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POLYPHENOLS IN THE LEAVES OF *EUCALYPTUS* L'HERIT: A CHEMOTAXONOMIC SURVEY—I.

INTRODUCTION AND A STUDY OF THE SERIES GLOBULARES

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Abstract—A brief description is given of the *Eucalyptus* genus showing the wide variations of morphological characters. The different classifications that have been proposed are outlined. A review of chemotaxonomic studies indicates that the polyphenols in eucalypt leaves could be the most useful compounds for taxonomic purposes. Over 80 per cent of the known species of eucalypts have been examined chromatographically for these compounds. The methods of examination are described and the chromatographic data of the primary polyphenols recorded. The composition of the polyphenols in the leaves of the species in the series Globulares are reported when it is clearly seen that *E. preissiana*, *E. megacarpa* and *E. coronata* have been wrongly classified.

INTRODUCTION

DURING a search for suitable experimental material to facilitate a study of the biosynthesis of certain polyphenols, the leaves of eucalypt species were examined. A few chemical variants—with obvious advantages for biosynthetic studies—were found,[1] but it was also evident from an examination of more than 80 per cent of the known species that the polyphenols in the leaves can assist taxonomic studies. In this and subsequent papers, the chemotaxonomic aspects of the work will be discussed.

Brief Description of the Genus

The genus *Eucalyptus* (Myrtaceae) is a relatively large one, containing about 500 species, varieties and sub-species, growing under a wide range of environmental conditions (for summary see Hillis[1]), and is one of the highly variable in the plant kingdom. Almost every morphological character shows great diversity. Heights of more than 300 ft have been recorded for *E. regnans* (369),* 200 ft for *E. marginata* (304) and 250 ft for *E. diversicolor* (57), whereas the maximum height for *E. redunca* (115) is about 8 ft, *E. kruseana* (243) 10 ft, *E. coronata* (247a) 6 ft, *E. stricta* (384) 12 ft, *E. burdettiana* (96a) 6 ft. The former group of species exemplifies the tall shaft-like eucalypts, whereas the latter group includes the mallees in which several stems arise from thickened woody rootstock (lignotuber) embedded in the upper layer of the soil. The mallees have a high survival potential in time of drought and this form appears in a number of the groups of eucalypts.

On bark characteristics, the eucalypts may be grouped broadly into the following six

* The botanical nomenclature and number (given after each name) of the species is that of Blakely[2] with the revisions of Johnston and Marryatt[3].

[1] W. E. HILLIS, *Phytochem.* **5**, 541 (1966).

[2] W. F. BLAKELY, *A Key to the Eucalypts*. Forestry and Timber Bureau, Canberra, 2nd Edition (1955).

[3] R. D. JOHNSTON and R. MARRYATT, *Taxonomy and Nomenclature of Eucalypts*. Forestry Research Institute Leaflet No. 92, Canberra (1965).

classifications. (*a*) *Gum or smooth bark*. Usually smooth but may be rough and dark at the butt of the tree. The bark is shed annually (in other types, bark layers are shed or fretted away at irregular intervals, these are regarded as persistent types). At certain seasons, the bark may be pale green (*E. viminalis*, 277), red (*E. rubida*, 235), white (*E. papuana*, 17), purple (*E. maculata*, 54), yellow with blue patches (*E. diversicolor*, 57) etc. Usually, however, the bark is a mottled dull grey-blue (*E. globulus*, 248; *E. pauciflora*, 394; *E. camaldulensis*, 197); (*b*) *Stringybark*. Soft and fibrous with a stringy structure, brown in colour, as with *E. macrorhyncha* (331), *E. baxteri* (337), *E. eugenioides* (318), *E. muelleriana* (308); (*c*) *Box*. A firm to hard bark without a characteristic structure. Yellowish (*E. melliodora*, 550) to dark grey (*E. moluccana*, formerly *E. hemiphloia*, 484; *E. polyanthemos*, 558); (*d*) *Peppermint*. Grey in colour, soft but not fibrous and the structure reveals definite trellis formation (*E. radiata*, 411; *E. dives*, 417); (*e*) *Ironbark*. A hard bark that is deeply furrowed near the base of the tree. It may be black and impregnated with small particles of kino (*E. sideroxylon*, 541; *E. crebra*, 514) or a dark grey colour (*E. paniculata*, 537); (*f*) *Bloodwood*. This group is typified by a persistent, rough, short-fibred tessellated bark which is rather friable, e.g. *E. gummifera* (45), *E. trachyphloia* (40), *E. calophylla* (32).

Almost all eucalypt species are evergreen, but some are more or less completely deciduous in the dry season: *E. brachyandra* (440), *E. confertiflora* (19a), *E. grandifolia* (18), *E. latifolia* (30). Others are partly deciduous, namely, *E. bigalerita* (209), *E. alba* (207), *E. apodophylla* (286a), *E. foelscheana* (29), *E. oligantha* (491) and *E. staigeriana* (520).

The leaves of eucalypts vary in shape and size at different stages in the life cycle of the tree. The degree of heterophylly found in the genus varies widely. In the Globulares series of the genus (223–263 see Table 1, Section Macrantherae–Normales) for example, the difference in leaf size, shape and appearance between juvenile and adult foliage is very pronounced. In *E. globulus* (248) the juvenile leaves are opposite, sessile to amplexicaul and markedly glaucous, whereas the mature leaves are alternate, petiolate, lanceolate and dark green. The juvenile and adult leaves of the Ironbarks (536–545) are quite similar but as is usual the former are opposite and the adult leaves alternate. The adult leaves exist in a variety of shapes and sizes: 5–15 cm × 2·2–10 cm (about twice as wide as long) ovate or elliptic for *E. confertiflora* (19a); 6–10 cm × 11–18 cm long ovate or elliptic for *E. foelscheana* (29); 3–4 cm × 10–30 cm (or longer) narrow lanceolate for *E. globulus* (248); 1–3·5 cm × 5–16 cm narrow lanceolate, acuminate for *E. tasmanica* (421).

The inflorescence of the eucalypts is a contracted dichasium and the number of flowers present, if all develop, falls into the series 1, 3, 7, 15, 31, . . . or if there is partial suppression into series such as 1, 3, 7, 11, 15 or 1, 3, 7, 13, 21 (Pryor[4]). *E. globulus* (248) possesses a single-flowered unit; *E. viminalis* (277) a three-flowered, *E. macarthurii* (273) seven-flowered, *E. macrorhyncha* (331) mostly an eleven-flowered and *E. pauciflora* (394) a fifteen or more flowered unit.

The size and shape of the receptacle and operculum also vary considerably. The mode of attachment of anthers differs and the lobes show great variation in shape and are used as the basis for the classification of Blakely[2] which is followed in this work.

The shape of the disc at the top of the fruit may be used to assist identification despite the marked variations within a single species. The shape and size of the fruit show a marked variation throughout the genus. The fruit of *E. tetraptera* (5) is 6 × 6 cm long; *E. miniata* (4) 2 × 6 cm; *E. macrocarpa* (599) 8 × 3 cm. On the other hand, the fruit of *E. blakelyi* (186) is 0·5 × 0·3 cm long; *E. deglupta* (437) 0·4 × 0·4 cm; *E. stellulata* (398) 0·3 × 0·4 cm.

[4] L. D. PRYOR, *Proc. Linnean Soc. N. S. Wales* **79**, 89 (1954).

The colour of the heartwood ranges from red to a very light tan and more of the pale-coloured species grow in the south-eastern portion of Australia than elsewhere. The intensity of colour also varies within the species, and a faster growth rate is one factor responsible for a lighter colour in the wood.

Some species frequently form kino (for description see Hillis[5]) when the cambium is injured. Bloodwoods such as *E. gummifera* (45) and *E. calophylla* (32) are notable in this regard. Other species such as *E. baxteri* (337), *E. sieberi* (371), *E. pauciflora* (394), and *E. dives* (417) commonly contain kino veins. On the other hand *E. microcorys* (314) rarely forms kino.

Although early workers considered it unlikely, hybridization between certain *Eucalyptus* species is now known to be of frequent occurrence.[6,7] This hybridization occurs between species within but not between the groups *Macrantherae* (Sections *Transversae* and *Bisectae*), *Renantherae* and *Corymbosae*, which were suggested as subdivisions of the genus by Pryor[8] and are considered to be of subgeneric status. Many of these naturally arising hybrids are known to be quite fertile. However, this ready hybridization does not lead to continuity of variation throughout the "subgenera" since ecological factors operate to conserve the integrity of the species. Also groups of characters resembling one or other parent tend to cohere in segregating populations.[7] Some of the forms originally designated as species[2] are now recognized as hybrids and their removal accounts for the absence of some of Blakely's numbers[2] in the various tables in this and subsequent papers. Clarification of the status of some of the former species accounts for the absence of the other numbers.[3] All the known species will be listed in the tables, whether they have been examined or not, so that the extent of the examination can be readily ascertained.

Classification of Eucalyptus *Species*

The classification in use today is Blakely's *A Key to the Eucalypts*[2] based on the so-called antheral system of Bentham. There is an over-emphasis on the value of the anthers in establishing the taxonomic groups of this Key. Whereas Blakely's major groups (Sections) appear generally to represent natural groups, the differences between some Subsections are often very slight, and sometimes the scheme is confusing and difficult to apply;[10] the Subsections will not be recorded in this and subsequent papers. A second classification (into Series) based on general morphology was also used by Blakely but in some instances it cuts across the first. The main subdivisions are given in Table 1. It is to be expected in such a prodigious task that a few anomalies in arrangement would occur, and this system has been subject to published and unpublished criticism. Nevertheless the Key has contributed a great deal to the systemization of the genus and still remains the only complete scheme evolved.

By giving greater attention to other morphological characters most of the anomalies in classification have been recognized and discussed in Australia for many years although little has been published. With this difficult genus, as many taxonomic criteria as possible are needed for reliable classification, as the dangers in using a single character on which to erect taxonomic groups are recognized. "It will be necessary to consider and evaluate all the evidence affecting each particular judgement and to distinguish between correlations of

[5] W. E. Hillis, In *Wood Extractives* (Edited by W. E. Hillis), p. 59. Academic Press, New York (1962).
[6] R. G. Brett, *Papers Proc. Roy. Soc. Tasmania* **71**, 75 (1937).
[7] L. D. Pryor, *Proc. Linnean Soc. N. S. Wales* **78**, 43 (1953).
[8] L. D. Pryor, *Australian J. Sci.* **22**, 45 (1959).
[9] J. Hartley, *Australian J. Biol. Sci.* **18**, 190 (1965).
[10] L. D. Pryor, *Proc. Linnean Soc. N. S. Wales* **78**, 43 (1953).

TABLE 1. BLAKELY'S CLASSIFICATION OF EUCALYPT SPECIES[2]

Section	Series (+Subseries)		No. of Species recorded
Macrantherae	Eudesmieae		12
	Miniatae		2
	Tetrapterae		2
	Corymbosae		17
	Corymbosae-peltatae		20
	Transversae		18
	Obliquae		7
	Cornutae		15
	Subcornutae		4
	Microcorythae		1
	Dumosae		33
	Anisomeleae		3
	Decurvae		3
	Elongatae		3
	Exsertae		16
	Subexsertae		8
Macrantherae-Normales	Microcarpae		8
	Globulares		31
	Semiunicolores		3
	Viminales		7
	Argyrophyllae		5
	Paniculatae		7
		Total	225
Renantheroideae	Diversiformae		4
Renantherae	Occidentales		4
	Ochroxylon		1
Renantherae-Normales	Pseudo-stringybarks		2
	White Mahoganies		3
	Steatoxylon		1
	Pachyphloiae		27
	Fraxinales		16
	Longitudinales		8
	Piperitales		16
	Psathroxyla		3
	Myrtiformes		3
		Total	84
Porantheroideae	Fructicosae		4
Porantheroideae-Normales	Subbuxeales		9
	Buxeales		23
	Siderophloiae		16
		Total	52
Terminales	(Rhodoxylae)		5
	(Leucoxylae)		1
	Melliodorae		1
	Heterophloiae		6
		Total	13

TABLE 1—*continued*

Section	Series (+Subseries)	No. of Species recorded
Graciles	Aridae	3
Micrantherae	Eremophilae	2
Platyantherae	Subulatae	13
	Leptopodae	9
	Contortae	1
	Quadricostatae	1
	Xylocardae	9
	Total	33

derived characteristics which are likely to be highly significant indications of evolutionary relationships, and unspecialised conditions which do not necessarily suggest close affinity in the same way."[11]

In recent years, increasing attention has been given to wood anatomy,[12] bark anatomy,[13] cytological studies,[14] pollen grains,[15] seed coat anatomy [16,17] and floral morphology.[18, 19, 20, 21]

On the basis of floral morphology,[19] Carr and Carr have divided the genus into two separate but very convergent genera that have been named *Symphomyrtus* and *Monocalyptus*,[18] the latter being renamed *Eucalyptus*.[21] They have proposed that the latter *Eucalyptus* genus contains:

Series Eudesmieae (of the Section Macrantherae, Table 1) except *E. lirata*
Series Miniatae (of the Section Macrantherae)
E. jacksoni, E. preissiana, E. megacarpa, E. coronata (*E. mitrata*), *E. gamophylla*
Section Renantheroideae
Section Renantherae except *E. guilfoylei, E. microcorys* and the Series Myrtiformes (of the Section Renantherae-Normales).

The separate and non-interbreeding *Symphomyrtus* genus contains the remainder of the species in Blakely's Key.

[11] L. D. PRYOR and L. A. S. JOHNSON, *Australian J. Botany* **10**, 129 (1962).
[12] H. E. DADSWELL and M. BURNELL, *C.S.I.R. Bull.* 62 (1932); H. E. DADSWELL, M. BURNELL and A. M. ECKERSLEY, *C.S.I.R. Bull.* 78 (1934); H. E. DADSWELL, unpublished (1964).
[13] M. M. CHATTAWAY, *Australian J. Botany* **1**, 402 (1953); **3**, 21, 28 and 29 (1955).
[14] S. SMITH WHITE, *Proc. Linnean Soc. N. S. Wales* **67**, 335 (1942).
[15] K. M. PIKE, *Australian J. Botany* **4**, 13 (1956).
[16] E. GAUBA and L. D. PRYOR, *Proc. Linnean Soc. N. S. Wales* **83**, 20 (1958).
[17] E. GAUBA and L. D. PRYOR, *Proc. Linnean Soc. N. S. Wales* **84**, 278 (1959).
[18] D. J. CARR and S. G. M. CARR, *Nature* **184**, 1549 (1959).
[19] D. J. CARR and S. G. M. CARR, *Australian J. Botany* **7**, 109 (1959); S. G. M. CARR and D. J. CARR, *Proc. Roy. Soc. Victoria* **77**, 207 (1963).
[20] D. J. CARR and S. G. M. CARR, In *The Evolution of Living Organisms* (Edited by G. W. LEEPER), p. 426. Melb. Univ. Press (1962).
[21] D. J. CARR and S. G. M. CARR, *Nature* **196**, 969 (1962).

Pryor[8, 22] suggested dividing the genus into the "sub-genera", *Macranthera* (Sections *Transversae* and *Bisectae*), *Renanthera*, *Corymbosa* and *Adnata*. The first three "subgenera" are reproductively isolated from each other, and frequently representatives of them grow mixed together in single stands. Pryor[22] divided parts of the non-renantherous groups into the Section "Bisectae" and the proposed subgenus "Adnata". Blakely's Cornutae, Subcornutae and Platyantherae (Table 1) are brought together (with certain exceptions), as the recently evolved West Australian unit the "Bisectae". The comparable but remotely related eastern Australian unit, the "Adnata", comprises most of the species in the Terminales and the Porantheroideae.

Chemotaxonomy with Particular Reference to the Eucalypts

The composition of the low molecular weight "secondary" compounds in a plant may not be a more important taxonomic criterion than a morphological character. However, the components in the mixture of these compounds can be accurately described, and their relative amounts more readily put on a quantitative basis than can the morphological characters. Because of these attributes, the chemical composition of a plant tissue could become a most valuable taxonomic criterion when used in association with morphological and anatomical criteria. The structures of the secondary compounds are as characteristic of the overall metabolism of the plant, as are the morphological features. Just as all the morphological features make up the characteristic visual aspects of the plant, so also does the complex composition of all the secondary constituents have a characteristic pattern. Chemical examination expresses its results in different terms from those of the classical biological methods, so that the chemical composition gives an independent check of the conclusions of taxonomists. Also the composition may possibly indicate associations of species that is not apparent from the morphological features currently studied.

The nature of different classes of secondary products (e.g. terpenes and polyphenols) may not change at the same time during evolution. Similarly the evolution of chemical features may not necessarily be synchronized with morphological and other botanical characteristics.

Just as some morphological features are more highly significant than others in determining taxonomic relationships so also could some secondary components have a high importance. The work of Bate-Smith has shown polyphenols in the leaves to be important when considering the taxonomy of plants at family level[23] and to a certain extent at genus level.[24] Hasegawa[25] has shown that the distribution of polyphenols in the heartwood of *Prunus* species is, in the main, in accordance with their classification and Erdtman[26] has shown an association between the distribution of several components in different woods and classification.

Eucalyptus was one of the first genera to be investigated chemically and Baker and Smith[27] commenced their 30-year-long study of the relationship of chemical composition and taxonomy of the eucalypts in the year 1890. These workers gave most attention to the essential oil composition of the leaves and their collaboration achieved a great deal. However, towards

[22] L. D. PRYOR, In *The Evolution of Living Organisms* (Edited by G. W. LEEPER), p. 446 Melb. Univ. Press (1962).
[23] E. C. BATE-SMITH, *J. Linnean Soc. London (Botany)* **58**, 95 (1962).
[24] E. C. BATE-SMITH, *J. Linnean Soc. London (Botany)* **58**, 39 (1962).
[25] M. HASEGAWA, *J. Japan. Forestry Soc.* **40**, 111 (1958).
[26] H. ERDTMAN, In *Chemical Plant Taxonomy* (Edited by T. SWAIN), p. 89. Academic Press, New York (1963).
[27] R. T. BAKER and H. G. SMITH, *The Eucalypts and their Essential Oils*. Govt. Printer, Sydney, N.S.W., 2nd Edition (1920).

the end of their study they gave to the chemical characteristics of the plant a status equal to, and occasionally greater than, that of the classical taxonomic features. At that time, natural hybridization of eucalypts was not considered and when they also encountered physiological forms with different essential oil composition of the leaves, they assigned either varietal or species rank to the new form in spite of the lack of morphological differences. As a result, their work fell into some disrepute. Their successors[28] have defined the sources of error in this type of work and have comprehensively investigated several species.[29, 30] There is often considerable difference in the composition of the oil from various species and sometimes from different samples of the same species. More than fifty compounds have been found in the oils of *Eucalyptus* species, such as cineole, citronellal, phellandrene, pinene, citral, borneol, eudesmol, geraniol etc.[29] However, the usefulness of the composition of the oil in taxonomic studies appears limited.[30] Similar conclusions have been made concerning *Pinus* turpentines,[31] and the diterpene hydrocarbons of the Podocarpaceae.[32] Whereas the terpenes in *Abies* balsams appear taxonomically significant in many cases,[33] there is a wide difference from tree to tree in the composition of wood oleoresin of *Pinus ponderosa*.[34]

The leaves of many eucalypt species, particularly during the juvenile stage of growth, are very glaucus. In most cases the appearance is due to a wax which has different properties when collected from different species.[35] A chemical investigation,[36] has shown that many of the waxes contained long-chain β-diketones, the amount being high in the Globulares. On the other hand, representatives of the Corymbosae and Corymbosae-peltatae (and other species) lack these compounds. The composition of the leaf wax is complex, and although the number of species examined was too small for any conclusions to be drawn, a more extensive survey may possibly reveal useful taxonomic information.

A chromatographic examination of the wood and phloem extractives of a few botanically dissimilar eucalypt species (unpublished) indicated a rather limited usefulness of these extractives in taxonomic studies, although the subsequent work of Hathway[37] has shown that eucalypt heartwood extractives have some value. One of the first papers to show that the judgement of the taxonomist could be supported by the chromatographic examination and the chemical composition of the leaves, reported a study of alcohol extracts of the shoots (consisting of two or three leaves and the growing apex) from the *Thea* and non-*Thea Camellias* before and after hydrolysis.[38] The composition of two varieties and one form of *Camellia sinensis* are appreciably different in their amounts of depsides, flavonol triglycosides and the compound "IC". This work indicated that some populations of cultivated teas could be species hybrids. More recently, chromatograms of leaf extracts have been used to reveal

[28] J. L. Willis, H. H. G. McKern and R. O. Hellyer, *J. Proc. Roy. Soc. N. S. Wales* **96**, 59 (1963).
[29] A. D. Penfold and J. L. Willis, *The Eucalypts*. Leonard Hill, London (1961).
[30] H. H. G. McKern, *J. Proc. Roy. Soc. N. S. Wales* **98**, 1 (1965).
[31] N. T. Mirov, *Composition of Gum Turpentines of Pines*. U.S. Dept. Agric. Tech. Bull. No. 1239 (1961).
[32] R. T. Aplin, R. C. Cambie and P. S. Rutledge, *Phytochem.* **2**, 205 (1963).
[33] E. Zavarin and K. Snajberk, *Phytochem.* **4**, 141 (1965).
[34] R. H. Smith, *Science* **143**, 1337 (1964).
[35] H. N. Barber, *Evolution* **9**, 1 (1955).
[36] D. H. S. Horn, Z. H. Kranz and J. A. Lamberton, *Australian J. Chem.* **17**, 464 (1964).
[37] D. E. Hathway, *Biochem. J.* **83**, 80 (1962).
[38] E. A. H. Roberts, W. Wight and D. J. Wood, *New Phytologist* **57**, 211 (1958).
[39] B. L. Turner and R. E. Alston, *Am. J. Botany* **46**, 678 (1959).
[40] R. E. Alston and J. Simmons, *Nature* **195**, 825 (1962).
[41] R. E. Alston and B. L. Turner, *Proc. Natl Acad. Sci. U.S.* **48**, 130 (1962).
[42] R. E. Alston, T. J. Mabry and B. L. Turner, *Science* **142**, 545 (1963).
[43] R. E. Alston and B. L. Turner, *Am. J. Botany* **50**, 159 (1963).
[44] B. G. Brehm and R. E. Alston, *Am. J. Botany* **51**, 644 (1964).

accurately the complex natural hybrids within the *Baptisia* genus[39–44] and hybrids of other genera[45–48] and in one case the biochemical data was presented as "profiles".[49] An attempt to assess the reliability of these profiles from plants grown under different ecological influences was made by growing *Spirodela oligorhiza* (duckweed) under different conditions.[50] The basic chromatographic patterns, particularly with regard to the flavonoids, were found to be similar among controls of different origins and differently treated cultures. For the most part the differences were quantitative and when qualitative differences occurred they nearly always involved minor components. Consequently, chromatographic patterns were considered to be reliable phenotype expressions.

It would appear from the above that the polyphenols in the leaves of eucalypts could have great value for comparative studies bearing on taxonomic studies.

Choice of Samples

The modern species concept takes into consideration the relation of the sample to the surrounding population and to other populations, and also to genetic, ecological, edaphic and other factors. Consequently for a complete examination it is necessary to compare individuals within a restricted and uniform habitat and then with individuals from populations in different areas. However, it is first necessary to obtain a broad view of the genus in order to gain some assessment of the relative importance of the different components of the polyphenolic mixture in the leaves.

A study of *E. camaldulensis* (197)[1] samples collected from a wide area showed that while the ratio of the components may vary, there were no significant qualitative differences except in those cases where hybridization was known or suspected. Also, samples of several other species were collected from different areas and in most cases there was little difference in composition between samples. Consequently, the examination of a small number of samples should be adequate at this stage, and in any case the work involved in a full examination would limit the extent to which the survey could be made and this could itself introduce difficulties.

An examination of a small portion of the genus can lead to undue emphasis as to the taxonomic importance of certain compounds. This is exemplified by a study[37] of the heartwood extractives of Blakely's heterogeneous Subsection Longiores which indicated that owing to the absence of stilbenes in the Transversae (56–87), the yellow bloodwoods (48–52), *E. cornutae* (96), *E. lehmanii* (97) and *E. gomphocephala* (98), these species differed from the rest of the Subsection. This tended to imply that they perhaps had some affinity with each other. It should be noted that, for many years the Transversae and the yellow bloodwoods have been separated, on the basis of morphological characters, from the rest of the Subsection and it has been found that Blakely's Subseries are much more homogeneous than his Subsections which are based entirely on antheral characters. In placing undue emphasis on stilbenes in a small portion of the genus, incorrect groupings have been overlooked in this case. Stilbenes have been found in the heartwood from species in several other parts of the genus,[51] so that the presence of stilbenes in *E. woodwardii* (89) and *E. griffithsii* (92) need not support the view that these species are correctly classified. (Hathway[37] refers to "*E. guilfoylei*

[45] A. M. TORRES and D. A. LEVIN, *Am. J. Botany* **51**, 639 (1964).
[46] D. M. SMITH and D. A. LEVIN, *Am. J. Botany* **50**, 952 (1963).
[47] G. L. STEBBINS, B. L. HARVEY, E. L. COX, J. N. RUTGER, G. JELENCOVIC and E. YAGIL, *Am. J. Botany* **50**, 830 (1963).
[48] P. M. HARNEY and W. P. GRANT, *Am. J. Botany* **51**, 621 (1964).
[49] W. M. ELLISON, R. E. ALSTON and B. L. TURNER, *Am. J. Botany* **49**, 599 (1962).
[50] J. W. MCLURE and R. E. ALSTON, *Nature* **201**, 311 (1964).
[51] W. E. HILLIS and K. ISOI, *Phytochem.* **4**, 541 (1965).

(Blakely No. 38)", whereas this Blakely number refers to *E. ficifolia* variety *guilfoylei*. The sample studied is probably *E. guilfoylei* (Blakely No. 305) and it is not unexpected that stilbenes are absent from the heartwood.)

Consequently, the approach in this present work has been to examine one or more samples from as many species as possible (botanical varieties have in the main been omitted) and to interpret the results on a broad basis. The intention is to indicate the possible key compounds in a group of species, so that in future confirmatory work the various species under consideration can be compared at the one time and attention focused on the key compounds. A semi-quantitative assessment of the polyphenolic composition has been made, and although it is of a subjective nature, reproducible results have been obtained. A quantitative determination of the absolute amounts present in the leaves was not made as this could be affected by environment, mineral deficiencies in the soil etc.

E. sieberi (371), *E. sideroxylon* (541) and *E. astringens* (112) leaves were examined at various stages of growth and at different intervals throughout the year but no significant differences in the composition were observed. The juvenile and mature foliage of many species differs considerably in shape, size and appearance. The leaves of both types from *E. accedens* (125), *E. goniocalyx* (229), *E. perriniana* (242), *E. ligustrina* (343), *E. dives* (417) and *E. polyanthemos* (558) have been examined and, in *E. accedens*, *E. dives* and *E. ligustrina*, there were significant differences in composition. The juvenile leaves of *E. accedens* and *E. dives* contain significant amounts of myricetin that is absent in mature leaves, whereas the juvenile leaves of *E. ligustrina* contain smaller amounts of myricetin but stronger amounts of leucodelphinidin than the mature leaves. Fully grown mature leaves were chosen as the tissue for study and in most cases fresh material was examined.

Composition of the Leaves

The polyphenolic components of leaves are largely polymeric materials which cannot be adequately chracterized, but, there is also a sufficient amount of monomeric compounds present to permit characterization of the leaf extractives. These extractives have been examined before and after acid hydrolysis and the chromatographic properties of the commonly encountered polyphenols are given in Table 2 and their formulae in Fig. 1. In addition, components found only in a species or groups of species were encountered. The method of scoring, which has been previously described[1] (see also Experimental), has the shortcomings of a subjective method but all observations were made by the author so that the results should be mutually comparable. This work has been conducted over a period of more than 5 years and checks have shown that very similar or identical scores were obtained when early samples were re-examined. It should be emphasized that the relative amounts are not absolute. Some compounds (e.g. ellagic acid) are only weakly visualized and they may be present in greater amounts than their score indicates. Unknown compounds A and B are difficult to detect and are best seen when the Forestal chromatograms are dry or partly neutralized with ammonia. Furthermore, other compounds (leucoanthocyanins and gallotannins) give low yields of end-products on hydrolysis.

Delphinidin, cyanidin, myricetin, quercetin, kaempferol, ellagic, gallic, caffeic, *p*-coumaric, ferulic, sinapic and gentisic acids, aromadendrin and taxifolin have been previously identified[52] in the acid hydrolysis products. "Pelargonidin" has chromatographic properties and colour reactions similar to authentic pelargonidin but its identity has not yet been

[52] W. E. Hillis and F. J. Hingston, *J. Sci. Food Agr.* **14**, 866 (1963).

TABLE 2. CHROMATOGRAPHIC PROPERTIES OF EUCALYPT POLYPHENOLS

Polyphenol*	$R_f \times 100$† Solvent					Appearance‡
	F	Be	HA	BA/HA		
e. Delphinidin	32	—	—	39	05	red
f. Cyanidin	49	—	—	68	02	red
g. "Pelargonidin"	68	—	—	—	—	red, pink fl.
h. Myricetin	26	—	—	43	00	or. fl.
i. Quercetin	40	—	—	64	00	y. fl.
j. Kaempferol	55	11	—	82	00	y. fl.
k. Ellagic acid	33	—	—	34	02	m. fl., s. m. fl. in u.v. (254 mμ), →dull y. fl.
l. Unknown cpd. A	48	—	—	54	02	m. fl., s. m. fl. in u.v. (254 mμ), →dull y. fl.
m. Unknown cpd. B	61	—	—	71	02	m. fl., s. m. fl. in u.v. (254 mμ), →dull y. fl.
n. Gallic acid	65	—	35	58	42	s. m. fl. in u.v. (254 mμ), brown with pNA
o. Gentisic acid	86	18	59	82	62	grey w. fl.→stronger, f. pink with pNA
p. Caffeic acid	86	13	40	75	38	bu. w. fl. →s. w. bu., pink with pNA
q. *p*-Coumaric acid	89	48	44	84	42	opaque→bu., or. with pNA
r. Sinapic acid	90	65	29	75	26	bu.–gr.→l. gr. fl., purple–pink with pNA
s. Ferulic acid	90	70	33	80	33	bu. fl. →l. bu. fl., purple–pink with pNA
t. Macrantherin	89	49	40	89	39	canary y. with pNA
u. Renantherin	92	34	57	89	68	canary y. with pNA
v. Unknown cpd. C	95	93	—	95	90	canary y. with pNA
w. Unknown cpd. D	93	83	05	92	07	l. y. with pNA
x. Taxifolin	75	03	40	73	42	opaque u.v. (254 mμ), or. with pNA
y. Aromadendrin	86	10	41	89	42	opaque u.v. (254 mμ), or. br. with pNA
z. Unknown cpd. E	95	65	50	90	60	y. with pNA
a. Astringin	—	—	—	22	07	i. bu. fl., →gr. bu. fl.
b. Rhapontin	—	—	—	30	10	i. bu. fl.,→l. magenta fl.
c. Piceid	—	—	—	44	15	i. bu. fl.,→duck-egg bu. fl.
d. Chlorogenic acid	—	—	—	55	60	bu. fl.,→i. gr. fl.
				55	74	
e. *p*-Coumarylquinic acid	—	—	—	68	72	nil→i. bu. fl. u.v.
				68	85	
f. Unknown cpd. F	—	—	—	47	12	s. l. y. fl.→fades u.v., tan with pNA
g. Unknown cpd. G	—	—	—	52	06	opaque u.v., tan with pNA
h. Unknown cpd. H	86	67	48	82	54	pink→or.→purple with pNA
i. Unknown cpd. I	47	—	—	55	00	brown in u.v.
j. Unknown cpd. J	79	16	45	73	49	pink→purple with pNA
k. Unknown cpd. K	89	60	67	95	66	y. or. with pNA

* Polyphenols *a–e* were observed in alcohol extracts, and the remainder in the acid-hydrolysed products.

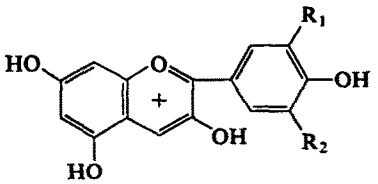
† R_f values (× 100) were taken from chromatograms of mixed components and may be slightly different from those of pure compounds.

Solvent F = Forestal (hydrochloric acid–acetic acid–water: 3:30:10); Be = benzene–acetic acid–water (6:7:3); HA = 6% acetic acid; BA/HA = two dimensional chromatograms with butanol-acetic acid–water then acetic acid.

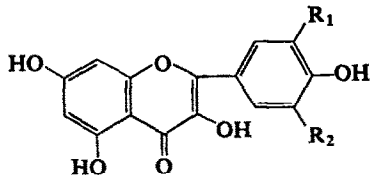
‡ Appearance: bu. = blue; f. = faint; fl. = fluorescence in u.v. light (366 mμ); gr. = green; i. = intense; l. = light; m. = mauve; or. = orange; pNA = diazotized *p*-nitroaniline; s. = strong; w. = white; y. = yellow; →= fluorescence after exposure to ammonia. Red and the colours with pNA were observed in daylight.

confirmed. In the alcohol extracts of different species, piceid [53] and rhapontin[53], engelitin (aromadendrin 3-rhamnoside), [51, 52] rutin (quercetin 3-rutinoside),[53, 54] various quercetin and kaempferol glycosides[51], chlorogenic and *p*-coumarylquinic acids[53] have been identified.

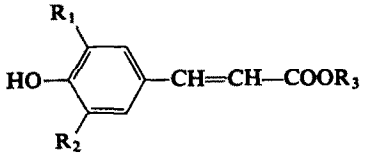
[53] W. E. HILLIS and M. HASEGAWA, *Biochem. J.* **83**, 503 (1962).
[54] F. R. HUMPHREYS, *Econ. Botany* **18**, 195 (1964).



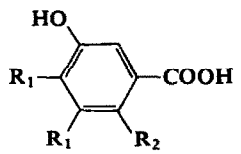
R_1, R_2=H; Pelargonidin
R_1=OH, R_2=H; Cyanidin
R_1, R_2=OH; Delphinidin



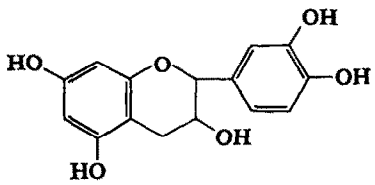
R_1, R_2=H; Kaempferol
R_1=OH, R_2=H; Quercetin
R_1, R_2=OH; Myricetin



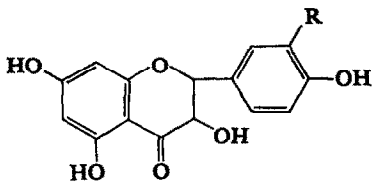
$R_1R_2R_3$=H; *p*-Coumaric acid
R_1R_2=H, R_3=quinic acid; *p*-Coumarylquinic acid
R_1=OH, R_2R_3=H; Caffeic acid
R_1=OH, R_2=H, R_3=quinic acid; Chlorogenic acid
R_1=OCH_3, R_2R_3=H; Ferulic acid
R_1R_2=OCH_3, R_3=H; Sinapic acid



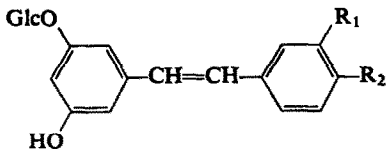
R_1=OH, R_2=H; Gallic acid
R_2=OH, R_1=H; Gentisic acid



Catechin



R=H; Aromadendrin
R=OH; Taxifolin



R_1=H, R_1=OH; Piceid
R_1R_2=OH; Astringin
R_1=OH, R_2=OCH_3; Rhapontin

FIG. 1. MAIN PHENOLIC COMPONENTS PRODUCED ON ACID TREATMENT OF EUCALYPT LEAVES.

Recent work[55] indicates that astringin contains 3,4,3′,5′-tetrahydroxystilbene glucoside and perhaps other stilbenes. In the present work, identity has been based on chromatographic data obtained under the same standard conditions used in the work just mentioned. The provisional identity of the compounds is fairly certain and is adequate for the purposes of this survey. However, when the scope of this present work is extended, it will be necessary to confirm or establish the identity of the key polyphenols by isolational procedures.

[55] M. HASEGAWA and W. E. HILLIS, unpublished data.

TABLE 3. POLYPHENOLS IN THE LEAVES OF THE GLOBULARES SERIES (No. XVIII) OF THE MACRANTHERAE-NORMALES SECTION

Blakely's No.	Species	No. of samples	Location*	Compound† c	f	g	h	i	j	k	l	m	n	o	p	q	r	s	t	u	v	w	x	y	z	*a*	*b*	*c*	*d*	*e*	*f*	*g*	*h*	*i*	*j*	*k*
				Subseries XXXV. Malacoxyla																																		
223.	*E. dunnii*	(1)	Lr	3	T	–	2	5	2	4	-	–	5	2	–	–	–	-	–	–	–	–	–	–	T	–	–	–	–	–	1	1	-	–	–	–
224.	*E. angophoroides*	(1)	Oo	–	1	T	3	1	–	5	1	-	5	2	-	-	1	–	1	–	–	-	–	–		5	–	2	3	2	–	–	–	–	–	–	-
		(1)	Z			–	–	3	–	5	–	–	5	2	–	–	–	–	–	–	–	–	–	–		2	5	5	–	–	1	-	–	–	–	–	–
225.	*E. bridgesiana*	(1)	Op	–	T	1	–	3	–	5	3	4	5	2	-		–	–	–	–	–			-		-	–	–	1	-	3	2	–	–		–
227.	*E. malacoxylon*			Not examined																																
228	*E. banksii*	(1)	Lq	–	-		–	5	2	5	3	2	5	1	2	-	1	1	T	-	–			–			–	–	T	–	3	–	–	–	-
229.	*E. goniocalyx*	(4)	On, Oo			-	–	5	–	5	3	5	5	1	2	-	1	1	–	–	–	–	–	–	-	–	–	3	–	1	–	–	–	–	–	–
230.	*E. nortonii*	(1)	Z		-	-	-	5	3	5	2	2	5	2	2	-	-	1	2	–	T	–	–	–	–		–	–	1	2	–	–	2	–	4	-
				Subseries XXXVI. Oritae																																		
233.	*E. mannifera* ssp. *mannifera*			Not examined																																
235.	*E. rubida*	(2)	Op, Ro	-	-	–	–	3	–	5	2	–	5	2	1	1	–	–	3	–	–	–	–	–	–	–	–	–	3	3	2	–	–	–	–	–	–
235a.	*E. chapmaniana*	(1)	Oo	–	1	-	–	5	2	5		-	5	2	1	–	–	–	1	–	–	-	–	–	–	–	–	–	2	–	1	–	–	–	–	–	–
236.	*E. dalrympleana* ssp. *dalrympleana*	(5)	Op, Z	-	-	3	–	5	T	5	–	–	5	1	–	–	–	–	2	–	–		–	–	–		4	5	2	3	3	2		-	–	–	–
236a.	*E. dalrympleana* ssp. *heptantha*	(1)	Z		-	-	–	5	3	3	-	-	3	1	3	-	-	-	2	–	–	–	–	–	1		–	–	–	4	3	T	–	2	–	1	–
237.	*E. glaucescens*	(1)	Z	–	–	3	T	5	-	5	1	–	3	1	–	–	–	-	3	–	–	–	–	–	–		5	–	2	1	3	1	–	-		–	-
		(1)	Z	–	–		–	3	–	5	1	-	3	1	–	–	–	–	3	–	–	–	–	–	–		–	–	–	3	1	1	–	–	–	–	–
238a.	*E. morrisbyi*	(1)	Rp	-	-		–	5	-	4		–	5	2	2	–	–	–	3	–	–	–	–	–	–		–	–	–	3	3	5	–	–	–	–	–
239.	*E. gunnii*	(2)	Rp, Z		T	-		5	–	5	-	2	5	2	2		-		–	–	–	–	–	–	–		-	-		3	2	2	-	1	–	–	-
240.	*E. urnigera*	(2)	Rp		T		–	5		5		-	4	2	3		–	2	2	–	–	-	–	–	–		1		–	4	2	1	–	1	-	–	–
				Subseries XXXVII. Perfoliatae																																		
242.	*E. perriniana*	(2)	Rp, Z	–	1			5	1	5	-		-	5	1	2	-	–	1			-	-	-	-					3	3		-	2	-	–	
				Subseries XXXVIII. Isophyllae																																		
243.	*E. kruseana*	(1)	Me				–	–	5	-	4	-	2	4	2	-	–	–	3	–	–	–	–	–	–		–	–	–	–	–	2	–	5	–	2	–
243a.	*E. brachyphylla*	(1)	Me	–			1	4	T	5	2	3	5	3	2	1	–	1	3	–	–	-	–	–	–	–		–	–	-	3	3	2	1	3	–	2	-
244.	*E. cordata*	(1)	Rp			–	–	5		4	2	–	4	1	–	–	–	–	2	–	–		-	-	–	–		–	–	–	1	1	1	–	1	–	–	–
244a.	*E. crenulata*	(1)	Z	-	–		T	5	1	5	1	1	4	2	–	-	–	2	3	–	–	–	–	–	–	–		–	–	–	3	–		–	–	5	–	–
245.	*E. pulverulenta*	(3)	Z		T		1	3		5	4	1	4	2	–		–	–	T	–	–	–	–	–	–		–	–	–	–	T	1	–	2	–	–	–

				Subseries xxxix. Pluriloculares				
246.	*E. preissiana*	(2)	Z	2 – – 5 2 – 5 5 3 5 2 – – – –	– 3 –	4 – T –	– – – – –	3 2 – – – –
				Subseries xl. Glandulares				
247.	*E. megacarpa*	(1)	Z	3 2 – 5 1 – 2 – – 5 3 – – – –	– 2 –	– T – –	– – – – –	1 – – – – –
247a.	*E. coronata*	(1)	Z	2 – – 5 1 – 4 3 3 5 3 – – – –	– 1 –	– T – –	– – – – –	1 – – – – 1
				Subseries xli. Euglobulares				
248.	*E. globulus*	(1)	Rp	– – – – 3 – 5 1 2 5 2 1 – – 2	2 – –	– – – –	– – – 2 2	5 – – – – –
250.	*E. bicostata*	(4)	Pn, Z	T 2 – – 2 – 5 4 – 5 3 1 – – –	2 – –	– – – –	– – – 2 2	3 1 – – – –
254.	*E. st.-johnii*	(1)	Po	T – – – 3 – 5 4 4 5 2 1 – – –	3 – T	– – – –	– – – 2 –	2 3 1 – – 1
				Subseries xlii. Pluriflorae				
261.	*E. maidenii*	(1)	Po	T – – – 4 – 5 4 2 5 2 T – – –	3 – –	– – T –	– – – 3 –	1 4 1 – – –
				Subseries xliii. Doleiformes				
262.	*E. cypellocarpa*	(5)	On, Pn	– – – – 2 – 5 3 4 5 2 – – 1 1	– – –	– – – –	– – – 2 1	3 3 – – – –
263.	*E. nitens*	(1)	Z	– T 2 – 5 – 4 – – 5 2 2 – – –	2 – –	– – – –	5 – 3 2 2	1 – – – – –

* See Map, Hillis[1]

† e = Leucodelphinidins	p = Caffeic acid	*a* = Astringin
f = Leucocyanidins	q = *p*-Coumaric acid	*b* = Rhapontin
g = "Leucopelargonidins"	r = Sinapic acid	*c* = Piceid
h = Myricetin	s = Ferulic acid	*d* = Chlorogenic acid
i = Quercetin	t = Macrantherin	*e* = *p*-Coumarylquinic acid
j = Kaempferol	u = Renantherin	*f* = Unknown compd. F
k = Ellagic acid	v = Unknown compd. C	*g* = Unknown compd. G
l = Unknown compd. A	w = Unknown compd. D	*h* = Unknown compd. H
m = Unknown compd. B	x = Taxifolin	*i* = Unknown compd. I
n = Gallic acid	y = Aromadendrin	*j* = Unknown compd. J
o = Gentisic acid	z = Unknown compd. E	*k* = Unknown compd. K

Routine examination of two-dimensional chromatograms of the alcohol extracts of the leaves revealed: (*a*) the ratio of the stilbenes to each other, (*b*) the relative amounts of chlorogenic and *p*-coumarylquinic acids. The yield of caffeic and *p*-coumaric acids from these acids on hydrolysis with hydrochloric acid is low and partly dependent on the conditions used; the relative amounts of the latter acids obtained from the hydrolysates can be misleading, (*c*) the presence of engelitin (which is probably important taxonomically) and rutin (which is possibly not important), (*d*) the presence of a number of flavonol glycosides [51] and the occurrence of certain glycosides appears to be associated with the provenance of certain species, (*e*) the almost consistent presence of catechin. Comparison of chromatograms of alcohol extracts showed it was often present in about the same amount, but owing to its wide distribution in both the eucalypts and the plant kingdom, no special record has been made here of its presence.

All the compounds resolved by the solvents used have been recorded. However, additional significant but unresolved components may exist, for example, the C-methyl flavones.[36, 51, 56] Similar flavones may exist in the Exserta and Subexserta (particularly species No. 207–209) and other Series. The opaque substance(s) which form a periphery around the waxy material in BAW (R_f 0·9–1·0) in the alcohol extracts of some species give a strong yellow with diazotized *p*-nitroaniline and may contain polyphenols.

The composition of the leaves given in the tables is arranged in the order of three leucoanthocyanins, three flavonols, ellagic acid and two similar compounds, six organic acids, three compounds giving a canary yellow and four compounds giving a yellow to orange colour with diazotized *p*-nitroaniline. The stilbenes and chlorogenic and *p*-coumarylquinic acids were detected in the alcohol extracts of the leaves, whereas the above compounds and the unknown compounds F–K were detected in the acid-hydrolysed products.

Examination of the Series Globulares

The Series Globulares of the Section Macrantherae includes mainly medium-sized trees although some can be as tall as 200 ft (*E. nitens* (263)) or less than 12 ft high (*E. kruseana* (243), *E. brachyphylla* (243a); *E. preissiana* (246) and *E. coronata* (247a)). They usually have a smooth deciduous bark except for a few feet at the butt, and the species are more markedly heteroblastic than those in other Series of the genus. Most species occur naturally in the eastern portion of Australia particularly in New South Wales, some are found only in Tasmania (*E. morrisbyi* (238a), *E. gunnii* (239), *E. urnigera* (240), *E. cordata* (244)) whereas *E. preissiana* (246), *E. megacarpa* (247), *E. coronata* (247a), *E. kruseana* (243) and *E. brachyphylla* (243a) occur naturally only in the southern portion of Western Australia. Blakely recognized the anomalous characters of *E. preissiana* (246) and *E. megacarpa* (247) when placed in this series. More recently, other workers have studied these two species and also *E. coronata* (247a) and on the basis of the anatomy of the ovule and seed and breeding behaviour,[11] the nature of the operculum,[57] and floral morphology,[18, 20, 21] have concluded these species are wrongly classified.

The composition of the polyphenols in the leaves of the Globulares (Table 3) is relatively uniform for such a large Series. The most important compounds in the Series are the leucoanthocyanins, myricetin, quercetin, ellagic, gallic and gentisic acids, macrantherin, renantherin, unknown compound D, the stilbenes and chlorogenic and *p*-coumarylquinic acids. These compounds are associated with taxonomy in the following way.

[56] J. A. LAMBERTON, *Australian J. Chem.* **17**, 692 (1964).
[57] L. A. S. JOHNSON (1956), quoted in Ref. 11.

(*a*) Leucodelphinidin is present in significant amounts in *E. dunnii* (223), *E. preissiana* (246), *E. megacarpa* (247), *E. coronata* (247a) and leucocyanidin in *E. megacarpa* (247) and *E. bicostata* (250).

(*b*) "Leucopelargonidin" is associated with the presence of stilbenes except with *E. bridgesiana* (225).

(*c*) Almost three-quarters of the species lack leucoanthocyanins.

(*d*) In most cases, the amount of quercetin relative to ellagic acid is high; kaempferol is present only rarely and apparently without taxonomic significance.

(*e*) Myricetin is present in significant amounts only in *E. dunnii* (223), a chemical variant of *E. angophoroides* (224), and particularly so in *E. preissiana* (246), *E. megacarpa* (247) and *E. coronata* (247a).

(*f*) Ellagic acid is present as a major component in all species except *E. megacarpa* (247). Gallic acid is present in large amounts in almost all species.

(*g*) Gentisic acid is present in all species.

(*h*) A distinctively coloured but unidentified component (named "macrantherin") is present in most species, but in *E. preissiana* (246), *E. megacarpa* (247) and *E. coronata* (247a) the compound "renantherin", with an identical appearance but different R_f values, is present.

(*i*) Unknown compound D is present only in *E. preissiana* (246).

(*j*) Stilbenes are present in five species.

(*k*) Chlorogenic and *p*-coumarylquinic acids are present in almost all species but absent in *E. preissiana*, *E. megacarpa* and *E. coronata*.

The presence of significant amounts of leucoanthocyanins, of very large amounts of myricetin, and low amounts of quercetin, and the absence of chlorogenic and *p*-coumarylquinic acid in *E. preissiana* (246), *E. megacarpa* (247) and *E. coronata* (247a) in contrast to the other species in this Series are consistent with the conclusions drawn from morphological and hybrid studies that these species are wrongly classified. The presence of unknown compound D in *E. preissiana* further supports these views. The possible reclassification into the Renantherae on the basis of the presence of renantherin will be discussed in the next paper.

Most of the remaining species in this Series show a high degree of similarity in the composition of polyphenols. *E. banksii* (228) is considered[57] to be the northern vicariad of *E. goniocalyx* (229) and chemically these species are very similar; *E. banksii* (228) and *E. nortonii* (230) are even more similar. Apart from the presence of leucocyanidin in *E. bicostata* (250) and the large amount of the unknown compound F in *E. globulus* (248), these two species and *E. st.-johnii* (254) and *E. maidenii* (261) have a very similar composition. This is in accordance with the morphological features. *E. cypellocarpa* (262) also has a similar composition but the only sample of *E. nitens* (263) was a stilbenoid chemovariety so that comparison is not possible. *E. morrisbyi* (238a), *E. gunnii* (239) and *E. urnigera* (240) have a very similar composition. Carr and Carr[18] consider *E. kruseana* (243) to be closely related to *E. crucis* (587); chemically also these two are similar (unpublished data) but the species do not appear to be misplaced in their present groupings.

No significant difference in composition of the small (Nos. 243, 243a) and the larger (Nos. 248, 261, 229) members of the Series was evident. Those (Nos. 243, 243a) growing in Western Australia have a very similar composition to those found in the Eastern states.

EXPERIMENTAL

The approximate origin of the samples can be ascertained by reference to the map in an earlier paper.[1] Samples labelled "Z" were collected from Botanic Gardens or Arboreta.

Small pieces of the leaves (about 0·5 g) were heated with 2 N aqueous HCl (5 ml) in a test-tube in a boiling water bath. The contents of the tube were shaken a few times and after 30 min the hot liquor was poured off into a narrow test-tube and shaken with a few drops of amyl alcohol. The extract was examined by one- and two-dimensional chromatography and the components scored as previously described[1] (see also Table 2).

The score of unknown compound D, the stilbenes, chlorogenic and *p*-coumarylquinic acids and a few of the specific components were obtained from the chromatograms of alcohol extracts of the leaves, and the score assessed, to an extent, in an arbitrary manner. Approximately the same amount of alcohol-soluble solids was added to each chromatogram and, in many cases, catechin assumed a spot of the same size and this was given a score of 5 and the latter group of compounds was related to this size of catechin.

Caffeic, *p*-coumaric, ferulic, sinapic and gentisic acids were added to the chromatograms as markers to assist in their identification. Some of these markers formed double spots in the 6% acetic acid and benzene solvents. When present in the acid hydrolysates of leaves, only the low R_f components of sinapic and ferulic acids were observed using 6% acetic acid as solvent and the high R_f components of sinapic, ferulic and caffeic acids in benzene solvent.

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[58] L. A. S. JOHNSON, *Contrib. N. S. Wales Herbarium* **3**, 111 (1962).