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POLYPHENOLS IN THE LEAVES OF *EUCALYPTUS*: A CHEMOTAXONOMIC SURVEY—II.

THE SECTIONS RENANTHEROIDEAE AND RENANTHERAE

W. E. Hillis

Division of Forest Products, C.S.I.R.O., South Melbourne, Australia

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Abstract—A paper chromatographic study has been made of the polyphenols in the leaves of seventy-five species and varieties belonging to the Sections Renantheroideae and Renantherae. An unidentified compound (renantherin) found in many of the species examined, but not in non-renantherous species, is especially diagnostic. An unknown compound *D* is found less frequently but in combination with other compounds it suggests certain interspecific relationships. Close agreement exists between the absence or presence of myricetin in large amounts and the existing classification into Series; low to medium ratios of ellagic acid are found in related species. The Pachyphloiae Series is characterized by distinctive compounds ("Factors") of limited occurrence and on the basis of their distribution an inter-relationship of the different species can be proposed. The composition of the polyphenolic mixture in certain species supports recently proposed reclassifications deduced from morphological characters.

INTRODUCTION

The Section of *Eucalyptus* which Blakely[1] named Renantherae is a distinctive group of species which do not hybridize with eucalypts outside the Section. On morphological grounds, the Renantherae could be classified as a sub-genus and Carr and Carr[2] have suggested that it be considered (together with certain other species) as a separate genus. The small Section Renantheroideae is very similar morphologically, although Blakely separated it on antheral characters.

The greater portion of the renantherous species grow naturally in the eastern coastal regions of Australia. The Sections include the world's tallest hardwood with heights of more than 300 ft and small shrubs of about 10 ft.

The present paper reports the results of a survey of the polyphenols in the mature leaves and an analysis of these results to ascertain their possible usefulness in taxonomic studies. Occasionally two or more species of the Sections are found in the same locality and hybridization can then occur. In order to ensure, as far as possible, that no hybrids were examined, the greater portion of the samples were obtained from representative areas by collectors familiar with the species in the field. The remainder were collected from trees grown in arboreta etc. from seeds of authentic species.

RESULTS AND DISCUSSION

Blakely's Series XXIX. Pachyphloiae

This Series, commonly known as the Stringybarks (315–369 on Blakey's numbering), is characterized by its strongly fibrous and persistent bark. The species are weakly differentiated and some of these are difficult to identify. The majority are confined to the eastern

[1] W. F. Blakely, *A Key to the Eucalypts*, 2nd edition. Forestry and Timber Bureau, Canberra (1955).
[2] S. G. M. Carr and D. J. Carr, *Proc. Roy. Soc. Victoria* 77, 207 (1963).

TABLE 1. POLYPHENOLS IN THE LEAVES OF THE PACHYPHLOIAE SERIES (NO. XXIX) OF THE RENANTHERAE SECTION OF THE GENUS *Eucalyptus*

*a	b	c	d	e 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*	Factor	
				Subseries xlvi. Odoratae						
315.	*E. tinghaensis*			Not examined						
318.	*E. eugenioides*	(1)	Nq	- - - - 5 - 2 - - 5 1 - - - -	- 5 1	- - - -	- - - - 1	3 - - - - -	Eugenioides:	3
		(1)	Lq	2 3 - - 5 - 3 - - 5 1 - - - 2	- - 3	3 - - -	- - - T -	T - 1 - - -	Eugenioides:	5
									Tindaleae:	2
		(1)	Z	- - - - 5 - 4 5 4 5 1 T T 1 3	- 1 -	- - - -	- - - - -	2 - - - - -	Muelleriana:	3
322.	*E. cameronii*	(1)	Mq	- 3 - - 5 - 5 - 3 5 3 - - - -	- - -	- - - -	- - - - -	1 - - - - -	Eugenioides:	7
323.	*E. nigra*			Not examined						
324.	*E. caliginosa*	(2)	Mq, Z	1 2 - 2 5 - 3 - - 3 T - - - -	- 1 -	1 - 1 -	- - - 3 2	2 2 - - - -	Eugenioides:	2
		(1)	Mq	- 1 - - 5 - 3 - 3 2 2 - - - -	- 3 2	2 - - -	- - - 4 3	2 3 - - - -	Caliginosa:	1
									Eugenioides:	7
325.	*E. phaeotricha*	(1)	Kq	5 T - - 2 - 5 - 2 5 2 - - - -	- T -	T - - -	- - - 2 2	1 - - - - -	Eugenioides:	3
		(1)	Lr	1 T - 1 2 2 4 - - 5 1 - - - -	- - 2	3 - 1 -	- - - T 1	3 1 - - - -	Phaeotricha:	4
									Tindaleae:	3
326.	*E. tindaleae*	(1)	Lr	- - - 1 4 3 5 3 3 5 2 - - - 1	- - -	2 - 1 -	- - - 2 2	3 - 1 - - -	Tindaleae:	5
									Phaeotricha:	5
327.	*E. laevopinea*	(3)	Mq	- T 2 - 5 5 5 - - 3 1 2 - - -	- 2 2	- - 1 -	- - - 3 2	1 - - - - -	Muelleriana:	5
									Caliginosa:	3
328.	*E. laevopinea* var. *minor*	(1)	Z	- T 2 - 3 3 5 1 - 3 1 - - - -	- 1 3	- - 1 -	- - - 3 2	1 - - - - -	Muelleriana:	4
									Caliginosa:	2
				Subseries xlvii. Macrorhyncha						
331.	*E. macrorhyncha*	(6)	Om, Pn, Z	- - - - 5 - 1 - - 3 1 - - - -	- 1 -	T - 1 -	- - - 1 -	T - - - - -	Tindaleae:	T
333.	*E. cannonii*	(1)	Z	- - - - 5 - 1 - - 3 1 - - - -	- T -	T - - -	- - - - 1	T - - - - -	Eugenioides:	2
									Muelleriana:	1
334.	*E. youmanii*	(2)	Lq, Mq	- - - - 5 - 1 - - 3 1 - - - -	- 3 -	- - - -	- - - T T	T - - - - -	Eugenioides:	2
				Subseries xlviii. Congestae						
336.	*E. blaxlandii*	(2)	Z	- - 2 - 3 3 5 1 1 4 3 2 - - -	- 2 2	- - 2 -	- - - 1 1	1 1 3 - - -	Eugenioides:	4
									Muelleriana:	2
									Blaxlandii:	4
									Caliginosa:	1
337.	*E. baxteri*	(3)	Ok, Pn, Ol	- - - - 5 - 5 - 3 3 2 - - 2 -	- 2 2	2 - 2 -	- - - T -	3 2 3 - - -	Eugenioides:	5
									Muelleriana:	T
									Blaxlandii:	3
									Caliginosa:	T
									Tindaleae:	2

339.	*E. alpina*	(1)	Z	– 3 – – 2 – 5 3 3 5 3 – – – –	– 3 2	T – – –	– – – – –	3 1 – – – –	Eugenioides:	4
		(1)	Om	– T – – 5 – 5 2 – 5 1 – – – –	– 1 –	2 – – –	– – – – –	3 – – – – –	Eugenioides:	4
340.	*E. capitellata*	(1)	Z	3 T – 1 5 – 4 – – 5 2 – – – –	– – 1	3 – – –	– – – – –	3 – – – – –	Eugenioides:	4
									Tindaleae:	3
342.	*E. camfieldii*	(1)	Nq	– – – – 4 – 5 2 4 3 2 T – 1 –	– T –	– – – –	– – – – –	3 2 2 – – –	Eugenioides:	2
									Caliginosa:	2
									Muelleriana:	T
343.	*E. ligustrina*	(1)	Kq	2 T – 2 4 – 4 1 – 5 2 – – – –	– 3 –	– – – –	– – – T –	2 – – – – –	Eugenioides:	7
344.	*E. deformis*			Not examined						
345.	*E. agglomerata*	(1)	Kq	2 – – T 4 – 5 T T 5 2 – – – –	– T 3	4 – T –	– – – – –	– – – – – –	Tindaleae:	4
									Muelleriana:	4
346.	*E. globoidea*	(3)	Oq	– – – – 5 – 5 2 4 3 2 – – – –	– T 3	1 – – –	– – – – T	1 1 – – – –	Muelleriana:	4
				Subseries xlix. Angustiores						
350.	*E. mckieana*			Not examined						
351.	*E. conglomerata*			Not examined						
353.	*E. oblonga*	(2)	Nq	– – – – 5 – 3 3 5 5 2 1 – 1 –	– T 3	– – 1 –	– – – 1 1	4 4 – – – –	Muelleriana:	3
				Subseries li. Prealtae						
362.	*E. obliqua*^{m}	(7)	Ol, Op, Pn, Po, Rp	2 1 – 2 4 – 3 T 1 5 2 – – – –	– – –	T – – –	– – – – –	2 1 – – – –		
368.	*E. fastigata*	(1)	Z	T 3 – – 4 – 2 – 1 5 1 – – – –	– T 3	– – – –	– – – 1 –	– – – – – –	Saligna:	2
369.	*E. regnans*	(4)	On, Ro	1 1 – 4 5 – 3 1 – 4 1 – – – –	– T 1	2 – – T	– – – – –	2 – 1 – – –		

*

a = Blakely's Number
b = Species
c = Number of samples examined
d = Origin (see map, Hillis[6])
e = Leucodelphinidins
f = Leucocyanidins
g = "Leucopelargonidins"
h = Myricetin
i = Quercetin
j = Kaempferol
k = Ellagic acid
l = Unknown compd. *A*
m = Unknown compd. *B*
n = Gallic acid
o = Gentisic acid
p = Caffeic acid
q = *p*-Coumaric acid
r = Sinapic acid
s = Ferulic acid
t = Macrantherin
u = Renantherin
v = Unknown compd. *C*
w = Unknown compd. *D*
x = Taxifolin
y = Aromadendrin
z = Unknown compd. *E*
a = Astringin
b = Rhapontin
c = Piceid
d = Chlorogenic acid
e = *p*-Coumarylquinic acid
f = Unknown compd. *F*
g = Unknown compd. *G*
h = Unknown compd. *H*
i = Unknown compd. *I*
j = Unknown compd. *J*
k = Unknown compd. *K*
m = Average of values given by Hillis.[6]

Numbers indicate relative amounts present.[6]

portion of New South Wales, although *E. phaeotricha* (325) is found in Queensland, *E. baxteri* (337) in South Australia and Victoria, and *E. alpina* (339) in a small area of western Victoria. The atypical *E. obliqua* (362) is found in Victoria, South Australia and Tasmania. Blakely[1] also included in this Series *E. regnans* (369; "mountain ash") and *E. fastigata* (368) which possess fibrous barks that tend to be stringy, although these species are usually included among the Series Fraxinales. Most are medium-sized trees about 80 ft high, although some are less than 20 ft high (*E. cannonii* (333), *E. alpina* (339), *E. camfieldii* (342) and *E. ligustrina* (343)) and others are very tall, namely, *E. cameronii* (322), *E. obliqua* (362), *E. fastigata* (368) and particularly *E. regnans* (369).

All the Pachyphloiae, except four species of rather limited distribution, have been examined. Most of them (Table 1) contain the compound we have named "renantherin" which is more frequently present in this Series than in others of the Renantherae (see later). The unknown compound *C*, which gives a similar colour to renantherin with diazotized *p*-nitroaniline, is present in many species of this Series but has been found in other Sections also.

About half of the species examined contain leucoanthocyanins and apparently these compounds have little taxonomic value in this Series. Myricetin is consistently present in large amounts in *E. regnans* (369) and in smaller amounts in *E. obliqua* (362). The presence of significant amounts of kaempferol in *E. tindaleae* (326), *E. laevopinea* (327) and its variety *minor* (328) is in agreement with their close taxonomic affinity. The consistently medium ratio of ellagic acid and the high ratio of gallic acid in the Prealtae (362–369) may prove, on further investigation, to be a suitable basis for the separation of this subseries.

Smith[3] first noticed that the leaves of *E. macrorhyncha* (331) contained rutin in amounts of about 10 per cent., although values as high as 23 per cent. have been recorded.[4] *E. cannonii* (333) and *E. youmanii* (334) also contain large amounts. Such large amounts of rutin (Table 1) apparently form at the expense of the other polyphenols, so that the biosynthetic pathway leading to ellagic and gallic acids is not completely independent from that of flavonols. Rutin was detected also in two of the samples of *E. eugenioides* (318), in *E. caliginosa* (324), *E. baxteri* (337) and *E. capitellata* (340). With the exception of *E. eugenioides*, Humphreys[4] has found rutin in all the above species and also in *E. blaxlandii* (336) and *E. alpina* (339), whereas our samples of these species did not contain it. Very large amounts of rutin have been observed in certain Western Australian species (unpublished data) and the significance of its presence is not yet evident.

An unkown compound *D* is found only in the renantherous species with the exception of *E. preissiana* (246).[5] Its presence in most of the species of the Pachyphloiae is probably significant and suggests a close relationship with some of the Fraxinales (see below) which contain the compound in large amounts.

The presence of aromadendrin and other features support Blakely's groupings of No. 325, 326, 327 and 328, and also No. 336 and 337 (Table 1).

Most of Blakely's Pachyphloiae are characterized by the presence, usually in large amounts, of one or more distinctive components ("Factors") of very limited distribution. These six Factors (Table 1) are named (except in the case of the Tindaleae Factor) after the species with the lowest Blakely number in which it was detected. Either the Muelleriana or the Eugenioides Factor or both have been found in all but two (*E. tindaleae* (326) and a chemical variety

[3] H. G. SMITH, *J. Proc. Roy. Soc. N.S. Wales* **31**, 377 (1897).
[4] F. R. HUMPHREYS, *Econ. Botany* **18**, 195 (1964).
[5] W. E. HILLIS, *Phytochem.* **5**, 1075 (1966).

of *E. phaeotricha* (325)) of the species examined. It would appear possible that the Pachyphloiae (except the sub-series Prealtae) could have resulted from complex hybridization among species during the early development of the genus. The Muelleriana and the Eugenioides Factors have been found alone in a number of species, but the other four Factors have been detected only in combination. It is tentatively proposed from these results that the species containing one only of the latter four Factors have become extinct and that the Pachyphloiae Series can be divided into a small number of groups, those containing more than one Factor being the result of hybridization during development of the genus. These proposals may require re-appraisal after the examination, by more refined methods, of more extensive collections. This necessity is exemplified by the existence of chemical variations in species No. 318, 324 and 325.

Most of the Factors have distinctive colour reactions which facilitate their detection. After hydrolysis, the Eugenioides Factor (which is sometimes present in very large amounts) is not readily detected in chromatograms freshly sprayed with diazotized *p*-nitroaniline although it is more apparent after storage. It has been chromatographically identified as hydroquinone, and the unhydrolysed Factor as arbutin. The unknown compound *D* and the Tindaleae Factor may be confused as they are not always satisfactorily resolved by the techniques used. However, this will not affect the results in Table 1, as both compounds are always present together, except in one of the varieties of *E. caliginosa* (324) in which the Tindaleae Factor is absent. Identification of the components will further indicate the relationship of the different groups.

Blakely placed *E. blaxlandii* (336) and *E. baxteri* (337) together in his classification, and both possess the distinctive Eugenioides Factor and also the Blaxlandii Factor found only in these two species. In recent times these species have been found only in two isolated areas about 700 miles apart. It would appear they formerly occupied larger contiguous areas, or a species from which they have evolved was formerly spread through this area. Only a trace of the Tindaleae Factor was observed in most samples of *E. macrorhyncha* (331) and the appreciable amounts of the Eugenioides and Muelleriana Factors in *E. cannonii* (333) and *E. youmanii* (334) indicate the possibility that these latter species are derived from ancestral crosses of *E. macrorhyncha* with some other species.

E. obliqua (362), *E. fastigata* (368) and *E. regnans* (369) do not contain the Factors found in the rest of the Pachyphloiae, and on chemical grounds could be included in the Fraxinales Series. The seven samples of *E. obliqua* (362) examined were variable[6] and the average values have been given in Table 1; one of the samples contained the Saligna Factor.

Blakely's Series XXX. Fraxinales

The Fraxinales (or Ashes, 370–389) (Table 2) contain very tall eucalypts (370–371) and small trees of mallee form less than 20 ft high (383, 384, 389) growing in the cool subalpine regions of Tasmania, Victoria and New South Wales. The proportion of persistent rough bark to smooth and deciduous bark varies considerably.

Species of the Prealtae (362–369, Table 1) resemble *E. delegatensis* (370, containing rutin, see also Ref. 4) and *E. sieberi* (371) more than other species in that they contain appreciable amounts of compound *D*, a relatively low amount of ellagic acid and the absence of Factors in large quantities. *E. fraxinoides* (381) shows several affinities with this group. The samples of *E. sieberi* (371) collected from areas 300 miles apart on the mainland showed little difference in composition. On the other hand, those from Tasmania were notably different. In contrast,

[6] W. E. Hillis, *Phytochem.* 5, 541 (1966).

TABLE 2. POLYPHENOLS IN THE LEAVES OF THE FRAXINALES (NO. XXX) AND LONGITUDINALES (NO. XXXI) SERIES OF THE RENANTHERAE SECTION

*a	b	c	d	e 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*	Factor	
				Series XXX. FRAXINALES						
				Subseries lii. Pyriformes						
370.	*E. delegatensis*	(6)	Oo, Ro, Z	– T – – 5 – 2 – – 3 1 – – – –	– – –	3 – – –	– – – – –	3 – – – – –		
371.	*E. sieberi*	(4)	Pp, Oq, Po	2 1 – 5 3 – 3 1 3 5 2 – – – –	– – –	7 – – –	– – – – –	3 – – – – –	Setosa:	T
		(2)	Ro	– – – T 3 – 5 2 4 5 2 – – – –	– – –	9 – – –	– – – – –	7 – – – – –		
373.	*E. consideniana*	(1)	Po	– – – – 3 – 5 3 3 5 1 – – – –	– – –	– – – –	– – – – –	3 3 – – – –		
374.	*E. multicaulis*			Not examined						
375.	*E. remota*	(1)	Ok	2 T – – 2 – 5 – – 5 2 – – – –	– – –	– – T –	– – – – –	1 – 1 – – –	Setosa:	1
				Subseries liii. Costatae						
377.	*E. planchoniana*	(1)	Mr	5 T – T 2 T 3 3 1 5 3 – – – –	– – –	– – – –	– – – – –	1 – – – – –		
				Subseries liv. Platydiscae						
378.	*E. luehmanniana*	(1)	Nq	1 T – 4 2 – 5 3 2 5 2 – – – –	– – –	1 – – –	– – – – –	1 – – – – –		
380.	*E. oreades*	(1)	Z	2 1 – 1 2 – 5 – 2 5 T – – – –	– – –	– – 2 –	– – – – –	1 – 1 – – –	Saligna:	T
				Subseries lv. Urceolares						
381.	*E. fraxinoides*	(1)	Z	3 2 – 2 5 2 3 – – 5 2 – – 1 –	– – –	– – – –	– – – – –	3 – – – – –		
382.	*E. triflora*	(1)	Nq	1 T – 4 1 – 5 2 2 4 T – – – –	– – –	– – – –	– – – – –	3 – 2 – – –	Saligna:	T
383.	*E. obtusiflora*	(1)	Nq	– – – – 3 – 4 4 5 5 2 2 – – –	– – –	– – – –	– – – 3 T	5 – – – – –	Saligna: Obtusiflora:	1 1
384.	*E. stricta*	(2)	Op, Oq	– T – T 5 – 5 3 5 5 2 – – – –	– – –	– – – –	– – – – 2	3 – – – – –	Saligna: Obtusiflora:	2 1
387.	*E. apiculata*			Not examined						
388.	*E. approximans*			Not examined						
				Subseries lvi. Apodae						
389.	*E. kybeanensis*	(1)	Op	2 – – 5 2 – 4 3 3 5 2 – – – –	– – –	– – – –	– – – 3 2	1 – – – – –	Caliginosa:	2
				Series XXXI. LONGITUDINALES						
				Subseries lvii. Claviformes						
394.	*E. pauciflora*	(5)	Po, Op	1 T – 2 5 – 2 – – 5 2 – – – –	– 2 –	T – – 1	– – – 1 1	– – – – – –		
394a.	*E. pauciflora* var. *nana*	(1)	Op	1 1 – 3 5 2 2 – 1 5 2 1 – – 1	– 4 1	T – – –	– – – 1 –	– – – – – –		

396.	*E. debeuzevillei*			Not examined							
397.	*E. niphophila*	(1)	Z	1 T – 2 5 – 1 – 1 5 2 – – – –	– – –	– – – –	– – – – –	– – – – – –	Setosa:	3
				Subseries lviii. Cylindriformes							
398.	*E. stellulata*	(4)	Op	1 1 – 2 4 – 2 1 2 5 2 – – – –	– T –	2 – – 1	– – – – –	1 – – – – –	Setosa:	1
399.	*E. mitchelliana*	(1)	Z	1 1 – 5 2 – 1 – 1 3 2 – – – –	– – –	2 – T –	– – – – –	– – 1 – – –	Tindaleae:	3
									Eugenioides:	T
400.	*E. moorei*	(1)	Z	– T – 2 5 – 2 – 2 5 3 – – – –	– – –	2 – – –	– – – T –	– – 2 – – –	Tindaleae:	2
401.	*E. moorei*[i] var. *latiuscula*	(1)	Z	1 – – 2 5 – 3 1 3 5 1 – – – –	– – 2	2 – – –	– – – 1 –	– – 4 – – –	Tindaleae:	2
									Tetraptera:	2

* See footnote, Table 1.

the mainland and Tasmanian samples of *E. regnans* and *E. delegatensis* showed no difference in composition. *E. consideniana* (373, Victoria) and *E. remota* (375, Kangaroo Island) are closely related chemically.

Leucoanthocyanins are more frequently present in this Series than in the stringybarks, as also is myricetin. Renantherin and compound *C* are absent and the Saligna Factor is present in small amounts. In the majority of species, chlorogenic and *p*-coumarylquinic acids are absent.

Blakely[1] pointed out that *E. planchoniana* (377) has an anomalous environment for this Series but morphologically is closer to this taxon than any other; chemically it is anomalous in that it contains a high ratio of leucodelphinidin, and a low ratio of flavonols and ellagic acid.

The Saligna Factor which is present in small amounts in some species is not very distinctive and is detected only in the Forestal solvent; its presence requires confirmation by other means. The composition of the basic components (columns e–s) and the Factors in *E. kybeanensis* (389) suggest a relationship to *E. sieberi* (371).

Blakely's Series XXXI. Longitudinales

Members of the Longitudinales (Snow Gums, 394–401, Table 2) are small mallees or medium-sized trees with smooth barks growing in the mountainous regions of Tasmania, Victoria and New South Wales. The trends indicated in the Fraxinales are more evident in that the leucoanthocyanins are weakly but consistently present and appreciable amounts of myricetin were found; ellagic acid however is consistently weak. The Claviformes (394–397), which are clineforms,[7] have a very similar polyphenol composition and are separate from the Cylindriformes (398–401) which contain appreciable amounts of unknown compound *D* indicating a possible relationship with some of the ashes. The high ratio of myricetin in *E. mitchelliana* (399) sets it apart from the other snow gums and suggests a possible affinity with the Piperitales.

Blakely's Series XXXII. Piperitales

Members of the Series Piperitales (405–427), which are endemic to Tasmania, Victoria and New South Wales and very occasionally to Queensland, are commonly known as "Peppermints" because of their very fragrant leaf oils containing cineole, phellandrene and piperitone. These small to medium-sized trees usually possess barks that are shorter-fibred than the "stringybarks" and more finely interlaced. The ecological requirements of peppermints are similar to those of the Fraxinales. Although the presence of leucoanthocyanins is not consistent, the trends noticed in earlier series are more noticeable in that myricetin has characteristically and almost uniformly a high ratio and ellagic acid a medium ratio (Table 3). The notable exception is that of *E. dives* (417) which is a typical peppermint morphologically and in essential oil composition, but samples from a number of areas did not contain myricetin and, in addition, the ratio of ellagic acid was high. A significant ratio (2) of myricetin was found in juvenile leaves but no explanation for the anomalous polyphenol composition of the adult leaves from the same trees can be given. *E. coccifera* (418) has a low ratio of myricetin and, in addition, is the only peppermint containing kaempferol; morphologically also this species can be placed apart from the rest of the Series.[8] *E. campanulata* (423) and one of the chemical forms of *E. andrewsii* (422) have a very similar polyphenol composition (Table 3);

[7] L. D. Pryor, *Proc. Linnean Soc. N.S. Wales* **81**, 299 (1956).
[8] L. D. Pryor, Personal communication.

TABLE 3. POLYPHENOLS IN THE LEAVES OF THE PIPERITALES SERIES (NO. XXXII)

*a	b	c	d	e 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*	Factor	
				Subseries lix. Angustifoliae						
405.	*E. linearis*	(2)	Ro, Z	1 T - 5 3 - 3 - - 5 2 - - - -	- - 1	- - - 1	- - - - -	1 - - - - -	Tindaleae:	1
406.	*E. andreana*	(3)	Op, Z	2 2 - 5 2 - 3 T - 5 2 - - - -	- - -	- - - 1	- - - - -	- - 1 - - -		
407.	*E. robertsonii*	(4)	Op, Z	T T - 5 3 - 3 1 - 5 2 - - - -	- - -	- - - T	- - - 1 -	3 - 3 - - -	Tindaleae:	2
408.	*E. amygdalina*	(2)	Ro, Z	T T - 5 3 - 4 1 3 5 2 - - - -	- - T	- - - T	- - - 1 1	3 - - - - -	Tindaleae:	1
									Amygdalina:	2
411.	*E. radiata*	(9)	Pn, On, Po	T 1 - 5 3 - 2 - - 5 2 - - - -	- - T	- - - -	- - - 1 1	- - - - - -	Amygdalina:	1
413a.	*E. radiata* var. *subplatyphylla*	(1)	Lq	- T - 5 5 - 3 - 1 3 1 - - 1 3	- - -	- 3 3 -	- - - - -	T - - - - -	Amygdalina:	2
414.	*E. tenuiramis*			Not examined						
				Subseries lx. Latifoliae						
415.	*E. simmondsii*			Not examined						
417.	*E. dives*	(8)	Oo, Po, Op	- 1 - - 5 - 5 - 3 5 2 - - - -	- - 1	- - - -	- - - 3 3	2 - - - - -		
418.	*E. coccifera*	(2)	Ro, Z	2 2 - 2 5 2 3 - - 5 2 - - - -	- - -	T - - -	- - - 1 -	- - 1 - - -	1 specific cpd.:	4
				Subseries lxi. Connatae						
420.	*E. risdonii*	(2)	Z	4 - - 5 - - 2 - - 5 2 - - - -	- - -	- - - -	- - - - -	1 - - - - -		
		(1)	Ro	1 - - 5 3 - 3 - - 5 3 - - - -	- - -	- - - -	- - - - -	1 - - - - -		
421.	*E. tasmanica*	(2)	Ro, Z	2 1 - 2 5 - 3 - - 5 2 - - - -	- 1 -	- - - 1	- - - - -	- - - - - -	Tindaleae:	1
				Subseries lxii. Paucijugae						
422.	*E. andrewsii*	(1)	Z	- - - 2 5 - 3 3 3 5 1 - - - -	- - -	- - - -	- - - - -	5 3 1 - - -	Obtusiflora:	3
									Tindaleae:	2
		(1)	Lr	- 3 - 2 5 - 3 - - 3 2 - - - -	- - -	- - - T	- - - - -	1 - - - - -	Tindaleae:	7
423.	*E. campanulata*	(2)	Nq	1 2 - 4 5 - 3 - - 5 3 - - - -	- - -	- - - -	- - - - -	1 - - - - -	Tindaleae:	7
427.	*E. piperita*	(1)	Nq	T T - 5 3 - 3 2 - 5 1 - - - -	- - -	- - - -	- - - - -	4 - - - - -	Amygdalina:	7
									Tetraptera:	2
									Tindaleae:	1

* See footnote, Table 1.

the essential oils of these species are also very similar[9] as is also the morphological characters. These two species are considered now to be identical.[10] *E. tasmanica* (421) is found in a few small areas in Tasmania and about 900 miles from the habitat of the above two species to which it is very similar in composition of the basic components and also in the presence of the Tindaleae Factor.

E. linearis (405), *E. andreana* (406), *E. robertsonii* (407) and *E. amygdalina* (408) are very similar chemically (Table 3) and morphologically.[8] On the basis of the Amygdalina Factor, species No. 408 is also closely related to *E. radiata* (411) (and its variety *subplatyphylla*, 413a) and to *E. piperita* (427). The colour reactions and R_f values of this factor are similar to those of the Eugenioides Factor indicating a relationship between the Stringybarks and Peppermints; the presence of the Tindaleae Factor in No. 421, 422 and 423, supports this view. It is noteworthy that *E. radiata* var. *subplatyphylla* (413a) contains large amounts of engelitin (yielding aromadendrin on hydrolysis) and although this compound has been found to be variably present in *E. sideroxylon* (541) leaves,[11] it will be shown in subsequent papers that it can be useful in taxonomic studies.

Blakely's Series XXXIII. Psathyroxyla

The Psathyroxyla or Snappy Gums (431–435) are found in New South Wales and to a limited extent in northern Victoria and southern Queensland. They are medium-sized trees with smooth barks. Chemically (Table 4) *E. racemosa* (434) and *E. rossii* (435) are closely related but differ from *E. haemostoma* (431). The Factors indicate a relationship of the first two species with some of the Pachyphloiae and the Fraxinales.

Blakely's Series XXVI. Pseudo-stringybarks

The Pseudo-stringybarks (306–308) are large trees mainly confined to the east coast of Australia. Although there are chemical differences between *E. pilularis* (306) and *E. muelleriana* (308) they resemble each other in that the ratio of gallic acid, and particularly the flavonols, to ellagic acid is low (Table 4). In these aspects they do not resemble the Pachyphloiae or any other Series. However, the distinctive Muelleriana Factor (possibly a flavonoid C-glycoside) is found also in species No. 327, 328, 336, 345, 346 and 353. *E. muelleriana* (308) has a widespread occurrence and it may be responsible for the gene controlling the formation of this compound because of hybridization with other species during the early development of the genus.

Blakely's Series XXVII. White Mahoganies

The White Mahoganies (311–313) are medium to large trees with a persistent stringy bark and confined to the coastal range of New South Wales and Queensland. Chemically the samples examined differ noticeably but the high ratios of quercetin, ellagic and gallic acids and the presence of renantherin (Table 4) in No. 311 and 313 indicate some affinity with the Pachyphloiae. However, the high ratio of leucodelphinidin, the presence of taxifolin in No. 311, the presence of compound *D* in No. 313, and the absence of Factors in all three samples support the view that these species should be kept separate from the Pachyphloiae.

Blakely's Series XXIII. Diversiformae and Series XXIV. Occidentales

Included in Blakely's Renantherae are four species found in the south-western portion of Australia and ranging in height from 10–20 ft (*E. buprestium*, 301) to 130 ft for *E. marginata*

[9] J. L. WILLIS, H. H. G. MCKERN and R. O. HELLYER, *J. Proc. Roy. Soc. N.S. Wales* 96, 59 (1963).
[10] R. H. ANDERSON, *The Trees of New South Wales*. N.S.W. Govt. Printer Sydney (1956).
[11] W. E. HILLIS and K. ISOI, *Phytochem.* 4, 541 (1965).

TABLE 4. POLYPHENOLS IN THE LEAVES OF THE PSATHROXYLA, PSEUDO-STRINGYBARKS AND WHITE MAHOGANIES SERIES

*a	b	c	d	e 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*	Factor	
				Series No. XXXIII. PSATHROXYLA						
431.	*E. haemastoma*	(1)	Z	– – – – 5 – 5 1 1 5 1 – – 1 –	– – –	– – 2 –	– – – – –	2 3 1 – – –	Tetraptera: Tindaleae:	2 1
434.	*E. racemosa*	(2)	Op, Lq	– 1 – 1 5 – 5 1 5 5 2 – – – –	– – –	– – – T	– – – – –	2 – 1 – – –	Tindaleae: Fastigata:	9 T
435.	*E. rossii*	(2)	Z	– – – 3 4 1 5 2 3 5 1 – – – –	– – –	– – 1 –	– – – – 1	4 3 – – – –	Tindaleae: 1 specific cpd.: Saligna:	7 5 3
				Series No. XXVI. PSEUDO-STRINGYBARKS						
306.	*E. pilularis*	(1)	Z	3 – – 1 1 – 5 3 2 3 2 – – – –	– – –	– – – –	– – – – –	1 1 – – – –		
308.	*E. muelleriana*	(3)	Op	– 1 2 – 1 1 5 2 1 3 1 – – – –	– 3 –	– – – –	– – – 3 –	2 – – – – –	Muelleriana: Setosa:	5 T
				Series No. XXVII. WHITE MAHOGANIES						
311.	*E. umbra* ssp. *umbra*	(1)	Kq	5 T – 1 5 1 3 – – 5 1 – – – –	– 5 –	2 – – –	– – – 1 –	1 1 – – – –		
312.	*E. umbra* ssp. *carnea*	(1)	Lr	2 1 – 3 5 – 5 – – 3 3 – – – –	– – –	– – – –	– – – – –	– – – – – –		
313.	*E. acmenioides*	(1)	Lq	– 3 – – 5 – 5 – – 5 2 – – – –	– 3 1	3 – 1 –	– – – – –	– – – – – –		

* See footnote, Table 1.

TABLE 5. POLYPHENOLS IN THE LEAVES OF THE DIVERSIFORMAE AND OCCIDENTALES SERIES

*a	b	c	d	e 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*	Factor	
				Series No. XXIII. DIVERSIFORMAE						
296.	*E. pachyloma*	(1)	Nd	– – – 5 4 – 4 2 3 5 2 – – – –	– – –	2 – – –	– – – – –	3 2 – – – –	Tindaleae:	1
297.	*E. diversifolia*	(2)	Ol, Z	– – – 5 2 – 5 3 – 5 2 – – 1 –	– 2 1	T – – –	– – – – –	4 – 2 – – –		
298.	*E. todtiana*	(1)	Z	2 1 – 3 T – 5 2 2 5 4 – – – –	– – –	– – – –	– – – – –	2 1 – – – –		
299.	*E. patens*	(1)	Z	1 2 – 5 2 – 5 – – 5 4 – – – –	– – –	– – – –	– – – – –	1 – – – – –		
				Series No. XXIV. OCCIDENTALES						
300.	*E. sepulcralis*	(1)	Z	2 – – 5 2 – 5 2 2 5 3 – – – –	– 3 –	– – – 1	– – – – –	3 – 1 – – –	1 specific cpd.:	3
301.	*E. buprestium*	(1)	Nd	1 – – 5 1 – 3 2 – 5 2 – – – –	– – –	– – – –	– – – – –	3 – – – – –		
303.	*E. staeri*	(1)	Z	T – – 5 3 – 4 3 5 5 T – – – –	– – –	– – – –	– – – – –	3 2 – – – –		
304.	*E. marginata*	(6)	Mc, Nc	3 T – 3 3 – 4 4 3 5 2 – – – –	– T –	– – – –	– – – – –	3 3 – – – –		

* See footnote, Table 1.

(304). The Series Diversiformae is the only Series in the very closely related Section Renantheroideae, and it contains three species found in this region and one (*E. diversifolia*, 297) in South Australia and with the same range in height (12 ft for No. 296; 100 ft for No. 299). The results in Table 5 show that the occurrence and amount of leucoanthocyanins and renantherin varies in a similar way to that found in other renantherous series. However, the high ratio of myricetin and gallic acid, the absence of caffeic, *p*-coumaric, sinapic and ferulic acids show a close resemblance to the Piperitales (Table 3). The ratio of ellagic acid is possibly significantly lower and the ratio of quercetin higher than that found in the Piperitales. Very little information on the essential oil composition of the West Australian group is available so that it is not possible to make further chemical comparisons.

The composition of the polyphenols in the Renantheroideae is very similar to that in the Occidentales of the Renantherae and there is no apparent reason to establish a separate Section. The results support the view of Carr and Carr[12] (based on floral morphology) that the Occidentales (300–304) are possibly akin to the peppermints, but are contrary to their conclusions that *E. todtiana* (298), *E. diversifolia* (297) and *E. patens* (299) are close to the stringybarks. It is interesting that these eight species and the Series Piperitales, which is geographically widely separated, either should retain the same composition of polyphenols as the primitive species from which these groups originated or have evolved separately in a very similar way with respect to these characters.

Anomalous Species in Blakely's Renantherae

It has been pointed out,[5] that the high ratio of myricetin and the presence of renantherin in the West Australian species *E. preissiana* (246), *E. megacarpa* (247) and *E. coronata* (247a) support the view that these species have been incorrectly included in the Globulares. The presence of leucodelphinidin, a low ratio of quercetin, a high ratio of ellagic and gallic acids, and the presence of unknown compounds *D*, *F* and *G* in *E. preissiana* (246) indicate a relationship with the Diversiformae (Table 5). The appreciably higher ratio of compound *D* in this latter species may be indicative of a line of development that differs from the other West Australian species. The lower ratio of ellagic acid and the absence of compound *D* in *E. megacarpa* and *E. coronata* differentiate these species from *E. preissiana* and they may be more closely affiliated with the Occidentales (300–304). However, recent taxonomic studies have shown these species to be wrongly classified,[2,13] and it appears that these species are closely related, belonging to the Diversiformae.

Pryor and Johnson[2,13] consider *E. preissiana* (246) and *E. megacarpa* (247) to be survivors of an evolutionary line which separated before the modification of the anthers which took place in the eastern and western members of the Renantherae.

The traces of macrantherin (Table 6) in *E. guilfoylei* (305), a tree of medium height growing in a small area of Western Australia, suggests an incorrect classification in the Renantherae. This view is supported by the presence of stilbenes (which have not been detected[6] in true members of this Section) and, to a lesser extent, by the large amounts of *p*-coumarylquinic and chlorogenic acids. The unknown compound *J* has not been detected in members of the Renantherae. These conclusions agree with those drawn from studies of seed-coat anatomy[14] and floral morphology.[15]

[12] D. J. CARR and S. G. M. CARR, *Australian J. Botany* **7**, 109 (1959).
[13] L. D. PRYOR and L. A. S. JOHNSON, *Australian J. Botany* **10**, 129 (1962).
[14] E. GAUBA and L. D. PRYOR, *Proc. Linnean Soc. N.S. Wales* **83**, 20 (1958).
[15] D. J. CARR and S. G. M. CARR, *Nature* **184**, 1549 (1959).

TABLE 6. POLYPHENOLS IN THE LEAVES OF ANOMALOUS SPECIES

*a	b	c	d	e 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*	Factor
				Series No. XXV. OCHROXYLON					
305.	*E. guilfoylei*	(3)	Nc, Z	– 2 2 – 3 – 5 – – 4 2 4 1 – –	T – –	– – T –	5 – 1 5 3	1 – 2 – 2 –	
				Series No. XXVIII. STEATOXYLON					
314.	*E. microcorys*	(2)	Mq, Z	– – – – 1 2 5 4 2 5 T – 1 – –	1 – –	– – T –	– – – – –	5 5 – – – –	
				Series No. XXXIV. MYRTIFORMES					
437.	*E. deglupta*	(3)	Do	– 2 – T 5 2 5 2 – 5 1 1 – – –	2 – –	– – T –	– – – 1 1	1 2 1 – – –	Specific cpd.: 3
438.	*E. raveretiana*	(1)	Jq	– 3 – 1 5 2 2 – – 3 2 – 2 – 2	– – –	– – – –	– – – T 1	– – – – – –	
440.	*E. brachyandra*			Not examined					

* See footnote, Table 1.

The presence of macrantherin in the leaves of *E. microcorys* (314), a tree of medium height found on the east coast, also suggests an incorrect classification (cf. the evidence drawn from seed-coat anatomy[14] and floral morphology[12]). The low ratio of flavonols and the unusual proportion of kaempferol to quercetin further show this species to be anomalous.

The presence of appreciable amounts of macrantherin in *E. deglupta* (437), a tall tree found in the islands to the north of Australia, supports the views drawn from seed-coat anatomy[14] and floral morphology[16] that this species is wrongly classified. *E. raveretiana* (438), a tree of medium height found in a small area of Queensland, has been grouped[1, 16] with this species, but the ratios of ellagic and gallic acids are appreciably different.

The Relationship of Polyphenols with Classification

Renantherin has been found only in those species mentioned in this paper. The value of this distinctive compound as a taxonomic marker is limited as it is not invariably present. About 65 per cent of the Pachyphloiae contain renantherin while it is absent or almost so in the Fraxinales, Piperitales and Psathroxyla. The proportion of the species containing this compound in the remaining Series lies between these extremes.

The leucoanthocyanins are erratic in distribution. Most of the Longitudinales contain leucodelphinidins, whereas about one-half the Fraxinales contain these compounds. About one-half of the Pachyphloiae contain either leucodelphinidins or leucocyanidins. Present information indicates that leucoanthocyanins have little taxonomic use in the Renantherae.

In the Renantheroideae and Renantherae Sections there is close agreement between Blakely's classification and the absence or presence of myricetin. Less than 20 per cent of the Pachyphloiae (Table 1) contain myricetin and then only in medium amounts. On the other hand, all the Piperitales, with one exception, contain myricetin and usually in large amounts (Table 3). The Longitudinales occupy a median position. All the West Australian members of these Sections contain medium to large amounts of myricetin. Kaempferol is almost always absent and has little taxonomic value in this part of the classification, although it supports Blakely's association of species No. 325, 326, 327 and 328.

Most of the eucalypt species containing small or medium ratios of ellagic acid are found in the Renantherae. Almost all the species in the Longitudinales (394–401) and Piperitales (405–427) Series are of this type, so that a low amount of ellagic acid can be used as a taxonomic feature. Moreover, most of the species in which the amount of ellagic acid is much lower than that of gallic acid are found here and usually in the Pachyphloiae and Longitudinales.

Chlorogenic and *p*-coumarylquinic acids were found to be absent from most of the renantherous species, particularly those in the Occidentales, Fraxinales and Longitudinales. These acids are usually present in other Sections. Compound *C* is possibly a taxonomically significant compound in the Pachyphloiae (315–369) and compound *D* (found only in the Renantherae) is important in indicating the association of numerous renantherous species.

Although renantherin is a characteristic of the Renantherae, the composition of the polyphenols do not give data which could be used to support or oppose Carr and Carr's thesis[2] that the Renantherae should be treated as a separate genus.

There are great variations in the height of species in the different series but the differences in polyphenol composition are not consistently associated with height. For example in the series Pachyphloiae *E. cannonii* (333) is less than 20 ft high and very similar to *E. macrorhyncha* (331) 80–110 ft; *E. alpina* (339) is less than 20 ft high and very similar to *E. cameronii* (322)

[16] D. J. Carr and S. G. M. Carr, *Nature* **196**, 969 (1962).

which is more than 150 ft. In the Fraxinales Series, *E. sieberi* (371, Tasmanian form) is about 200 ft and similar in composition to *E. stricta* (384) of less than 20 ft height, except for the absence of compound *D* in the latter.

E. sepulcralis (300) is a small willow-like tree with slender drooping branches but this form is not noticeably different in polyphenol composition when compared with other members of the Occidentales. Also the composition of *E. radiata* (411) and its high altitude variety *subplatyphylla* (413a) do not differ appreciably, and neither does that of the snow gums (394–397) and others growing at warmer altitudes.

EXPERIMENTAL

The methods of examination have been reported in Part I of this Series.[5] When more than one sample of a species was examined and the score of a component differed by more

TABLE 7. CHROMATOGRAPHIC PROPERTIES OF SECONDARY EUCALYPT POLYPHENOLS DETECTED IN THE SECTIONS RENANTHEROIDEAE AND RENANTHERAE

Polyphenol	$R_f \times 100$* Solvent F	Be	HA	BA/HA		Appearance†
Factors						
Muelleriana	–	05	75	72	85	s. or. pNA
Eugenioides	90	10	–	83	75	Opaque u.v., bleaches pNA then turns br. after 3 days (hydroquinone)
Eugenioides‡	–	–	–	42	84	Opaque u.v., bleaches pNA then turns red–br. (arbutin)
Tindaleae	91	85	–	89	25	or. pNA
Caliginosa	–	–	77	67	78	y. pNA
Phaeotricha	–	90	–	60	25	Opaque → bu. fl., or. pNA
Phaeotricha‡	–	–	–	58	83	Faint m. u.v. (254 nm), or. pNA
Blaxlandii‡	–	–	–	59 64	19 51	→l. gr. fl. u.v.
Saligna	97	–	–	–	–	y. fl. u.v.
Amygdalina	97	68	69	92	71	Opaque u.v., bleaches pNA
Setosa	90	–	70	55	74	or. pNA
Obtusiflora‡	–	–	–	20	16	m. → l. y. fl. u.v.
Tetraptera	–	–	–	45	28	s. or. y. fl. u.v.
Specific compounds						
300. *E. sepulcralis*	12	–	–	18	02	br.
418. *E. coccifera*	82	–	63	80	65	y. daylight, opaque, u.v., or. pNA
435. *E. rossii*	95	–	–	84	11	l. bu. fl.→s. l. y. fl. u.v.
437. *E. deglupta*	–	–	–	67	78	or. pNA

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

Solvents: F=Forestal solvent (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 first with butanol: acetic acid:water (6:1:2) then 6% acetic acid.

† Appearance: bu.=blue; br.=brown; f.=faint; fl.=fluorescence in u.v. light (365 nm); gr.=green; l.=light; m.=mauve; or.=orange; pNA=*diazotized p-nitroaniline*; s.=strong; y.=yellow; →=fluorescence after exposure to ammonia.

The colours formed with pNA were observed in daylight.

‡ Properties before acid hydrolysis; other data obtained after hydrolysis.

than 2 units the composition of the sample was recorded separately. In other cases the average composition is recorded.

The properties of the compounds not previously recorded[5] are given in Table 7.

An alcohol extract of *E. cameronii* was streaked on to No. 3 Whatman paper and resolved with butanol:acetic acid:water (6:1:2). The band containing the Eugenioides Factor was eluted with methanol and further resolved with 6% acetic acid. Final purification was carried out on layers of silica gel using chloroform:ethyl acetate:formic acid (5:4:1) as solvent. The band (R_f 0·10) was extracted with methanol and after evaporation the residual needles were compared chromatographically with authentic arbutin (Koch-Light) using the above solvents (and chloroform:acetic acid:formic acid (5:4:1) on silica gel) and diazotized *p*-nitro-aniline as chromogenic reagent. The two materials behaved identically. The needles were recrystallized from water, m.p. and mixed m.p. 196–197°. Hydrolysis yielded hydroquinone which was identified by chromatographic comparison.

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