Phytochemistry, 1969, Vol. 8, pp. 1713 to 1727. Pergamon Press. Printed in England

A PHYTOCHEMICAL SURVEY OF THE AUSTRALIAN SPECIES OF *ACACIA*

MARY D. TINDALE

Royal Botanic Gardens and National Herbarium of New South Wales, Sydney, Australia

and

D. G. ROUX

Department of Chemistry, University of the Orange Free State, Bloemfontein, South Africa

(*Received* 28 *November* 1968, *in revised form* 29 *March* 1969)

Abstract—Examination by paper chromatography of the flavonoid content of the heartwoods and barks of sixty-one species of *Acacia* native to Australia, permits broad subdivision into four groups, depending on variations of the phenolic hydroxyl pattern (3′,4′,7-trihydroxy; 4′,7-dihydroxy; 3′,4′,7,8-tetrahydroxy or 4′,7,8-trihydroxy). Although these chemical subdivisions do not correspond with the broad morphological grouping of Australian *Acacia* into Phyllodineae and Bipinnatae, certain correlations are evident. All species of Botryocephaleae and Racemosae contain 3′,4′,7-trihydroxyflavonoids, associated in rare instances with 4′,7-dihydroxyflavonoids. Similarly most Plurinerves and Juliflorae contain 3′,4′,7,8-tetrahydroxyflavonoids, but with notable exceptions amongst certain of the Juliflorae which contain only 4′,7,8-trihydroxyflavonoids.

INTRODUCTION

THE TAXONOMIC significance of the flavonoid mixtures in the heartwoods and barks of three wattles that are closely related to black wattle (*Acacia mearnsii* De Wild., formerly known as *A. mollissima* Willd.) was previously evaluated by Roux *et al.*[1] and by Roux.[2] Recently Clark-Lewis and Dainis[3] summarized current knowledge regarding flavonoid content of heartwoods, leaves and flowers of a group of Australian *Acacia* species belonging mainly to the Phyllodineae.

These earlier studies have now been extended to a wider range of *Acacia* species of Australian origin (except *A. auriculiformis*). These belong to the Phyllodineae and Bipinnatae sect. Botryocephaleae (cf. Refs. [4–7]), comprising most of the exceptionally large group (*ca.* 600 species) native to Australia.

RESULTS AND DISCUSSION

The flavonoid and condensed tannin contents of the heartwood and bark of each species were examined by two-dimensional paper chromatography, using reference compounds

[1] D. G. ROUX, E. A. MAIHS and E. PAULUS, *Biochem. J.* **78**, 834 (1961).
[2] D. G. ROUX, *S. African J. Sci.* **58**, 389 (1962).
[3] J. W. CLARK-LEWIS and I. DAINIS, *Australian J. Chem.* **20**, 2191 (1967).
[4] G. BENTHAM and F. MUELLER, *Flora Australiensis*, p. 301, Lovell Reeve, London (1864).
[5] G. BENTHAM, *Trans. Linn. Soc. Lond.* **30**, 444 (1875).
[6] P. TAUBERT, in *Die natürlichen Pflanzenfamilien* (edited by A. ENGLER and K. PRANTL), Engelmann, Leipzig (1894).
[7] J. H. MAIDEN and E. BETCHE, *A Census of New South Wales Plants*, p. 89, Government Printer, Sydney (1916).

TABLE 1. RELATIVE CONCENTRATIONS OF HEARTWOOD COMPONENTS*

(*a*) 3′,4′,7-*Trihydroxyflavonoid* (*Mollisacacidin*) *Group*

Acacia species	(+)-Mollisacacidin	(+)-Fustin	(−)-Fisetinidol	(−)-Butin	Butein	Fisetin	Bileucofisetinidins	Trileucofisetinidins	High† polymers
1. *A. adunca*	+++++	+(+)				(+)	(+)		
2. *A. baileyana*	+++++	++	(+)	(+)	(+)	(+)			
3. *A. binervata*	+++++	+++(+)	(+)	(+)	(+)				
4. *A. botrycephala*	+++++	+(+)	(+)	(+)	(+)			+(+)	+++++
5. *A. buxifolia*	++	++	+	+++++	(+)	+			
6. *A. calamifolia*	+++++	++	+	(+)	+	(+)	(+)	++	
7. *A. cardiophylla*	+++++	++		+(+)	+	(+)	(+)	+	
8. *A. chrysotricha*	+++++	++(+)	+	(+)	(+)	(+)	+		+++(+)
9. *A. cluniesrossiae*	+++++	+(+)	+	(+)	(+)	(+)	++	++	+++++
10. *A. constablei*	+++++	++	(+)	(+)		(+)	(+)	(+)	
11. *A. dealbata*	+++++	++(+)	(+)	++	(+)	(+)			
12. *A. decora*	+++++	++(+)	(+)	(+)	+	(+)			++
13. *A. decurrens*	+++++								
14. *A. elata*	+++++	+(+)				(+)			++
15. *A. falciformis*	+++++	++(+)	(+)	(+)	(+)	(+)			+++++ ++ +++++
16. *A. filicifolia*	+++++	++	(+)	(+)	(+)	(+)	+(+)	+(+)	+(+)
17. *A. fimbriata*	+++++	(+)	(+)				++(+)		
18. *A. irrorata* ssp. *irrorata*	+++++	+(+)	+	(+)	(+)	(+)		++++	+++++
19. *A. irrorata* ssp. *velutinella*	+++++	++		(+)	(+)	(+)			
20. *A. kettlewelliae*	+++++	+++++		++	++	+			++
21. *A. lanigera*	+++++	+	(+)	(+)		(+)		++	++(+)
22. *A. leucoclada* ssp. *argentifolia*	+++++	+	(+)	(+)	(+)	(+)			(+)
23. *A. leucoclada* ssp. *leucoclada*	+++++	+++	(+)	(+)		(+)	(+)	+++	
24. *A. mabellae*	+++++	++(+)	+		+++	++			+++
25. *A. mearnsii*	+++++	++(+)	(+)	(+)	(+)	(+)			++++
26. *A. mollifolia*	+++++	++	(+)	+		(+)	(+)	+++	
27. *A. o'shanesii*	+++++	++	(+)	(+)	(+)	(+)	++	++	+++++
28. *A. parramattensis*	+++++	++(+)	+(+)	(+)	(+)	(+)	(+)	+++++	++
29. *A. pycnantha*	++	+++++	+(+)	+(+)	(+)	++++			+++++ +++++
30. *A. rubida*	+++++	++(+)	(+)	++(+)	+	++(+)	+(+)	+++	++++
31. *A. silvestris*	+++++	++	+	(+)	(+)	(+)	+++	+(+)	++
32. *A. trachyphloia*	+++++	+(+)	+	+	(+)	(+)	+(+)	+(+)	+

TABLE 1—*Continued*

(*b*) 3′,4′,7-*Trihydroxyflavonoid* + 4′,7-*Dihydroxyflavonoid* (*Mollisacacidin–Guibourtacacidin*) *Group***

	(+)-Mollisacacidin	(+)-Guibourtacacidin	(+)-Fustin	(−)-Fisetinidol	(−)-Butin	Butein	Fisetin	Bileucofisetinidins	Trileucofisetinidins	High† polymers
1. *A. cultriformis*	+++++	+++++	+++	+					+++++	
2. *A. deanei* ssp. deanei	+++++	+(+)	+(+)	+	+	(+)	(+)	(+)	++	+
3. *A. deanei* spp. *paucijuga*	+++++	trace	+	+	(+)	(+)	(+)	+	++	++++
4. *A. neriifolia*	+++++	(+)	+	(+)	(+)	(+)		+(+)	++	+++++
										+++++
5. *A. vestita*	+++++	(+)	+		(+)	+(+)				

(*c*) 3′,4′,7,8-*Tetrahydroxyflavonoid* (*Melacacidin*) *Group*§

	(−)-Melacacidin	(−)-Isomelacacidin	2,3-*trans*-3,4-*cis* Isomer	Dihydroflavonol	3′,4′,7,8-Tetrahydroxyflavanone	Chalcone (okanin)	Flavonol
1. *A. acuminata*	++++	+++++		+++(+)	+++	(+)	++++
2. *A. aneura* var. *aneura*	++++	+++++		++++			++
3. *A. aneura* var. *latifolia*	++++	+++++		++++	(+)		+
4. *A. aulacocarpa*	+++	+++++		+++++	+++		+(+)
5. *A. burrowii*‡	+++++	+++++		+++++	(+)		+++++
6. *A. cheelii*	+++++	+++++	(+)	+++++	+++		+++
7. *A. cunninghamii*	+++++	+(+)	+(+)	++(+)	+++	++	+++
8. *A. doratoxylon*	+++++	++++	(+)	++++	+++	+(+)	++++
9. *A. excelsa*	+++++	+	(+)	++	+	(+)	+++
10. *A. floribunda*	+++++	++	+	+++	+++		+
11. *A. glaucescens*	+++++	++++	(+)	++	++	(+)	+++
12. *A. harpophylla*	+(+)	++		++	++++		+++++
13. *A. holosericea*	+++++	++	+	+	+		++
14. *A. homalophylla*	+++++	++++	(+)	++++	+++	++	+++
15. *A. implexa*	+++++	+		++	+	++	(+)
16. *A. longifolia*	+++++	(+)	++(+)	++++	+		+
17. *A. melanoxylon*	+++++	+++++		++(+)	+		++
18. *A. obtusifolia*	+++++	++(+)	++	+++	+		+
19. *A. oswaldii*	+++++	+++(+)		+++(+)	+++		+++++
20. *A. pendula*	+++++	++++		++++	++++	(+)	++++
21. *A. pubifolia*	+++++	+++++	(+)	++	++		++
22. *A. pycnostachya*	+++++	++(+)	(+)	+++	+++		++
23. *A. rigens*	+++++	+(+)	(+)	+++++	++++		++
24. *A. trineura*	+++++	++		++	+		++
25. *A. verniciflua*	+++++	+++		+	++++		(+)

TABLE 1—*continued*

(*d*) 4′,7,8-*Trihydroxyflavonoid* (*Teracacidin*) *Group*‖

	(−)-Teraca-cidin	(−)-Isoteraca-cidin	2,3-*trans*-3,4-*cis* Isomer	Dihydro-flavonol	4′,7,8-Tri-hydroxy-flavanone	Chalcone	Flavonol
1. *A. auriculiformis*	+++++	++(+)	+	+++			(+)
2. *A. maidenii*	+++++	++	+++++	+(+)			+
3. *A. orites*	+++++	+++	++	++++	(+)		++

* Subdivisions confirmed by generation of anthocyanidins.

† Leucofisetinidin tannins near or on origin.

‡ Associated with five leucocyanidins.

§ Melacacidin or its analogues have been isolated from *A. cambagei*, *A. excelsa* and *A. salicina*.[31, 45] Heartwood flavonols of the same (3′,4′,7,8-tetrahydroxyl) pattern have been identified in *A. flavescens*, *A. havilandii*, *A. oswaldii*, *A. pendula*, *A. shirleyi*.[3]

‖ (−)-Teracacidin, and isoteracacidin have been obtained from *A. orites*.[32, 44] (−)-Teracacidin and 4′,7,8-trihydroxyflavonol from *A. sparsiflora*, *A. maidenii* and *A. obtusifolia*.[3, 44] *A. sparsiflora* also contains melacacidins.

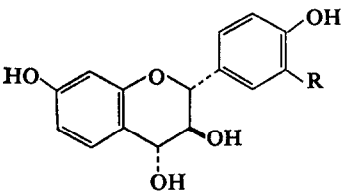
** Considerable variation in the concentration of guibourtacacidin in *A. cultriformis* occurs.

derived from previous studies.[1] The identities of the flavandiols were confirmed and the more complex leucoanthocyanidins characterized by generating anthocyanidins and subsequent identification by paper chromatography. The heartwood and bark components of sixty-one species and eight subspecies or varieties of the genus *Acacia* (mimosaceae) were examined (Tables 1 and 2).

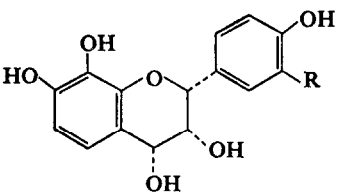
The species are classified according to Maiden and Betche,[7] their scheme (cf. Scheme 1) being essentially based upon that outlined by Bentham[5] but with a few minor variations. With regard to the Australian species of this genus these classifications have stood the test of time reasonably well, being based on strong morphological characteristics which are easily recognizable, although it is evident that some of the minor groups are not defined with a great deal of precision.

The chemical examination of the components of the heartwoods emphasizes that the species examined, although members of different sections, belong to the same genus, since they have the unusual group of 5-deoxyflavonoids in common. However, it will be shown that the chemical evidence in some instances, is at variance with the morphological grouping.

The Australian species of *Acacia* under consideration are classified into two main groups, namely the Phyllodineae and the Bipinnatae sect. Botryocephaleae. In the former the petiole



(I) R = OH, (+)-Mollisacacidin
(II) R = H, (+)-Guibourtacacidin



(III) R = OH, (−)-Melacacidin
(IV) R = H, (−)-Teracacidin

is modified and expanded into a leaf-like organ, the bipinnate foliage persisting in adult trees or shrubs only in rare instances, e.g. *A. rubida* and *A. linearifolia*. In the Botryocephaleae the bipinnate foliage is always present but phyllodes are never formed.

Such grouping is not reflected in the chemical composition of their heartwood and bark components. Thus, four basic patterns of phenolic hydroxylation of heartwood flavonoids based on the pairs 3′,4′,7-trihydroxy and 4′,7-dihydroxy, 3′,4′,7,8-tetrahydroxy and 4′,7,8-trihydroxy, suggest a broad basis for chemical subdivision (Table 1). The heartwood-containing stems and roots are here assumed to be the phylogenetically oldest portions of these *Acacia* (cf. Ref. 8). The most common group of flavonoid types is the 3′4′,7-trihydroxy pattern with the flavan-3,4-diol, (+)-leucofisetinidin or (+)-mollisacacidin (I), as the most prominent representative and precursor of the associated condensed tannins. In four instances, (+)-mollisacacidin is accompanied by the flavan-3,4-diol, probably (+)-guibourtacacidin (II), which gives the anthocyanidin guibourtinidin chloride (X). This represents the first recognition of the rare 4′,7-dihydroxy pattern amongst *Acacia* species. Other isomers of II have hitherto been identified only in *Guibourtia coleosperma* (Rhodesian copalwood) by Saayman and Roux.[9] The next most common type is (—)-melacacidin (III), its stereoisomers and analogues, while (−)-teracacidin (IV), its stereoisomers and analogues occur in separate heartwoods, forming a minor group.

[8] H. Erdtman, *Sci. Proc. R. Dublin Soc.* **27**, 129 (1956).
[9] H. M. Saayman and D. G. Roux, *Biochem. J.* **97**, 794 (1965).

TABLE 2. RELATIVE CONCENTRATIONS OF SOME BARK COMPONENTS, AND LEUCOANTHOCYANIDINS IN *Acacia* SPECIES

(*a*) 3′,4′,7-*Trihydroxyflavonoid* (*Mollisacacidin*) *Group*

	(−)-Robinetinidol	(+)-Catechin	(−)-Epicatechin	(−)-Epicatechin gallate	(+)-Gallocatechin	(−)-Epigallocatechin	(−)-Epigallocatechin gallate
1. *A. adunca**		+ + + + +	+	+ +	+ + +	+	+ + + + +
2. *A. baileyana*	(+)	+ + + + +	+		+ +	(+)	
3. *A. binervata*		+ + + + +			+ + +		
4. *A. botrycephala*	(+)	+ + + + +			(+)		
5. *A. buxifolia**							
6. *A. calamifolia*		+ +	+ + + + +	+	+	+ + + +	
7. *A. cardiophylla*	(+)	+ + + + +			+ +		
8. *A. chrysotricha*	(+)	+ + + + +			+		
9. *A. cunies-rossiae*		(+)	(+)	+(+)	+	+ +(+)	+ + + +
10. *A. constablei*		+ + + +			+		
11. *A. dealbata*	(+)	+ + + + +	+ +		+ +	(+)	
12. *A. decora**							
13. *A. decurrens*	+ +	+ + + + +			+ +		
14. *A. elata*	+ + + + +	+ + + + +			+ +		
15. *A. falciformis*	+ + +(+)	+ + + + +	+ + +		+ + +	+ + +	
16. *A. filicifolia*		+ +			+ + + +		
17. *A. fimbriata*	+ +	(+)	+ + + +		(+)		
18. *A. irrorata* ssp. *irrorata*		+			+ + + +		
19. *A. irrorata* ssp. *velutinella*	+	+			+		
20. *A. kettlewelliae*		+ + + +			+ + + + +		
21. *A. lanigera*		+ + + + +	+		+ + + +	+ +(+)	
22. *A. leucoclada* ssp. *argentifolia*	(+)	+ + + + +			+		
23. *A. leucoclada* ssp. *leucoclada*							
24. *A. mabellae*		+(+)	+ +(+)		+	+ + + + +	
25. *A. mearnsii*	+ +	+ + + + +			+ + +		
26. *A. mollifolia*		+ +	+ + + +		+	+ +	
27. *A. o'shanesii*	(+)	+ + + + +			+ +		
28. *A. parramattensis*	+ +	+ + + + +			+(+)		
29. *A. pycnantha*		+ + +	+ + + + +	+ +	+(+)	+ + +	+ + +
30. *A. rubida**							
31. *A. silvestris*	(+)	(+)			+ + + + +		
32. *A. trachyphloia*	+(+)	+ + + +			+ +		

(*b*) 3′,4′,7-*Trihydroxyflavonoid* + 4′,7-*Dihydroxyflavonoid* (*Mollisacacidin–Guibourtacacidin*) *Group*

1. *A. cultriformis*	(+)	+ + + + +			+ +		
2. *A. deanei* ssp. *deanei*		+ + + + +			(+)		
3. *A. deanei* ssp. *paucijuga*		+	+ + + + +				
4. *A. neriifolia*		+ + (+)			+ + + + +		
5. *A. vestita*		+ + + + +			+		

(*c*) 3′,4′,7,8-*Tetrahydroxyflavonoid* (*Melacacidin*) *Group*

	(+)-Catechin	(−)-Epicatechin	(−)-Epicatechin gallate	(+)-Gallocatechin	(−)-Epigallocatechin	(−)-Epigallocatechin gallate
1. *A. acuminata*						
2. *A. aneura* var. *aneura*	+ + +	+ +		(+)	+ + + + +	
3. *A. aneura* var. *latifolia*	+ + + + +	+ + + + +		(+)	+ + +	
4. *A. aulacocarpa*	+ + + +	+	+ +	+ + +	+	+ + + + +
5. *A. burrowii*	+ +	+ + +		+	+ + + +	
6. *A. cheelii*	(+)	+		+ + (+)	+ + + +	
7. *A. cunninghamii*	+(+)	+ +(+)		+ + +	+ + + +	
8. *A. doratoxylon*	+ + +	+ + + + +	+	+ +	+ + + +	+ +
9. *A. excelsa*						
10. *A. floribunda*	+ + + + +	(+)		+ +	(+)	
11. *A. glaucescens*		+ +	+ +		+ +	+ +
12. *A. harpophylla*						
13. *A. holosericea*	+ + + + +			+ + + + +		
14. *A. homalophylla*	+ + +	+ + + +	+			
15. *A. implexa*	+ +	+ + +		+ + +	+ + + + +	
16. *A. longifolia*	+ + + + +			+ +		
17. *A. melanoxylon*	+(+)	+ + + +		+(+)	+ + + +	
18. *A. obtusifolia*	+ +	+ + + + +		+	+(+)	
19. *A. oswaldii*	+ + + + +	+ +	(+)	+ + + +	+ + +	
20. *A. pendula*	(+)	(+)				
21. *A. pubifolia*	(+)	(+)	+ + + +	+(+)	+ + + + +	
22. *A. pycnostachya*		(+)		+ +	+ + + + +	
23. *A. rigens*	+ + + +	+ + + + +		+ +	+ + + +	
24. *A. trineura*	+ + + + +	+ + + +	+	(+)	+	(+)
25. *A. verniciflua*	(+)	+ + + + +		+	+ +	

(*d*) 4′,7,8-*Trihydroxyflavonoid* (*Teracacidin*) *Group*

1. *A. auriculiformis*
2. *A. maidenii*
3. *A. orites*

* These barks contain major components of unknown structure. The reaction with bis-diazotized benzidine gives the following colours: *A. adunca* (ochre), *A. buxifolia* and *A. decora* (yellow), and *A. rubida* (pale mauve).

† Biflavonol B: Leucorobinetinidin-(+)-catechin; biflavonol D: leucorobinetinidin-(+)-gallocatechin; biflavonol F: leucofisetinidin-(+)-catechin.

TABLE 2—*Continued*

Biflavonols†			Phloroglucinol-derived leucoanthocyanidins	Leucoanthocyanidins‡			
B	D	F		LD	LC	LR	LF
			3	+++++	(+)		
++	+	+	3	++(+)	+++++	(+)	+
++		++++	2	+++	+++++		++
++	(+)	++(+)			+++++		+++++
			5		++++		
+++	+++	++	6	+++++	++(+)	++	(+)
+++++	++	++		+++++	+++++	+	(+)
+	(+)	++		+++++	+++++	+	(+)
			2	+++++			
+++++	+++++	++++		+++++	++	+++++	+
++(+)	+	+	2	++		+++++	+
					+++++		
+++	+++	++		+++++	+++	++	+
++++	+++++			++		+++++	(+)
++++	+++++	+		+++++	+	+++	(+)
			2	++	+++++		+
+++++	+++++	+		++++		+++++	(+)
++				+++++	+++++	(+)	(+)
++++	+++++	++		+++++	+++	+++	+(+)
					++	+++	+
+++	+++++	++++		+++++	++++	+(+)	+(+)
				(+)	++	(+)	++++
+++	++++			+++++	+++	+++	+
+++++	+++	++		++++	(+)	+	+
				+++++	+	+++++	+
+++++	+	++(+)		+++++	++(+)		
+++++	+++	+++++		+++++	+++++	+	+
				+++	+++	+++	+
				+++++	+(+)	++(+)	(+)
			5		+++++		(+)
+++++	+++		2	+++++	+++	(+)	
+++++	+++	+++++		++		+++++	++
+++(+)	+++++	+++				+++++	++
++	++	+	4	+++++	+++++	(+)	(+)
+++			2	+++++	++++	(+)	(+)
			3	+++++			
+++	++++	+		+++++	+++++	+++++	+(+)

Biflavonols†			Phloroglucinol-derived leucoanthocyanidins	Leucoanthocyanidins‡				
B	D	F		LD	LC	LP	LR	LF
			5		+++++			
			8	+++++	+(+)			
			6	+++++	+(+)			
				+++++	+			(+)
			3	+++++	++			
				+++++	(+)			
			6	+++++	(+)		+	(+)
				+++++	+(+)			
			6		+++++	+++++		
			6	+++++	++			
++	+++		5	+++++	++		(+)	+
			2	+++++				
					+++++			
			6		+++++			
			6	+++++	+(+)			
			4	+++++	+			
			6	+++++	++(+)			
			6	+++	++++			
			6	+++++	++++			
			5		++++			
			3	+++++	(+)			
			5	+++++			(+)	
			5	+++++	+(+)			
			6	+++++	+++++			
			5	+(+)	+++++			
			1	++++	+++			
				++++(+)	+++++			
					+++++			

‡ Leucoanthocyanidin concentrations are estimated from the concentrations of anthocyanidins generated: LD=leucodelphinidins, LC=leucocyanidins, LP=leucopelargonidins, LR=leucorobinetinidins, LF=leucofisetinidins.

In every heartwood, the flavan-3,4-diols are accompanied by dihydroflavonol, flavonol, flavan-3-ol (mollisacacidin series only), flavanone, and chalcone analogues. Whereas (+)-mollisacacidin and guibourtacacidin are apparently presented as single isomers, (−)-melacacidin and (−)-teracacidin are accompanied by their 2,3-*cis*-3,4-*trans* isomers, isomelacacidin and isoteracacidin respectively, and in some instances also by the 2,3-*trans*-3,4-*cis* isomer as in *A. auriculiformis*.[10] Polymeric condensed tannins accompany (+)-mollisacacidin, the dimeric bileucofisetinidins XI, XII and XIII having been characterized,[11,12] while trimeric, pentameric, and decameric or undecameric forms are known.[13–15] By contrast (−)-teracacidin (IV) is not associated with tannins (cf. Ref. 10), while those tannins which apparently accompany (−)-melacacidin (III) may be largely *post mortem* oxidation products.

Consideration of the association of mollisacacidin (I) and guibourtacacidin (II) in *A. cultriformis*, *A. deanei*, *A. neriifolia* and *A. vestita* indicates that these compounds are more closely related biogenetically to each other than to either melacacidin (III) or teracacidin (IV). Similarly melacacidin and teracacidin are associated in the heartwood of *A. sparsiflora* showing their close relationship. Although exceptions are known, these examples indicate that, amongst the *Acacia*, variation in the pattern of phenolic hydroxylation of the B-ring occurs more readily than similar variation of the A-ring, and consistency in the latter coupled with variation of the former are extant in heartwoods of other genera of the Leguminosales, for example the association of 4′,7-dihydroxy-, 3′,4′,7-trihydroxy- and 3′,4′,5′,7-tetrahydroxy-flavonoids in *Robinia pseudacacia*.[16] The above suggests that *Acacia* species containing flavonoids with identical A-ring substitution patterns have close chemical and possibly close taxonomic relationships, as illustrated in Scheme 1.

SCHEME 1. FLAVONOID PATTERNS OF HEARTWOODS OF *Acacia* spp. MOSTLY ENDEMIC TO AUSTRALIA

Arranged according to the classification of Maiden and Betche[7] (1916) which is a later modification of Bentham's[4] scheme (1864).

Phyllodineae

Section	Subsection	Species	Flavonoid* pattern
III: Pungentes	ii: Plurinerves	*A. lanigera*	3′,4′,7
IV: Calamiformes		*A. calamifolia*	3′,4′,7
		A. rigens	3′,4′,7,8
VI: Uninerves	iv: Angustifoliae	*A. verniciflua*	3′,4′,7,8
	v: Racemosae	*A. adunca*	3′,4′,7
		A. buxifolia	3′,4′,7
		A. clunies-rossiae	3′,4′,7
		A. cultriformis	3′,4′,7+4′,7
		A. decora	3′,4′,7
		A. falciformis	3′,4′,7
		A. fimbriata	3′,4′,7
		A. kettlewelliae	3′,4′,7
		A. mabellae	3′,4′,7

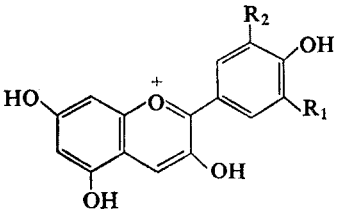
[10] S. E. DREWES and D. G. ROUX, *Biochem. J.* **98**, 493 (1966).
[11] S. E. DREWES, D. G. ROUX, J. FEENEY and S. H. EGGERS, *Chem. Commun.* 368 (1966).
[12] S. E. DREWES, D. G. ROUX, S. H. EGGERS and J. FEENEY, *J. Chem. Soc.* (C), 1217 (1967).
[13] D. G. ROUX and E. PAULUS, *Biochem. J.* **80**, 476 (1961).
[14] D. G. ROUX and E. PAULUS, *Biochem. J.* **82**, 320 (1962).
[15] S. E. DREWES and D. G. ROUX, *Chem. Commun.* 1 (1968).
[16] D. G. ROUX and E. PAULUS, *Biochem. J.* **82**, 324 (1962).

SCHEME 1—*continued*

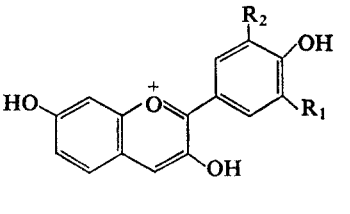
Section	Subsection	Species	Flavonoid* pattern
		A. neriifolia	3′,4′,7+4′,7
		A. pycnantha	3′,4′,7
		A. rubida	3′,4′,7
		A. vestita	3′,4′,7+4′,7
VII: Plurinerves	ii: Oligoneurae	*A. trineura*	3′,4′,7,8
	iii: Microneurae	*A. homalophylla*	3′,4′,7,8
		A. oswaldii	3′,4′,7,8
		A. pendula	3′,4′,7,8
	iv: Nervosae	*A. excelsa*	3′,4′,7,8
		A. harpophylla	3′,4′,7,8
		A. implexa	3′,4′,7,8
		A. melanoxylon	3′,4′,7,8
	v: Dimidiatae	*A. binervata*	3′,4′,7
VIII: Juliflorae	iii: Rigidulae	*A. pubifolia*	3′,4′,7,8
		A. pycnostachya	3′,4′,7,8
	iv: Tetramerae	*A. floribunda*	3′,4′,7,8
		A. longifolia	3′,4′,7,8
		A. obtusifolia	3′,4′,7,8
		A. orites	4′,7,8
	v: Stenophyllae	*A. aneura* var. *aneura*	3′,4′,7,8
		A. aneura var. *latifolia*	3′,4′,7,8
	vi: Falcatae	*A. acuminata*	3′,4′,7,8
		A. aulacocarpa	3′,4′,7,8
		A. auriculiformis	4′,7,8
		A. burrowii	3′,4′,7,8
		A. cheelii	3′,4′,7,8
		A. cunninghamii	3′,4′,7,7
		A. doratoxylon	3′,4′,7,8
		A. glaucescens	3′,4′,7,8
		A. maidenii	4′,7,8
	vii: Dimidiatae	*A. holosericea*	3′,4′,7,8
Bipinnatae			
IX: Botryocephaleae		*A. baileyana*	3′,4′,7
		A. botrycephala	3′,4′,7
		A. cardiophylla	3′,4′,7
		A. chrysotricha	3′,4′,7
		A. constablei	3′,4′,7
		A. dealbata	3′,4′,7
		A. deanei ssp. *deanei*	3′,4′,7+4′,7
		A. deanei ssp. *paucijuga*	3′,4′,7
		A. decurrens	3′,4′,7
		A. elata	3′,4′,7
		A. filicifolia	3′,4′,7
		A. irrorata ssp. *irrorata*	3′,4′,7
		A. irrorata ssp. *velutinella*	3′,4′,7
		A. leucoclada ssp. *leucoclada*	3′,4′,7
		A. leucoclada ssp. *argentifolia*	3′,4′,7
		A. mearnsii	3′,4′,7
		A. mollifolia	3′,4′,7
		A. o'shanesii	3′,4′,7
		A. parramattensis	3′,4′,7
		A. silvestris	3′,4′,7
		A. trachyphloia	3′,4′,7

* Phenolic hydroxylation patterns of heartwood components correspond to the following groups: Mollisacacidin (3′,4′,7-trihydroxy); Guibourtacacidin (4′,7-dihydroxy); Melacacidin (3′,4′,7,8-tetrahydroxy); Teracacidin (4′,7,8-trihydroxy).

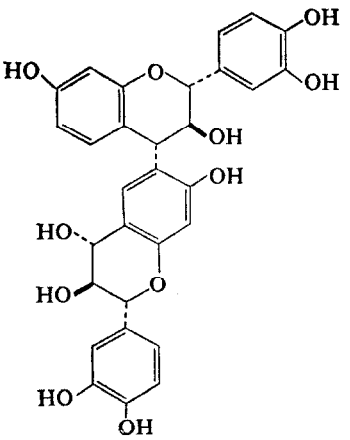
Barks contain far more complex flavonoid mixtures than the corresponding heartwoods (cf. *A. mearnsii*,[17]). The action of hydrochloric acid on bark extractives usually gives complex mixtures of anthocyanidins amongst which delphinidin (V), cyanidin (VI), pelargonidin (VII), robinetinidin (VIII), and fisetinidin (IX) chlorides may be recognized by chromatographic means (Table 2). Broad differences exist between the barks of those species which contain 3′,4′,7-trihydroxyflavonoids [predominantly (+)-mollisacacidin], 3′,4′,7,8-tetrahydroxyflavonoids [(−)-melacacadin] and 4′,7,8-trihydroxyflavonoids [(−)-teracacidin] in their heartwoods (Table 2). Barks of species of the first-mentioned group contain (+)-catechin (XIV), (+)-gallocatechin (XV), their respective (−)-epimers (XVI, XVII) and rarely (−)-



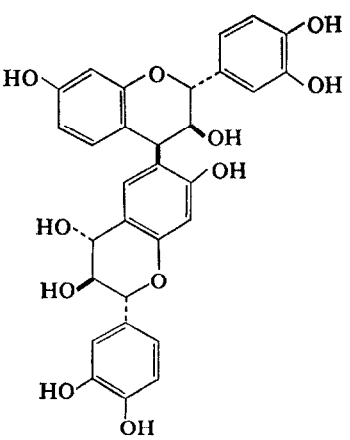
(V) $R_1 = R_2 = OH$, Delphinidin
(VI) $R_1 = OH$, $R_2 = H$, Cyanidin
(VII) $R_1 = R_2 = H$, Pelargonidin



(VIII) $R_1 = R_2 = OH$, Robinetinidin
(IX) $R_1 = OH$, $R_2 = H$, Fisetinidin
(X) $R_1 = R_2 = H$, Guibourtinidin



(XI)



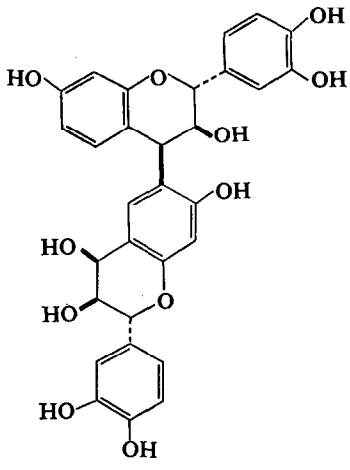
(XII)

epimer gallates (XVIII, XIX), as well as bimolecular leucorobinetinidin (D, B) and bimolecular leucofisetinidin (F) tannins (XXI–XXIII). The bimolecular flavonoids B, D and F are absent from barks of species of the (−)-melacacidin group, but the catechins remain prominent. Both leucorobinetinidins, leucofisetinidins and the catechins are absent from barks of species of the (−)-teracacidin group. Bark flavonoid content seems to be influenced to some degree by heartwood flavonoid content, although exceptions to the above exist among the (+)-mollisacacidin group.

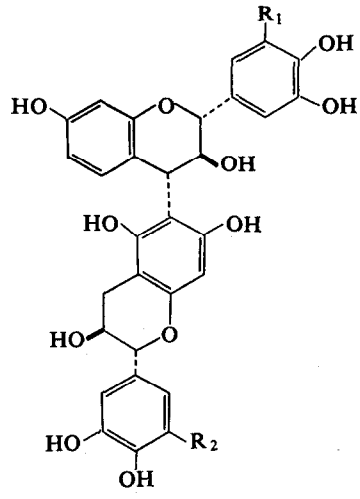
Examination of the bark content of the (+)-mollisacacidin and (+)-mollisacacidin-guibourtacacidin groups (Table 2) indicates that the following are closely related on a

[17] S. E. DREWES and D. G. ROUX, *Biochem. J.* **87**, 167 (1963).

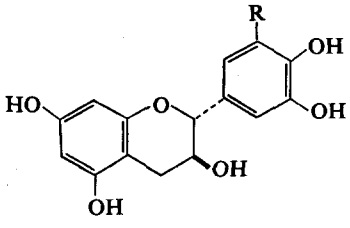
chemical basis to *A. mearnsii* (cf. Ref. 1) from their relatively high content of leucorobinetinidins and leucofisetinidins: *A. constablei*, *A. cultriformis*, *A. dealbata*, *A. decurrens*, *A. elata*,



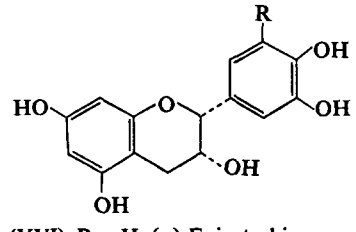
(XIII)



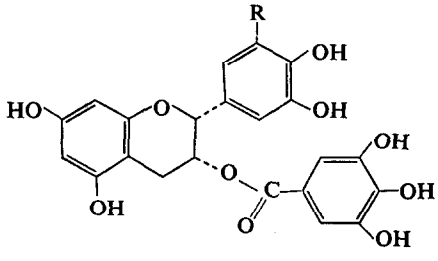
D. (XXI) $R_1 = R_2 = OH$, Leucorobinetinidin-(+)-gallocatechin
B. (XXII) $R_1 = OH$, $R_2 = H$, Leucorobinetinidin-(+)-catechin
F. (XXIII) $R_1 = R_2 = H$, Leucofisetinidin-(+)-catechin



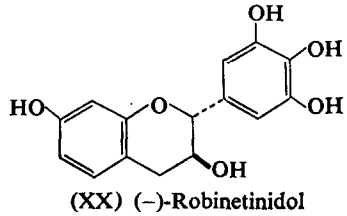
(XIV) R = H, (+)-Catechin
(XV) R = OH, (+)-Gallocatechin



(XVI) R = H, (−)-Epicatechin
(XVII) R = OH, (−)-Epigallocatechin



(XVIII) R = H, (−)-Epicatechin gallate
(XIX) R = OH, (−)-Epigallocatechin gallate



(XX) (−)-Robinetinidol

A. falciformis, *A. fimbriata*, *A. irrorata* ssp. *velutinella*, *A. kettlewelliae*, *A. lanigera*, *A. leucoclada*, *A. parramattensis*, *A. pycnantha*, *A. trachyphloia* and *A. vestita*. Most of these also show the presence of (−)-robinetinidol (XX), a flavan-3-ol related to the leucorobinetinidins. Also amongst the (+)-mollisacacidin and (+)-mollisacacidin-guibourtacacidin

groups are those species the barks of which do not yield leucorobinetinidins or leucofisetinidins, and are therefore chemically closer in bark content to the (−)-melacacidin group on account of their residual leucodelphinidin and leucocyanidin content: *A. adunca*, *A. buxifolia*, *A. clunies-rossiae*, *A. decora*, *A. mollifolia*, and *A. neriifolia* (cf. Table 2).

With minor exceptions, the barks of the (−)-melacacidin and (−)-teracacidin groups (Table 2) lack leucorobinetinidin and leucofisetinidin. The former contain mainly leucodelphinidins, leucocyanidins and, in one instance, *A. excelsa* leucopelargonidins. Monomeric forms of these structural types are particularly common in barks of the (−)-melacacidin group. By contrast, barks of the (−)-teracacidin group lack significant amounts of the catechins (XV)–(XVIII).

The bark and heartwood components of eighteen species and six subspecies of the economically important section Botryocephaleae were examined. This section is characterized by bipinnate foliage, globular inflorescences and non-spiny inflorescences. With the exception of *A. botrycephala* and *A. elata* which have larger pinnules, the other species under consideration belong to a closely allied group listed in Scheme 1. The heartwood components of these members of the Botryocephaleae are almost identical.

Their affinities are also reaffirmed by the bark analysis which has been approached from two angles, firstly chromatographic examination and secondly the conversion of the tannins into anthocyanidins and the study of the latter by paper chromatography (cf. Table 2). The first method shows that *A. mearnsii*, *A. decurrens*, *A. constablei*, *A. trachyphloia*, *A. irrorata* ssp. *velutinella* and *A. parramattensis* are closely allied, also that *A. silvestris*, *A. irrorata* and *A. filicifolia* as well as *A. o'shanesii*, *A. chrysotricha*, *A. botrycephala*, *A. cardiophylla* and *A. leucoclada* ssp. *argentifolia* form small groups almost equally removed from the *A. mearnsii* group. There is increasing divergence in *A. dealbata* and *A. baileyana* which are a pair of closely related species. The second method reaffirms the close similarity between the first six species cited and suggests that *A. o'shanesii* is related to them but the other species are allied but more removed.

The chemical evidence from both the bark and heartwoods (cf. Tables 1 and 2) reaffirms the alliance of some morphologically similar groups e.g. *A. decurrens–A. constablei–A. mearnsii–A. parramattensis*, *A. dealbata–A. baileyana* and *A. filicifolia–A. silvestris*. However it separates the closely allied *A. irrorata* ssp. *irrorata* and ssp. *velutinella*, *A. o'shanesii* and *A. trachyphloia* as well as grouping together the more divergent *A. chrysotricha*, *A. o'shanesii* and *A. leucoclada* ssp. *argentifolia* with *A. botrycephala*.

Other species of *Acacia* examined for bark and heartwood content belong to the Phyllodineae, to which the majority of the Australian species of this genus belong. There are eight sections in the Phyllodineae, species being examined from the following five sections, namely the Pungentes, Calamiformes, Uninerves, Plurinerves and Juliflorae (cf. Scheme 1). In the first four sections the inflorescences are globular as in the Botryocephaleae, whereas in the Juliflorae they are spike-like or rarely shortly oblong.

This morphological subdivision correlates with the observation that the Juliflorae (exclusively melacacidin and teracacidin group) differ in heartwood content from the Botryocephaleae (exclusively mollisacacidin and guibourtacacidin), whereas species from the remaining sections of the Phyllodineae contain both main groups of heartwood components (melacacidin and mollisacacidin).

Generalizations cannot be made about the first two sections of the Phyllodineae as an examination was made of the bark and heartwood components of only one species of the Pungentes, namely *A. lanigera*, which is in the mollisacacidin series (cf. Scheme 1). Two

species of the section Calamiformes were examined, namely *A. calamifolia* and *A. rigens*, the former being in the mollisacacidin series and the latter in the melacacidin series.

In section Uninerves the only member of the subsection Angustifoliae examined, namely *A. verniciflua*, is referred to the melacacidin group, whereas in the ten species of subsection Racemosae listed (Scheme 1) the heartwood and bark components are of the mollisacacidin group. Whereas *A. falciformis* agrees fairly well with *A. dealbata* and *A. baileyana* in the heartwood components and analysis of the bark, *A. pycnantha* has the same heartwood components as the *A. decurrens* group, but the bark analysis shows that there is an increasing divergence from the other species, although it is closest to the *A. falciformis–A. dealbata–A. baileyana* group. However, in another three species of the subsection Racemosae, namely *A. cultriformis*, *A. neriifolia* and *A. vestita*, the heartwood and bark components belong to the very closely related mollisacacidin–guibourtacacidin group.

Section Plurinerves is well represented in this investigation by species from four subsections, i.e. the Oligoneurae, Microneurae, Nervosae and Dimidiatae. The bark and heartwood of representatives of the first three subsections belong to the melacacidin series (cf. Scheme 1). However, in *A. binervata*, belonging to the subsection Dimidiatae, the heartwood components are identical with the *A. decurrens* group, i.e. in the mollisacacidin group, but the analysis of the bark shows that there is some divergence from them. Species in the latter subsection are morphologically dissimilar from those in the three other subsections.

Examination was also made of a number of species of the Juliflorae, a section characterized by inflorescences which are spike-like or rarely, when sessile, shortly oblong. They are members of five different subsections, viz. the Rigidulae, Tetramerae, Stenophyllae, Falcatae and Dimidiatae, within which the chemical components of closely allied species is fairly consistent. The heartwood and bark components of most of the species examined in this section belong to the melacacidin group and the remainder to the related teracacidin group, namely *A. auriculiformis* and *A. maidenii* (subsection Falcatae) and *A. orites* (subsection Tetramerae). In addition *A. sparsiflora* Maiden (subsection Falcatae) contains (−)-teracacidin and melacacidins, thus emphasizing the close relationship between the latter.

Although only fifteen of the 150 species in the Juliflorae have been studied, the chemical evidence based on the analysis of the heartwood components suggests that, in a new classification of the genus, greater emphasis should perhaps be placed on the different type of inflorescence occurring in the Juliflorae, as opposed to the globular heads found both in the Botryocephaleae and the other seven sections of the Phyllodineae. Maiden and Betche[7] transferred certain species with cylindrical inflorescences to the Juliflorae from the Continuae and Pungentes where Bentham and Mueller[4] had placed them.

Recent cytological and pollen analytical studies[18–26] have indicated that the Phyllodineae and Botryocephaleae appear to be closer in affinities than is apparent in Bentham's, Taubert's

[18] P. GUINET, *C.R. Acad. Sci. Paris* **258**, 4823 (1964).
[19] I. C. COOKSON, *Australian J. Botany* **2**, 52 (1954).
[20] C. D. DARLINGTON and E. K. JANAKI-AMMAL, *Chromosome Atlas of Cultivated Plants*, Allen and Unwin, London (1945).
[21] E. ATCHISON, *Am. J. Botany* **35**, 651 (1948).
[22] C. D. DARLINGTON and A. P. WYLIE, *Chromosome Atlas of Flowering Plants*, p. 151, Allen and Unwin, London (1955).
[23] A. J. PRITCHARD and K. F. GOULD, *Div. Trop. Pastures Tech. Pap. C.S.I.R.O.* No. 2, 8 (1964).
[24] B. G. BRIGGS, in *Contrib. N.S.W. Nat. Herb.* **3**, 127 (1962).
[25] B. G. BRIGGS, in *Contrib. N.S.W. Nat. Herb.* **4**, 56 (1966).
[26] B. G. BRIGGS, in *Proc. Linn. Soc. N.S.W.* **91**, 148, 150 (1966).

and Maiden and Betche's classifications. The chemical data certainly support this assumption, especially as the heartwood components mollisacacidin (I), guibourtacacidin (II), melacacidin (III) and teracacidin (IV) and their analogues are of the relatively rare 5-deoxyflavonoid type.

EXPERIMENTAL

Origins and Preparation of Samples

Samples were collected mainly by Messrs. E. F. Constable, D. J. McGillivray and A. N. Rodd, and Dr. Mary Tindale of the Royal Botanic Gardens, Sydney, N.S.W., Australia, as well as by Messrs. J. A. Brennan, E. Chiswell, A. G. Floyd and R. J. Turner, and Miss Joyce Lanyon of the Forestry Commission of New South Wales, and Dr. Elizabeth Phillips of the Botanic Gardens, Canberra, A.C.T. These were obtained in South Eastern Queensland and on the coast, tablelands, western slopes and western plains of New South Wales. Special collections of *Acacia cultriformis* were by Mr. B. Scott, of *A. cheelii* by Mr. W. Mactier and of *A. leucoclada* ssp. *leucoclada* by Mr. R. Cameron, whereas the Forestry Service of Western Australia supplied *A. acuminata*. Other special collections were by Dr. J. H. Leigh and Mr. W. E. Mulham. *A. auriculiformis* was kindly supplied by Mr. I. D. Nicholson, Sandakan Forest Department, Sandakan, Sabah, Malaysia. *A. mearnsii*, *A. decurrens* and *A. dealbata* were collected by Mr. S. P. Sherry of the Wattle Research Institute, Pietermaritzburg, and *A. pycnantha* by the Government Forester, Cape Town, from plantations at Elsies River, Cape, and by Mr. R. H. Kuchel, State Herbarium of South Australia, at Belair, South Australia.

The following were examined for flavonoid components of the mature heartwood and bark: *A. acuminata* Benth. (NSW 100988), *A. adunca* A. Cunn. ex G. Don (NSW 101290), *A. aneura* F. Muell. ex Benth. var. *aneura* (NSW 95677), *A. aneura* F. Muell. ex Benth. var. *latifolia* J. M. Black (NSW 95670), *A. aulacocarpa* A. Cunn. ex Benth. (NSW 101047), *A. auriculiformis* A. Cunn. ex Benth., *A. baileyana* F. Muell., *A. binervata* DC. (NSW 53517), *A. botrycephala* (Vent.) Desf. (NSW 53879, 97448), *A. burrowii* Maid. (NSW 95625), *A. buxifolia* A. Cunn. (NSW 101290), *A. calamifolia* Sweet ex Lindl. (NSW 101252, 101322), *A. cardiophylla* A. Cunn. ex Benth. (NSW 101336, 101234), *A. cheelii* Blakely (NSW 95626, 100985), *A. chrysotricha* Tindale (NSW 34451), A. *cluniesrossiae* Maid. (NSW 75426), *A. constablei* Tindale MS. (NSW 78964), *A. cultriformis* A. Cunn. ex G. Don (NSW 101238, 101494), *A. cunninghamii* Hook. (NSW 84077), *A. dealbata* Link (NSW 101327), *A. deanei* (R. T. Bak.) Welch, Coombs et McGlynn ssp. *deanei* (NSW 95444, 101329), *A. deanei* (R.T. Bak.) Welch, Coombs et McGlynn ssp. *paucijuga* (F. Muell. ex N.A. Wakef.) Tindale (NSW 101230), *A. decora* Reichb. (NSW 101279, 101261, 101316), *A. decurrens* (J. Wendl.) Willd. (NSW 97449), *A. doratoxylon* A. Cunn. (NSW 101235, 101259, 101273, 101233, 101334), *A. elata* A. Cunn. ex Benth. (NSW 91604), *A. excelsa* Benth. (NSW 101267), *A. falciformis* DC. (NSW 55272, 95439), *A. filicifolia* Cheel et Welch (NSW 53945, 97076, 55298), *A. fimbriata* A. Cunn. ex G. Don (NSW 95440, 101295, 95443, 101283), *A. floribunda* (Vent.) Willd. (NSW 82738, 101286), *A. glaucescens* Willd. (NSW 92964), A. *harpophylla* F. Muell. ex Benth. (NSW 84520, 101274, 95674), *A. holosericea* A. Cunn. ex G. Don (NSW 84923), *A. homalophylla* A. Cunn. ex Benth. (NSW 95442, 95438, 101260), *A. implexa* Benth. (NSW 84005, 101321, 84243), *A. irrorata* Sieber ex Spreng. ssp. *irrorata* (NSW 97080, 53896), *A. irrorata* Sieber ex Spreng. ssp. *velutinella* Tindale (NSW 53516), *A. kettlewelliae* Maid. (NSW 82139), *A. lanigera* A. Cunn. (NSW 101263), *A. leucoclada* Tindale ssp. *argentifolia* Tindale (NSW 63198, 72116), *A. leucoclada* Tindale ssp. *leucoclada* (NSW 101482), *A. longifolia* (Andr.) Willd. (NSW 101512), *A. mabellae* Maiden (NSW 90346, 90350), *A. maidenii* F. Muell. (NSW 82137), *A. mearnsii* De Wild. (NSW 53870, 97079, 85022), *A. melanoxylon* R. Br. (NSW 55275, 53618), *A. mollifolia* Maiden et Blakely (NSW 101271), *A. neriifolia* A. Cunn. ex Benth. (NSW 95437, 95441, 93663, 93996). *A. obtusifolia* A. Cunn. (NSW 78911, 55274), *A. orites* Pedley (NSW 78909), *A. o'shanesii* F. Muell. et Maid. (NSW 97077, 53899), *A. oswaldii* F. Muell. (NSW 84078, 101399), *A. parramattensis* Tindale (NSW 97447, 101489), *A. pendula* A. Cunn. ex G. Don (NSW 101268, 101287), *A. pubifolia* Pedley (NSW 95436), *A. pycnantha* Benth. (NSW 101457, 90347), *A. pycnostachya* F. Muell. (NSW 101289), *A. rigens* A. Cunn. ex G. Don (NSW 101330, 101347, 101254, 101253), *A. rubida* A. Cunn. (NSW 101281), *A. silvestris* Tindale (NSW 53778, 85026), *A. trachyphloia* Tindale (NSW 53897, 55299, 97078), *A. trineura* F. Muell. (NSW 101240), *A. verniciflua* A. Cunn. (NSW 101272, 101335) and *A. vestita* Ker (NSW 101200).*

The air-dried bark was ground to powder in a Wiley mill. The powder (3 g) was covered with methanol (10 ml) and extracted for 24 hr at room temperature (solution A). The extract was decanted and 45 ml dry ether added. The tannin precipitate was filtered off and the filtrate taken to dryness under reduced pressure (solid A). Heartwoods were sampled by drilling. The drillings (3 g) were covered with methanol (10 ml) and the mixture kept for 24 hr at room temperature (solution B).

* Herbarium voucher specimens are located at the National Herbarium of New South Wales, Royal Botanic Gardens, Sydney. Duplicates of most of these are at the Herbarium, Royal Botanic Gardens, Kew, and at the Queensland Herbarium, Indooroopilly, Queensland.

Chromatographic Examination of Bark and Heartwood Extracts

The methanolic extracts of the heartwoods (solution B) (0·4 ml) were applied to two-dimensional paper chromatograms (Whatman No. 1, 42·5 × 28 cm). The chromatograms were developed first in water-satd. butan-2-ol (25 cm migration) and then in 2% acetic acid (36 cm). They were sprayed with ammoniacal $AgNO_3$, and the colours rendered permanent by washing three times with distilled water, then with very dilute (0·01%) sodium thiosulphate, and finally with tap water.

Compounds were identified (Table 1) by their concurrence in R_f with authentic specimens.[11,12,17,27–32] Use of the spray reagents, toluene-*p*-sulphonic acid,[33] *bis*-diazotized benzidine[34] and $NaBH_4$–$AlCl_3$[35] assisted in these identifications.

The methanolic extracts of barks (solid A, 0·5–2 mg), from which tannins had been removed by precipitation with ether, were dissolved in methanol (0·2 ml) and examined by paper chromatography as above. Duplicate chromatograms were sprayed with *bis*-diazotized benzidine and washed with distilled water to preserve the rich ochre colours of the phloroglucinol-derived catechins and flavan-3,4-diols. Other chromatograms were sprayed with vanillin-toluene-*p*-sulphonic acid[36] and ferric alum.[37] The relative concentrations of the bark constituents[17,27,38,39] are recorded in Table 2.

Generation of Anthocyanidins from Bark and Heartwood Components

The methanolic extract of the bark (solution A, 0·25–0·5 ml) was transferred to a pressure tube, propan-2-ol (4 ml) and 3 N HCl (1 ml) added, and the enclosed mixture heated for 1 hr on a steam bath according to the method of Pigman *et al.*[40] The mixtures of anthocyanidins formed were examined by paper chromatography on Whatman No. 1 paper using 3 N HCl–90% (w/w) formic acid (1:1 v/v) mixture.[41,42] The R_fs of the anthocyanidins, their absorption maxima, and shifts produced on complexing with $Al_2(SO_4)_3$ were compared with those of authentic reference compounds.[41,43] The anthocyanidins generated correlated with the presence of flavan-3,4-diols of the 3′,4′,7-trihydroxy, 4′,7-dihydroxy, 3′,4′,7,8-tetrahydroxy and 4′,7,8-trihydroxy patterns as in Table 1. Anthocyanidin mixtures from the barks were more complex, delphinidin, cyanidin, pelargonidin, robinetinidin and fisetinidin being recognized, as indicated in Table 2.

[27] D. G. Roux and E. A. Maihs, *Biochem. J.* **74**, 44 (1960).
[28] D. G. Roux and E. Paulus, *Biochem. J.* **77**, 315 (1960).
[29] D. G. Roux and E. Paulus, *Biochem. J.* **78**, 120 (1961).
[30] D. G. Roux and E. Paulus, *Biochem. J.* **80**, 62 (1961).
[31] J. W. Clark-Lewis and P. I. Mortimer, *J. Chem. Soc.* 4106 (1960).
[32] J. W. Clark-Lewis, G. F. Katekar and P. I. Mortimer, *J. Chem. Soc.* 499 (1961).
[33] D. G. Roux, *Nature* **180**, 973 (1958).
[34] G. Lindstedt, *Acta Chem. Scand.* **4**, 448 (1940).
[35] B. H. Koeppen, *J. Chromatogr.* **18**, 604 (1965).
[36] E. A. H. Roberts, R. A. Cartwright and D. J. Wood, *J. Sci. Food Agr.* **7**, 637 (1956).
[37] D. G. Roux and E. A. Maihs, *J. Chromatogr.* **4**, 65 (1960).
[38] S. E. Drewes, D. G. Roux, H. M. Saayman, J. Feeney and S. H. Eggers, *Chem. Commun.* 370 (1966).
[39] S. E. Drewes, D. G. Roux, H. M. Saayman, S. H. Eggers and J. Feeney, *J. Chem. Soc.* (C) 1302 (1967).
[40] W. Pigman, E. Anderson, R. Fischer, M. A. Buchanan and B. L. Browning, *Tappi* **36**, 4 (1953).
[41] D. G. Roux, *Nature* **199**, 305 (1957).
[42] D. G. Roux and S. R. Evelyn, *Biochem. J.* **69**, 530 (1958).
[43] T. Geissman and L. Jurd, *Archs Biochem. Biophys.* **56**, 259 (1955).
[44] J. W. Clark-Lewis and I. Dainis, *Australian J. Chem.* **17**, 1170 (1964).
[45] J. W. Clark-Lewis and V. Nair, *Australian J. Chem.* **17**, 1164 (1964).