ward	+	−	−	+	−	−	+	−	−
Subsection Edgeworthii									
R. edgeworthii Hook.	−	−	+	−	−	−	+	−	−
Subsection Maddenia									
R. ciliatum Hook.	+	+	+	+	−	−	−	−	+
R. crassum Franch.	+	+	−	−	−	−	+	−	−
R. fletcherianum Davidian	+	+	−	−	−	−	−	−	−
R. manipurense Balf. & Watt	−	+	−	−	−	−	+	−	−
Subsection Micranthum									
R. micranthum Maxim.	+	+	−	−	−	−	−	−	−
Subsection Saluensia									
R. calostrotum Balf. & Ward	+	+	−	+	−	−	+	−	−
R. chamaeunum Balf. & Forr.	+	−	+	+†	−	+	−	−	−
R. charidotes Balf. & Farrer	+	−	+	−	−	−	+	−	−
R. keleticum Balf. & Forr.	+	+	−	+	−	−	+	−	+
R. nitens Hutch.	+	−	+	+	−	+	+	−	−
R. radicans Balf. & Forr.	−	−	+	−	−	−	−	−	−
R. riparium Ward	+	−	+	−	−	−	+	−	−
R. saluenense Franch.	+	−	+	+	−	−	+	−	+
R. saluenense X prostratum	+	−	+	+	−	+	−	−	−

TABLE 1 *cont.*

*Rhododendron** Subgenus, Section, Subsection and Species	Compound								
	Gossypetin	Kaempferol	Myricetin	Azaeatin	Caryatin	Dihydromyricetin	Dihydroquercetin	Dihydrokaempferol	Coumarins
Subsection Moupinensia									
R. moupinense Franch.	+	+	−	−	−	−	−	−	−
Subsection Cinnabarina									
R. cinnabarinum Hook.	+	−	−	−	−	+	+	−	−
R. cinnabarinum cv. (Lady Chamberlain)	+	+	+	−	−	−	+	−	−
R. concatenans Hutch.	+	−	−	−	−	−	+	−	+
Subsection Ferruginea									
R. kotschyi Simon K.	+	+	+	−	−	−	+	−	−
Subsection Lapponica									
P. acroanthum Balf. & Smith	+	−	+	+	−	+	+	−	−
R. cantabile Balf. ex Hutch.	+	−	+	−	−	+	+	−	−
R. capitatum Maxim.	+	+	+	+	−	+	+	−	+
R. chrysum Balf. & Ward	+	+	+	−	−	−	+	−	−
R. complexum Balf. & Smith	+	+	+	−	−	−	+	−	−
R. cuneatum W. W. Smith	+	−	+	−	−	−	+	+	−
R. dasypetalum Balf. & Forr.	+	−	+	+	−	+	+	−	+
R. drumonium Balf. & Ward	−	−	+	+	−	−	+	−	−
R. edgarianum Rehder & Wilson	+	−	−	−	−	−	+	−	+
R. fastigiatum Franch.	+	−	+	+	−	−	−	−	+
R. flavidum Hook. var *album*	+	−	+	−	−	−	−	−	+
R. hippophaeoides Balf. & Smith	+	−	−	−	−	−	+	−	−
R. idoneum Balf. & Smith	+	−	+	+	−	−	+	−	−
R. impeditum Balf. & Smith	+	−	+	+	−	+	+	−	+
R. intricatum Franch.	+	−	−	−	−	−	+	−	−
R. litangense Balf.	+	−	+	−	−	−	+	−	−
R. luridum Ward	+	+	+	−	−	−	−	−	−
R. lysolepis Hutch.	+	+	+	+†	−	−	−	−	+
R. microleucum Hutch.	+	−	+	+	−	−	+	−	−
R. orthocladium Balf. & Forr.	+	+	+	−	−	−	+	−	−
R. paludosum Hutch. & Ward	+	+	+	−	−	−	+	−	−
R. ramosissimum Franch.	+	−	+	+	−	−	+	−	+
R. rupicola Smith	+	+	+	−	−	−	+	+	−
R. russatum Balf. & Forr.	+	−	−	−	−	−	+	−	−
R. scintillans Balf. & W. W. Smith	+	−	−	−	−	+	+	−	−
R. stictophyllum Balf.	+	−	+	+†	−	−	−	−	−
Subsection Caroliniana									
R. minus Michx.	+	−	−	+	−	−	+	−	−
R. carolinianum Rehder	+	+	+	−	−	−	+	−	−
Subsection Heliolepida									
R. brevistylum Franch.	−	−	+	−	−	−	+	−	−
R. desquamatum Balf. & Forr.	+	+	+	−	−	−	+	−	+
R. heliolepis Franch.	−	+	+	−	−	−	−	+	+
R. rubiginosum Franch.	+	−	+	−	−	+	+	−	−

Table 1 *cont.*

*Rhododendron** Subgenus, Section, Subsection and Species	Compound: Gossypetin	Kaempferol	Myricetin	Azaleatin	Caryatin	Dihydromyricetin	Dihydroquercetin	Dihydrokaempferol	Coumarins
Subsection Triflora									
R. ambiguum Hemsl.	+	−	+	−	−	−	+	−	−
R. augustinii Hemsl.	+	−	−	−	−	−	+	−	−
R. chasmanthum Diels.	+	−	−	−	−	−	+	−	−
R. lanceanum Hemsl. var *nanum*	+	−	−	+	−	−	−	−	+
R. lutescens Franch.	+	+	+	−	−	−	−	−	−
R. keiskei Miq.	+	−	−	−	−	−	+	−	−
R. oreotrephes W. W. Smith	+	+	−	−	−	−	+	+	+
R. pseudoyanthinum Balf.	−	−	+	−	−	−	+	−	+
R. rigidum Franch.	+	+	−	−	−	−	+	−	−
R. tatsienense Franch.	+	−	−	−	−	−	+	−	−
R. villosum Roth.	+	+	+	+	−	−	−	−	−
R. yunnanense Franch.	+	+	+	−	−	−	+	−	−
R. xanthocodon Ward	+	−	+	+†	−	−	−	−	+
Section–Poganthum									
R. anthopogon D. Don.	+	−	+	−	−	−	+	−	−
R. cephalanthum Franch. var *crebreflorum*	+	−	+	−	−	−	+	−	+
R. kongboense Ward ex Rothschild	+	+	+	−	−	−	+	−	−
R. primulaeflorum Bureau & Franch. var *cephalanthoides*	+	−	+	−	−	−	+	−	+
R. sargentianum Rehd. & Wils.	+	−	+	−	−	−	+	−	+
R. sphaeranthum Balf. & Smith	+	−	+	−	−	+	+	−	−
R. trichostomum Franch. var *radinum*	+	−	−	−	−	+	+	−	+
PSEUDOVIREYA									
Subsection Euvireya									
R. lochae Muell.	−	+	+	−	−	−	−	−	+
PSEUDAZALEA									
R. lepidostylum Balf. & Forr.	−	−	−	−	−	−	−	−	+
HYMENANTHES									
Section—Hymenanthes									
Subsection Auriculata									
R. auriculatum Hemsl.	+	−	+	+	−	−	−	−	+
R. griersonianum Balf. & Forr.	+	−	+	+	+	−	−	−	−
Subsection Barbatum									
R. barbatum Wall.	+	+	−	+	−	−	−	−	−
Subsection Maculifera									
R. monosematum Hutch.	+	−	−	+	+	−	+	−	−
R. pachytrichum Franch.	+	−	−	−	−	+	+	+	−
Subsection Arborea									
R. arboreum Smith	+	+	−	+	+	−	−	−	−
R. niveum Cowan	−	−	+	−	−	+	+	−	−
R. sylvaticum Ward	+	−	+	−	−	+	−	−	−

TABLE 1 *cont.*

*Rhododendron** Subgenus, Section, Subsection add Species	Compound								
	Gossypetin	Kaempferol	Myricetin	Azaleatin	Caryatin	Dihydromyricetin	Dihydroquercetin	Dihydrokaempferol	Coumarins
Subsection Thomsonii									
R. cerasinum Tagg.	+	−	−	−	−	−	−	−	−
R. hylaeum Balf. & Farrer	−	+	−	−	−	−	−	−	−
R. meddianum Forr.	+	−	−	−	−	−	−	−	+
R. stewartianum Diels.	+	−	−	−	−	−	−	+	−
R. thomsonii Hook.	+	−	−	−	−	−	−	+	−
R. thomsonii hybrid	+	−	−	−	−	−	−	+	−
Subsection Neriiflora									
R. albertsenianum Forr. ex Balf.	+	−	+	−	−	−	−	−	−
R. aperantum Balf. & Ward	+	−	−	+†	+	−	−	−	−
R. beanianum Cowan	+	−	−	−	−	−	−	+	−
R. catacosmum Balf. ex Tagg.	+	−	−	+	+	−	+	−	−
R. chaetomallum Balf. & Forr.	−	−	+	+	−	−	−	−	−
R. chamaethomsonii (Tagg & Forr.) Cowan & Davidian	+	−	−	+	−	−	−	−	+
R. coelicum Balf. & Farrer	−	−	−	−	−	−	+	+	+
R. eudoxum Balf. & Forr.	+	−	+	+†	+	−	+	−	−
R. floccigerum Franch.	+	+	−	−	−	−	−	−	−
R. forrestii Balf. ex Diels	+	−	−	+	+	−	−	−	−
R. forrestii Balf. ex Diels var *repens*	+	−	−	+	+	−	+	−	−
R. hemidartum Balf. ex Tagg.	+	−	−	−	−	−	−	−	−
R. herpesticum Balf. & Ward	+	−	−	+	+	−	−	−	−
R. mallotum Balf. & Ward	+	−	+	−	−	−	−	−	−
R. neriiflorum Franch.	+	+	−	+†	−	−	−	−	−
R. pothinum Balf. & Forr.	−	−	−	+†	+	−	−	−	−
R. scyphocalyx Balf. & Forr.	+	−	−	+	+	−	−	−	−
R. sperabile Balf.	+	−	−	+	−	−	−	−	−
R. temeneum Balf. & Forr.	+	−	−	+†	+	−	−	−	−
Subsection Pontica									
R. degronianum Carv.	+	−	−	+	+	−	+	−	−
R. makinoi Tagg. ex Nak. & Koidy	+	−	−	+	−	−	+	−	−
R. ponticum L.	+	−	+	+†	−	−	−	−	−
R. smirnowii Trant.	+	−	−	−	+	−	+	−	−
Subsection Fortunea									
R. calophytum Franch.	−	+	−	−	−	−	+	−	+
R. decorum Franch.	−	+	+	−	−	+	−	+	−
R. fargesii Franch.	+	−	−	−	−	−	−	−	+
R. orbiculare Decne.	−	−	+	+	−	−	−	−	−
R. sutchuense Franch.	−	−	−	+	−	−	+	−	−
R. vernicosum Franch.	−	−	+	+†	−	+	−	−	+
Subsection Selensia									
R. erythrocalyx Balf. & Forr.	+	−	+	−	−	−	+	−	−
R. setiferum Balf. & Forr.	+	−	−	−	−	−	+	−	−
R. selense Franch. aff *probin*	+	−	−	−	−	−	+	−	−

TABLE 1 *cont.*

*Rhododendron** Subgenus, Section, Subsection and Species	Compound								
	Gossypetin	Kaempferol	Myricetin	Azaleatin	Caryatin	Dihydromyricetin	Dihydroquercetin	Dihydrokaempferol	Coumarins
Subsection Campylocarpa									
R. callimorphum Balf. & W. W. Smith	+	–	–	–	–	–	+	–	+
R. caloxanthum Balf. & Farrer	+	–	–	–	–	–	+	–	–
R. campylocarpum Hook.	+	–	–	–	–	–	+	–	–
R. myiagrum Balf. & Forr.	+	–	–	–	–	–	+	–	–
Subsection Argyrophylla									
R. argyrophyllum Franch.	–	–	+	+	–	–	–	–	+
R. coryanum Tagg. & Forr.	–	–	–	+†	–	–	+	–	–
R. insigne Hemsl. & Wils.	+	–	–	–	–	–	+	–	–
R. rirei Hemsl. & Wils.	+	–	–	+†	–	–	+	+	–
Subsection Parishia									
R. elliottii Watt. Lace & W. W. Smith	–	–	+	+†	–	+	–	+	–
Subsection Irrorata									
R. aberconwayi Cowan	+	–	–	+	–	–	–	–	–
R. araiophyllum Balf. & W. W. Smith	+	–	–	+	+	–	+	–	+
R. anthosphaerum Diels.	–	–	+	+	–	–	–	–	+
R. irroratum Franch.	–	–	+	–	–	–	–	–	–
Subsection Souliea									
R. croceum Balf. & Smith	+	–	+	–	–	–	+	+	–
R. juncundum Balf. & Smith	+	–	–	–	–	–	+	–	+
R. puralbum hybrid?	–	–	+	–	–	–	–	–	–
R. soulei Franch.	+	–	–	–	–	–	–	–	–
R. wardii W. W. Smith	+	–	–	–	–	–	+	–	–
R. williamsianum Rehd. & Wils.	+	–	–	–	–	–	+	–	–
Subsection Martiniana									
R. martinianum Balf. & Forr.	+	–	–	–	–	–	–	–	+
Subsection Lactea									
R. dryophyllum Balf. & Forr.	+	–	+	+	–	–	+	–	–
R. lacteum Franch.	+	–	–	+	+	–	+	–	–
R. traillianum Forr. & W. W. Smith	+	–	–	–	–	–	+	–	–
R. wightii Hook.	+	–	–	–	+	+	+	+	–
Subsection Falconera									
R. arizelum Balf. & Forr.	–	–	+	–	+	–	+	–	–
R. coriaceum Franch.	–	–	+	–	–	–	–	–	–
R. eximum Nutt.	–	+	–	+	+	–	+	–	–
R. falconeri Hook.	+	–	–	–	–	–	–	–	–
R. fictolacteum Balf.	–	–	–	–	–	–	+	–	–
R. hodgsonii Hook.	–	+	+	+	–	–	–	–	–
R. lanigenum Tagg.	–	–	+	–	–	+	–	–	–
R. rex Levl.	–	–	+	–	–	+	+	–	–

TABLE 1 *cont.*

*Rhododendron** Subgenus, Section, Subsection and Species	Compound								
	Gossypetin	Kaempferol	Myricetin	Azaleatin	Caryatin	Dihydromyricetin	Dihydroquercetin	Dihydrokaempferol	Coumarins
Subsection Grandia									
R. coryphaeum Balf. & Forr.	−	−	+	−	−	−	+	−	−
R. macabeanum Watt ex. Balf.	−	−	−	+	+	−	+	−	−
R. praestans Balf. & Smith	+	−	+	−	−	−	+	−	−
R. siderum Balf.	−	+	+	+†	−	−	−	−	−
R. semnoides Tagg. & Forr.	−	−	+	+	−	−	+	−	−
R. sinogrande Balf. & W. W. Smith	−	+	+	+†	−	+	+	−	−
Subsection Fulva									
R. fulvum Balf. & Smith	−	+	+	−	−	+	+	−	+
R. uvarifolium Diels.	+	−	+	−	−	−	−	−	−
Subsection Campanulatum									
R. aeruginosum Hook.	+	−	−	−	−	+	−	−	−
R. campanulatum Don.	−	−	−	−	−	−	+	−	+
R. fulgens Hook. L & S Form	−	−	−	−	−	−	+	−	+
R. wallichii Hook.	+	−	−	−	−	−	+	−	+
Subsection Taliensia									
R. adenophorum Balf. & Smith	+	−	+	−	−	−	−	−	+
R. phaeochrysum Balf. & Smith	+	−	+	+†	−	−	+	+	−
R. roxieanum Forrest	+	−	−	−	−	−	−	−	−
R. vellereum Hutch. ex Tagg.	−	−	−	−	−	−	−	−	−
Subsection Floribunda									
R. floribundum Franch.	−	−	−	−	−	−	+	−	+
PENTANTHERA									
Section—Rhodora									
R. canadense (L.) Torr.	−	−	+	−	−	−	+	−	−
R. albrechtii Maxim.	−	−	−	−	−	−	−	−	−
R. vaseyi A. Gray	−	−	−	−	−	−	+	−	−
Section—Pentanthera									
R. calendulaceum Torr.	−	−	+	−	−	−	+	−	−
R. luteum Sw.	−	−	+	+	−	−	−	−	−
R. occidentale A. Gray	−	−	+	+†	−	−	+	−	−
R. occidentale A. Gray var. *sonomenses*	−	−	+	+†	+	−	−	−	−
R. viscosum Torr.	−	+	+	−	−	−	−	−	−
R. viscosum Torr. var *stancum*	−	+	+	−	−	−	−	−	−
TSUTSUSI									
Section—Brachycalyx									
R. schlippenbachii Maxim.	−	−	−	−	−	−	+	+	−

TABLE 1 *cont.*

*Rhododendron** Subgenus, Section, Subsection and Species	Compound								
	Gossypetin	Kaempferol	Myricitin	Azaleatin	Caryatin	Dihydromyricetin	Dihydroquercetin	Dihydrokaempferol	Coumarins
Section—Tsutsusi									
R. amoenum Planch.	+	–	–	–	–	–	+	–	–
R. indicum Sweet var. *balsaminaeflorum*	+	–	+	–	–	–	+	–	+
R. mucronatum (Bl.) Don.	+	+	+	+	–	–	+	–	–
R. pulchrum Sweet	+	–	–	–	–	–	–	–	–
R. yedoense (Maxim) Regal var. *poukhanense*	+	–	+	+	–	–	+	–	+
R. kaempferi Planch.	+	–	–	–	–	–	+	–	+
R. kuisanum Mak.	+	–	–	+	–	–	–	–	–
AZALEASTRUM									
R. ovatum Maxim.	+	–	+	–	–	–	+	–	–
PSEUDORHODORASTRUM									
Section—Trachyrhodion									
R. hemitrichotum Balf. & Forr.	–	–	–	+	–	–	+	–	–
R. spiciferum Franch.	+	–	–	–	–	–	+	–	+
Section—Rhodobotrys									
R. racemosum Franch.	+	–	–	+	–	–	+	–	–
RHODORASTRUM									
R. dauricum L.	+	–	+	+	–	–	+	–	+
R. mucronulatum Turcz.	+	–	–	+†	–	–	+	–	+
HYBRIDS									
Cilpinense (*R. ciliatum* × *R. moupinense*)	+	+	+	–	–	–	–	–	+
Damaris var Logan (Cv. Dr. Stocker × *R. campylocarpum*)	+	–	+	+	–	+	+	–	–
Fittra (*R. dauricum* × *R. racemosum*)	+	–	+	+†	–	–	+	–	–
Radmosum (*R. racemosum* × *R. radicans*)	+	+	+	–	–	–	+	–	–

* Classified according to Sleumer.[4] Sources of material are given in the Experimental. Quercetin is omitted from the Table since it occurs in all taxa.

† Also contains 5-methylkaempferol and/or 5-methylmyricetin.

The distribution and significance of the different flavonoid and phenolic constituents will now be considered in turn.

The Common Flavonols

Quercetin has already been reported frequently in the flowers of *Rhododendron* and is also recorded in the leaves of some 10–20 species (see Hegnauer[5] and also the more recent

TABLE 2. MEANS OF IDENTIFICATION OF FLAVONOID AGLYCONES IN *Rhododendron* LEAF

Flavonoid*	Forestal	R_f (× 100) in 50% HOAc	PhOH	BAW	Colours in UV light	Other means of detection
Common Flavonols						
Kaempferol	62	49	58	91	Bright yellow	—
Quercetin	45	31	28	76		
Myricetin	29	21	10	41		
Flavonol 5-methyl ethers						
Kaempferol 5-methyl ether	70	43	78	82	Intense fluorescent yellow-green to yellow-brown	—
Quercetin 5-methyl ether (azaleatin)	53	29	42	55		
Myricetin 5-methyl ether	37	21	23	27		
Flavonol 3,5-dimethyl ethers						
Caryatin	78	56	88	72	Blue	Changes to yellow + NH_3
8-Hydroxyflavonol						
Gossypetin	26	18	12	31	Dull black	Yellow in visible, blue + NaOAc
Dihydroflavonols†						
Dihydrokaempferol	78	79	71	87	Dark absorbing	Purple colours with Zn dust and 5 N HCl
Dihydroquercetin	72	74	51	82		
Dihydromyricetin	64	60	31	71		

* Detected after acid hydrolysis of leaf (2 N HCl at 100° for 30 min) and extraction of the aglycones into ethyl acetate. For solvent details, see Experimental. Leucoanthocyanins, universally present, are converted to anthocyanidins during acid treatment and appear on chromatograms as coloured spots (R_f cyanidin 50 in Forestal, delphinidin R_f 30).

† More readily detected by chromatography in water, when all the other flavonoids mentioned here are immobile (R_fs (× 100), dihydrokaempferol, 32; dihydroquercetin, 29; dihydromyricetin, 26).

Russian literature, reported in *Chem. Abs.* 1966 to date). Our survey shows, in fact, that quercetin is ubiquitous, since it was detected in every single taxon studied. Myricetin, previously reported in only a handful of species, is likewise common, having been found in about 50 per cent of the 206 taxa surveyed; it also occurs in some further species (see Table 1) as its dihydro derivative. By contrast, kaempferol is rather less common, being present in about 25 per cent of the present sample.

On the basis of general leaf surveys among the angiosperms,[15,16] it has been possible to place the three common flavonols in a phylogenetic series, with myricetin representing a 'primitive' woody character and kaempferol probably being more advanced over quercetin. The fact that the frequency of occurrence of the common flavonols is in the order: quercetin > myricetin > kaempferol thus confirms the general taxonomic opinion that *Rhododendron* is phylogenetically a very ancient plant group.

Myricetin, quercetin and kaempferol occur in *Rhododendron* leaves as simple glycosides, most usually as the 3-galactoside but also as the 3-rhamnoside, 3-arabinoside and 3-glucoside. A detailed study, carried out with *R. ciliatum* as a representative species, revealed

TABLE 3. DISTRIBUTION OF PHENOLIC ACIDS AND OTHER SIMPLE PHENOLS IN *Rhododendron* LEAF

Phenol	Occurrence in 55 species sample (%)	Identified in
Hydroxy cinnamic acids		
Caffeic acid	68	Widespread
Ferulic acid	57	Widespread
Sinapic acid	62	Widespread
p-Coumaric acid	79	Widespread
o-Coumaric acid	19	*R. saluenense*, *R. sperabile*, Damaris var Logan, *R. degronianum*, *R. forrestii repens*, *R. insigne*, *R. yunnanense*, *R. cinnabarinum*, *R. ciliatum* and *R. calophytum*.
Hydroxybenzoic acids*		
Gallic acid	84	Widespread
Gentisic acid	80	Widespread
Simple phenols		
Hydroquinone	9	*R. russatum*, *R. saluenense*, *R. degronianum*, *R. dauricum*, *R*. *forrestii repens*,
Orcinol	7	*R. decorum*, *R. spiciferum*, *R. thomsonii*, and *R. traillianum*
Rhododendrol	37	*R. aeruginosum*, Damaris var Logan, *R. dauricum*, *R. degronianum*, *R. fastigiatum*, *R. forrestii repens*, *R. hyperythrum*, *R. kaempferi*, *R. x loderi*, *R. makinoi*, *R. mucronatum*, *A. pachytrichum*, *R. roxieanum*, *R. soulei*, *R. scintillans*, *R. thomsonii*, *R. williamsianum*

* The four common phenolic acids, *p*-hydroxybenzoic, protocatechuic, vanillic and syringic, were detected in all taxa.

that the following glycosides were present: the 3-galactoside of all three flavonols, the 3-rhamnosides and the 3-arabinosides of both quercetin and myricetin and the 3-glucoside of kaempferol. The position of these various glycosides on a two-dimensional chromatogram is indicated in Fig. 2; with the aid of two-dimensional surveys of this type, it was found that quercetin, for example, was present in practically all the samples studied as the 3-rhamnoside and the 3-galactoside.

Nearly all the above glycosides detected in *Rhododendron* are widespread in the angiosperms generally. The one exception is myricetin 3-arabinoside, previously only reported in two sources, in the fruits of the cranberry *Vaccinium macrocarpon* (Ericaceae)[17] and in leaves of *Lysimachia punctata* (Primulaceae).[18]

Gossypetin

One of the most striking results of this survey is that the yellow flavonol gossypetin occurs in the leaf of 155 species (i.e. in 76 per cent of the sample) and is thus a characteristic constituent of the genus. Since gossypetin is presumably produced in the plant by a selective

[17] O. PUSKI and F. J. FRANCIS, *J. Food Sci.* **32,** 527 (1967).
[18] J. MENDÉZ, *Experientia* **26,** 108 (1970).

oxidation of quercetin (or a related precursor) in the 8-position, it is surprising that the corresponding derivatives of myricetin and kaempferol do not occur also. In fact, these other derivatives were looked for during this survey, but there was no evidence of their presence in any of the plant species studied.

As a taxonomic marker within the Ericaceae, gossypetin is of considerable interest since it occurs in *Erica*, *Kalmia*, *Ledum*, *Phyllodoce* and *Rhodothamnus*[8] and has also been found widely in the Empetraceae.[19] Within the genus *Rhododendron*, it is really too widespread to be of much interest. It is, however, perhaps worth noting that it is infrequent in some subsections of the subgenus Hymenanthes (e.g. Fortunei, Falconera, Grandia) and is absent from all taxa of the subgenus Pentanthera and from *R. lepidostylum* and *R. lochae*.

Gossypetin functions in *Rhododendron* as a yellow flower pigment, but it does not occur as such in many species. It was earlier noted in the primrose-yellow corollas of *R. campylocarpum*, *R. chryseum*, *R. herpesticum*, *R. telopium*, *R. trichocladum* and *R. wardii*. During the present work, it was found in corollas of only four further species: *R. ambiguum*, *R. adenophorum*, *R. anthosphaerum* and *R. ponticum*. It is, however, much more widespread in the leaf, where it clearly has a different and as yet undefined function.

O-*Methylated Flavonols*

Three 5-*O*-methylated flavonols have been isolated from flowers of *Rhododendron*: 5-*O*-methylquercetin (azaleatin) first reported in *R. mucronatum*[20] and later found in 44 of 83 species surveyed;[6] 5-*O*-methylmyricetin, reported by Egger[9] in *R. catawbiense*, *R. japonicum* and *R. obtusum;* and 5-*O*-methylkaempferol, discovered in *R. racemosum* and *R. russatum* petals by Harborne.[10] These rare flavonoid constituents have now been found variously in the leaves of 78 species, i.e. in 38 per cent of the sample under examination (Table 1). The quercetin analogue, azaleatin, is by far the most common, being accompanied by one or both the other compounds in only a few species. Azaleatin occurs in leaves, as it does in flowers, mainly as the 3-rhamnoside azalein. This is hardly surprising since, in many species, azaleatin occurs in both leaf and flower. The correlation between leaf and flower occurrence is, however, by no means complete, since the frequency in flower is greater (53 per cent) than that in the leaf (38 per cent).

As leaf characters, 5-*O*-methylated flavonols are of taxonomic interest, since they are more consistent in their distribution patterns at the subsectional level than, say, gossypetin. Thus, they are completely lacking in a number of subsections, e.g. Thomsonii, Poganthum and Campylocarpa. On the other hand, when they do occur in a subsection, they are present in most taxa, e.g. Neriiflora 12/18, Pontica 3/4, Argyrophylla 3/4, Grandia 4/6 and Saluensia 5/7. It was earlier noted[6] that as a floral character, azaleatin was much more frequent in lepidote than in elepidote species. This correlation between chemistry and what is an important taxonomic character in the genus also holds true for the leaves, although there are a number of exceptions.

A fourth methylated flavonol, namely caryatin or 3,5-di-*O*-methylquercetin, has recently been reported in the genus, in *R. monosematum*.[10] It is, however, much rarer than azaleatin, as it occurs in the leaves of only 21 species (10 per cent of taxa surveyed). All species containing it, however, belong to one subgenus, namely Hymenanthes. It is presumably synthesized in the plant via the 5-*O*-methyl derivative azaleatin, and, indeed, is usually accompanied by this compound when it is present.

[19] D. M. Moore, J. B. Harborne and C. A. Williams, *J. Linn. Soc.* (*Bot.*) **63**, 277 (1970).
[20] E. Wada, *J. Am. Chem. Soc.* **78**, 4725 (1956).

Dihydroflavonols

The presence of dihydroflavonols in *Rhododendron* was established by Arthur and Tam,[21] who isolated dihydrokaempferol (aromadendrin) from leaves of a Malaysian species, *R. simiarum*. We have now surveyed leaves for these substances and find that the related dihydroquercetin and dihydromyricetin are also present and that the compounds are widespread in the genus (Table 1). One or other of them occurs in 68 per cent of the sample, i.e. in 138 species. In fact, they are so common that they do not provide any use for taxonomic purposes within the genus.

It is interesting that the relative frequency of the three dihydroflavonols is in the order dihydroquercetin (124), dihydromyricetin (27) and dihydrokaempferol (18), i.e. is in the same order of frequency as the corresponding flavonols (see above). The glycosidic pattern of the dihydroflavonols seems, however, to be much simpler than that of the flavonols. The only glycoside of dihydroquercetin that could be detected in a number of species examined was the 3-arabinoside.

Dihydroflavonols belong to the flavanone class of flavonoid, i.e. they are 3-hydroxyflavanones, and are detected on chromatograms using the same colour reagents. Two flavanones without 3-hydroxyl groups have been reported in the genus, there are the C-methyl derivatives matteucinol and farrerol, found in *R. simsii* and *R. kaempferi*.[21] Without having authentic markers of these compounds, we have been unable to survey for their presence/absence but, in our experience, flavanones other than the three dihydroflavonols mentioned above, do not occur in any quantity in more than a few species of our sample.

Simple Phenols

Hydroxycoumarins are relatively widespread in the angiosperms, so the discovery during the present survey of two common constituents of this type, namely umbelliferone and scopoletin in *Rhododendron* leaves (in 25 per cent of the species) is to be expected. Their distribution is rather sporadic (Table 1), so that they do not appear of great interest as taxonomic markers. They are, however, notably absent from the subgenus Pentanthera, which is already distinctive in lacking gossypetin.

Of the five hydroxycinnamic acids detected in *Rhododendron* leaf (Table 3), four are universal in higher plants and their frequency of occurrence (57–79 per cent) in the genus is similar to that in the dicotyledons generally. The fifth, *o*-coumaric acid, is relatively uncommon in angiosperms but its presence in the Ericaceae is predictable, in view of the number of other *o*-hydroxy substituted phenols present in the family. Besides the four common plant benzoic acids, two others are regularly present, namely gentisic (2,5-dihydroxybenzoic) and gallic (3,4,5-trihydroxybenzoic) acids. Salicyclic acid (2-hydroxybenzoic acid) is surprisingly absent, since it is present as the methyl ester, oil of wintergreen, in several other Ericaceae, notably in *Gaultheria*. If present, it gives rise to a very characteristic odour, in acid-treated leaf, besides being easily recognized on chromatograms, but it was not detected in any of the *Rhododendron* samples being surveyed.

Two simpler phenols found during this leaf survey are hydroquinone and orcinol (1,3-dihydroxy-5-methylbenzene). Hydroquinone, a typical Ericaceous constituent present, for example, in *Arbutus* as the glucoside arbutin, was found in 11 per cent of our sample (Table 3). Orcinol is an interesting new taxonomic marker in the family, occurring as it does

[21] H. R. ARTHUR and S. W. TAM, *J. Chem. Soc.* 3197 (1960).

in *Phyllodoce*, *Erica* and *Pieris* (see Harborne and Williams[22]). In *Rhododendron* it was detected in 4 out of 55 species surveyed.

Another distinctive *Rhododendron* phenol not so far mentioned is rhododendrol (4-(*p*-hydroxyphenyl) butan-2-ol) which was first detected in *Rhododendron chrysanthum* by Archangelsky in 1901.[23] Very recently, Thieme and Winkler[24] surveyed some *Rhododendron* species and cultivars, reporting its presence in 11 species and its absence from 17 other species. Some correlation with the older 'series' classification of the genus was noted by these authors. Thus, it was found in all species studied of the 'series' Ponticum (8), Thomsonii (1), Virgatum (1) and Ferrugineum (2), but was absent from Dauricum (2), Micranthum (1), Triflorum (4) Azalea (5), Fortunei (4) and Lapponicum (1). In our own survey, we found it in 17 of 55 species examined (Table 3). Our results do not agree entirely with those of Thieme and Winkler, since we detected it in both *R. dauricum* (Dauricum) and *R. scintillans* (Lapponicum), two species reported as being negative by them. However, our other results fit in with their conclusions; for example, *R. williamsianum* and *R. thomsonii* (both 'series' Thomsonii) were positive. Clearly, further, wider surveys of *Rhododendron* leaves are necessary to confirm the taxonomic utility of this particular character.

Finally, it is worth mentioning that a number of unidentified and thus apparently novel phenols were detected during the present survey. Some of these may well prove to be of systematic interest when they have been identified. Some species–specific constituents were also noted and two, with quite remarkable colour and spectral properties, present in *R. roxeanum*, are under further investigation.

DISCUSSION

The results of the present survey show for the first time that a relatively unusual pattern of flavonoid pigmentation, characterised by the production of gossypetin, dihydroflavonols and 5-*O*-methylated flavonols, is widely distributed in the leaf tissue of the genus *Rhododendron*. From the taxonomic point of view, none of the chemical characters studied is of diagnostic value for distinguishing subgenera or sections. There are, however, some correlations with subgeneric classification, i.e. the subgenus Pentanthera is the only one lacking both gossypetin and coumarins and the subgenus Hymenanthes is the only one to contain caryatin.

Probably the present data are of most immediate interest at the level of the species, since they suggest that certain taxa may not be placed in the correct subsection. For example, *R. radicans* differs from the other six taxa of subsection Saluensia in no less than three chemical characters. Also, one may note that *R. pseudoyanthinum* is the only species in the subsection Triflora without gossypetin (13 species studied). Again, *R. falconeri*, the type species of the subsection Falconera, differs chemically from the other taxa placed in this subsection. These and other examples can be found from a study of Table 1, in which the species are classified according to Sleumer's system. Since the definition of a species and species classification are very difficult matters in *Rhododendron*, it is suggested that chemical data of this type might be taken into consideration in future taxonomic revisions.

From the phyletic viewpoint, this chemical study (summarized in Table 4) confirms the position of *Rhododendron* as an ancient relic genus, centred as it is in S.E. Asia, the area in

[22] J. B. Harborne and C. A. Williams, *Phytochem.* **8**, 2223 (1969).
[23] K. Archangelsky, *Arch. Exptl. Pathol. Pharmakol.* **46**, 313 (1901).
[24] H. Thieme and H. J. Winkler, *Die pharmazie* **24**, 703 (1969).

TABLE 4. SUMMARY OF THE DISTRIBUTION OF FLAVONOID AND OTHER PHENOLIC CONSTITUENTS IN *Rhododendron* LEAF

Phenolic character	Distribution (by species) (%)	Presence/absence in other Ericaceae
Leucoanthocyanidins	100	Widespread
Gallic Acid	84	
Gossypetin	76	In 9 other genera
Dihydroflavonols	68	Widespread
Myricetin	51	
5-Methyl flavonols	38	In 7 other genera
Rhododendrol	37	Not detected
Coumarins	26	
Caryatin	10	
Hydroquinone	11	In 6 other genera
Orcinol	7	In 3 other genera

which the angiosperms are thought originally to have evolved from the gymnosperms.[25] Thus, *Rhododendron* retains the whole gamut of primitive flavonoid characters—presence of leucoanthocyanidin, myricetin, gallic acid, dihydroflavonol and readily falls into Bate-Smith's unspecialized *ab* category.[15] Furthermore, both the more unusual *Rhododendron* flavonoid characters—presence of gossypetin and of azaleatin—characteristically occur elsewhere in the plant kingdom only in relatively primitive angiosperm groups.[9,10]

Variations in the distribution pattern of flavonoids within the genus may, therefore, be of most interest as indicating the pathway of evolutionary progression in these plants: for example, plants lacking one or more of these primitive flavonoid characters can be presumed to be advanced over those retaining them all. Indeed, this suggestion is confirmed by finding that *R. lochae*, the only known Australasian species which must have evolved away from the centre of origin of the genus in S.E. Asia, lacks nearly all these flavonoid characters (Table 1).

Many of the unusual flavonoids and phenols found in *Rhododendron* have been found elsewhere in the Ericaceae (Table 4) and the results of a survey of the family for these constituents will be discussed in a separate paper.

EXPERIMENTAL

Plant Sources

Over half the leaf samples were obtained from the *Rhododendron* species collection maintained at the University of Liverpool Botanic Gardens at Ness, Neston, Cheshire. These plants have been identified by J. K. Hulme and Dr. J. Cullen, and voucher specimens are deposited in the Garden herbarium for inspection. In general, mature fresh leaves were collected and analysed within 24–48 hr of collection. Whenever possible, plants were sampled 2–3 times at different times of the year and, when available, flowers were also collected and analysed separately.

The remaining leaf samples were obtained from plants growing at or near the Savill Gardens, Windsor Great Park. These plants nearly all originated in the collection of the late J. B. Stevenson of Tower Court, Ascot, and the numbers are those of the appropriate plant collectors (F = Forrest, KW = Kingdon Ward): *R. achroanthum* F.25532, *R. albertsenianum* F.14195, *R. anthopogon* type form, *R. aperantum* F.26934, *R. brevistylum* F.30977, *R. calendulaceum*, *R. caloxanthum* 937, *R. campanulatum* SSEW 9106, *R. cantabile* F.21032, *R. capitatum* F.13600, *R. carolinianum*, *R. catacosmum* R.59548, *R. cerasinum* KW.11011, *R.*

[25] A. TAKHTAJAN, *Flowering Plants, Origin and Dispersal*. Oliver & Boyd, Edinburgh and London (1968).

charidotes F.25560, *R. chryseum* R.59049, *R. coelicum* F.25625, *R. complexum* 15392, *R. coriaceum* F.25872, *R. coryanum* F.20322, *R. coryphaeum* F.25717, *R. crassum*, *R. croceum* F.16321, *R. cuneatum* F.21375, *R. dasypetalum* F.13905, *R. desquamatum*, *R. drumonium* F.15370, *R. edgarianum* F.16450, *R. erthyrocalyx* R.59149, *R. eudoxum* F. 21738, *R. eximum* T.C. *R. floribundum* type form, *R. fulgens* L. & S. form 2846, *R. fulvum* RO.3953, *R. heliolepis*, *R. hemidartum* F.21709, *R. herpesticum* F.27012, *R. hodgsonii*, *R. hylaeum* K.W. 9322, *R. idoneum* S.1313, *R. irroratum* R.59582, *R. juncundum* F.15579, *R. kongboense* K.W. 5700, *R. kotschyi*, *R. lacteum*, *R. lepidotum*, *R. litangense* 16277, *R. ludlowii* L. & S. form 6600, *R. luridum* K.W. 7048, *R. lutescens*, *R. lysolepis* type form, *R. manipurense* K.W. 10928, *R. martinianum* type form, *R. meddianum* F.24104, *R. microleucum*, *R. minus*, *R. occidentale*, *R. occidentale* var *somonenses*, *R. orthocladium*, *R. ovatum* Wilson 1391, *R. paludosum* K.W. 7058, *R. pothinum* F.21734, *R. praestans* R.59234, *R. primulaeflorum* var *cephalanthoides* YU15139, *R. ramosissimum* R.23310, *R. riparium* RO3954, *R. ririei* Wilson 1539, *R. rupicola*, *R. selense* aff *probin* F.21874, *R. semnoides* F.21870, *R. setiferum* F14066, *R. sperabile* F.26446, *R. sphaeranthum* K.W. 3998, *R. stictophyllum*, *R. tatsienense* F.21270, *R. temeneum* F.21914, *R. tephropeplum* RO.3914, *R. uvarifolium* F.21817, *R. villosum*, *R. viscosum* var *stancum*, *R. viscosum*, *R. wallichii* type form, *R. wardii* 59530, *R. xanthocodon* K.W. 6026.

Identification of Flavonoids

Flavonoid aglycones were identified in acid hydrolysed leaf (and flower) extracts of *Rhododendron* using standard procedures[11,15] by comparison with authentic markers. Identification of the rarer flavonoid components in *Rhododendron* is described in earlier papers.[6,8,10] The chromatographic and colour properties of all the *Rhododendron* constituents are collected in Table 2. Solvents used for chromatography on Whatman No. 1 paper were: BAW, *n*-butanol-acetic acid–water (4:1:5); Forestal, conc. HCl–acetic acid–water (30:3:10); 50% HOAc, acetic acid–water (1:1); and water. Direct 95% EtOH extracts of all tissues were chromatographed two dimensionally in BAW and 5% HOAc, a typical chromatogram being shown in Fig. 2.

Individual flavonoid glycosides were isolated from leaf extracts of appropriate species (see Text) by paper chromatography and purified and identified by standard methods. The dihydroquercetin 3-arabinoside appears to be a new glycoside. It has R_f 0·63 in BAW and 0·51 in 5% HOAc and gave arabinose and a mixture of dihydroquercetin and quercetin on acid hydrolysis. It has the spectral and colour properties of a dihydroflavonol 3-glycoside: λ_{max} 218, 230 (infl.) and 294 mn in EtOH, positive spectral shifts with NaOH, NaOAc, $AlCl_3$ and H_3BO_3, dark absorbing colour on paper unchanged on NH_3 treatment.

Identification of Other Phenols

The hydroxycoumarins umbelliferone and scopoletin were identified during the flavonoid leaf survey (see above) by chromatographic and colour comparison with authentic markers. Simpler phenols were

TABLE 5. IDENTIFICATION OF SIMPLE PHENOLS IN *Rhododendron*

Phenol	R_f on Silica gel† 10% Acetic acid in chloroform	45% Ethyl acetate in benzene	Colour‡ with Folin
Gallic acid	5	40	Blue
3,4-Dihydroxybenzoic acid	19	44	Blue
2,5-Dihydroxybenzoic acid	33	44	—
Rhododendrol	33	62	—
Hydroquinone	18	58	Blue
Orcinol*	19	62	—
p-Hydroxybenzoic acid	55	80	—
Syringic acid	79	58	—
Vanillic acid	82	73	—
Salicylic acid	91	82	—

* Gives pink colour on spraying with vanillin/HCl.

† Hydroxycinnamic acids are immobile in these systems, so they were separated by two-dimensional TLC on microcrystalline cellulose using solvent 1, benzene–acetic acid –water (6:7:3) and solvent 2, 15% Acetic acid in water. They appeared as blue spots in UV light, the colours being intensified in the presence of NH_3.

‡ All give blue with Folin + ammonia.

identified in acid hydrolysates by TLC on silica gel and microcrystalline cellulose. R_f and colour properties of the *Rhododendron* phenols are indicated in Table 5. The identification of orcinol in *Rhododendron* is described elsewhere.[22] Authentic rhododendrol, for use in chromatographic comparison, was isolated from the bark of *Betula alba.*

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