

A CHEMOTAXONOMIC STUDY OF THE  
NEW ZEALAND ARALIACEAE : PHENOLIC COMPOUNDS

NANCY NG and J.R.L. WALKER

Department of Botany, University of Canterbury  
Christchurch, New Zealand

ABSTRACT

A preliminary chromatographic survey of phenolic compounds present in the leaves of New Zealand species of Araliaceae was attempted. With the exception of *Pseudopanax anomalum*, both woody and herbaceous species exhibited similar basic patterns of phenolic constituents. Contrary to previous reports leucoanthocyanidins were detected in some, but not all, woody species of Araliaceae. It was of special interest to find that the juvenile leaves of *P. simplex* showed a higher concentration of leucoanthocyanidin than did the adult leaves.

INTRODUCTION

Higher plants exhibit an enormous variation in the chemical manifestations of their secondary metabolism and, during the past two decades, systematists have made increasing use of phytochemical studies as an aid to classification. The rationale of biochemical systematics has been discussed by Swain (1963, 1966) and Harborne (1967, 1973), who have shown that phenolic and flavonoid compounds fulfil most of the requirements for taxonomic markers. Moreover, these compounds are readily separated and identified by relatively simple techniques of chromatography and UV spectroscopy (Harborne 1973). To date relatively little work has been done on the chemosystematics of New Zealand plants and this paper reports a preliminary study of the phenolic constituents of the New Zealand Araliaceae.

The Araliaceae are represented in New Zealand by three small genera: *Meryta* (1 sp.), *Schefflera* (1 sp.) and *Stilbocarpa* (3 spp.) together with the larger genus *Pseudopanax* which contains fourteen species. Philipson (1971) sub-divided the latter genus on a basis of shoot characteristics into three main groups as follows:

- Group I: *P. arboreum* ("five-finger"), *P. colensoi*,  
*P. mackintyreii*, *P. laetum*.  
Group IIa: *P. gilliesii*\*, *P. lessorii*, *P. discolor*.  
Group IIb: *P. crassifolium* ("lancewood"), *P. ferox*,  
*P. chathamicum*, *P. lineare*.  
Group IIc: *P. simplex*, *P. edgerleyi*\*.  
Group III: *P. anomalum*.

\*not available for this investigation.

The aim of the studies reported here was to attempt to examine the distribution of the phenolic constituents within these species as a possible support for the above classification.

## MATERIALS AND METHODS

### PLANT MATERIAL

Leaf material from twelve of the species of *Pseudopanax* listed above were investigated together with samples of *Meryta sinclairii*, *Schefflera digitata*, *Stilbocarpa polaris* and *S. robusta*. All plant material was preserved by freeze-drying in an Edwards (U.K.) 30P2 centrifugal freeze-dryer.

### EXTRACTION OF PHENOLIC COMPOUNDS

Freeze-dried leaf material was homogenised in 70% (v/v) methanol containing 1% conc. HCl and extracted for two hours. The extract was filtered through Whatman No. 1 paper, and extracted with a small quantity of petroleum ether to remove chlorophylls and carotenoids. It was then concentrated in a rotary vacuum evaporator.

Most flavonoid and phenolic compounds are present in plants as a multiplicity of glycosides and other derivatives so it is normal practice, in the initial stages of a phytochemical survey, to hydrolyse the crude extract so that only the parent phenolic aglycones are present. This was achieved by refluxing the concentrated extract with an equal volume of 2N-HCl for one hour in a boiling water bath.

### CHROMATOGRAPHIC PROCEDURES

The routine separation and identification of plant phenolics is best achieved by two-dimensional chromatography on paper or cellulose thin-layers using standard solvent systems (Mabry and Markham 1970; Harborne 1973) to facilitate comparison of  $R_f$  values.

In the present work the hydrolysed extracts were separated by this procedure on Whatman No. 1 paper using TBA solvent (tert-butanol/acetic acid/water, 3:1:1) for the first direction followed by 15% (v/v) acetic acid as the second solvent. The dry chromatograms were viewed under long wavelength UV light (365 nm), before and after exposure to  $NH_3$  fumes, to locate fluorescent spots. Relative concentrations of each component were judged visually by the size and intensity of fluorescence of each spot.

### SPECTROSCOPY OF PHENOLICS

Most phenolic compounds exhibit characteristic UV absorption spectra and the position of the absorption maximum may be shifted by the addition of group specific reagents such as borate and  $AlCl_3$ , thus providing valuable data for their identification (Harborne 1973).

Identification of phenolic compounds present in high concentration was attempted by eluting the spots from the chromatogram with methanol and then recording their spectra on a Pye-Unicam SP1800 recording UV spectrophotometer. Additions of sodium acetate, sodium tetraborate, or  $AlCl_3$  were made to test for vicinal phenolic hydroxyl groups and unsaturated side-chains.

## LEUCOANTHOCYANIDIN ASSAY

Leucoanthocyanidins (LA's) yield a red colour, the "phlobaphene reaction", when heated in the presence of strong mineral acid. Swain and Hillis (1959) adapted this reaction to give a quantitative assay for LA's and this procedure was used to determine the levels of LA in 20 mg samples of freeze-dried leaf material.

## RESULTS AND DISCUSSION

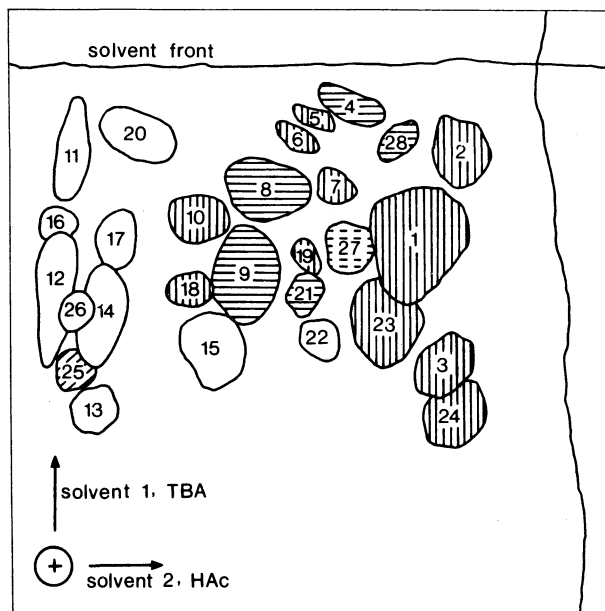
Examination of the individual two-dimensional chromatograms under UV light revealed the presence of 28 different UV-fluorescent or absorbing spots; these were most probably phenolic or flavonoid in nature. Figure 1 shows a master composite chromatogram derived from all the individual chromatograms. Table 1 gives the semi-quantitative distribution of the different phenolic compounds in the various species examined.

Considering first *Pseudopanax*, Group I, it may be seen that this group possesses a richer variety of phenolic constituents than the other groups. Species within the group appear to be closely related except for *P. laetum* which lacks spot 2 but possesses additional spots 18, 19 and 23, absent from the remainder of Group I.

Similarly the composition of the plants of Group II appears to correlate reasonably well with the classification based on morphological and other characteristics. Of particular interest is the absence of spot 3 from Groups IIa and IIb and the restriction of spot 28 to the plants of Group IIb.

One of the most interesting observations from the chemosystematic point of view is the uniquely different array of compounds present in *P. anomalum*. For example, spots 21, 22, 23, 24, 25 and 26 were observed only in this species whereas the commonly occurring spots 1 and 11 were absent. Spot 25 is also unique in that it was the only compound to exhibit a pink fluorescence. Thus both chemically and morphologically *P. anomalum* is anomalous! Moreover, greater chemical diversity was found within the single genus *Pseudopanax* than between such unrelated genera as *Stilbocarpa*, *Meryta* and *Schefflera*.

Attempts were made to identify some of the compounds separated on the chromatograms by comparison of R<sub>f</sub> values with those of authentic compounds and from spectroscopic data. Compounds 1 and 2 appeared to be very similar and may be *cis* and *trans* isomers; the spectral data suggested *ortho*-dihydroxy groups and an unsaturated side-chain but the compound(s) were not identical with caffeic acid. Spots 8 and 9 were of interest since their occurrence appeared to be complimentary; spot 8 was normally more prevalent but absent when 9 was dominant, as in *P. anomalum*, *P. chathamicum* and *P. laetum*. Spots 8 and 9 both yielded similar spectra and the present data suggests that they might be the *cis* and *trans* isomers of caffeic acid since 15% (v/v) acetic solvent separates these isomers. It may also be significant that spot 8 was one of the few compounds present in all the genera examined. Spots 12 and 16 were tentatively identified as quercetin derivatives and spot 11 was probably kaempferol. Bate-Smith (1958) has reported that flavonoids together with hydroxy- and methoxy-substituted phenolic acids are



Fluorescence colour:






	UV light:	UV light plus NH <sub>3</sub> vapour:
	blue	blue
	blue	green or yellow-green
	dark yellow-brown	bright yellow-brown
	pink	pink
	absorbing spot	orange-brown

Fig. 1. Composite two-dimensional chromatogram of phenolic compounds extracted from leaves of New Zealand Araliaceae.

TABLE 1. DISTRIBUTION OF PHENOLICS IN LEAVES OF NEW ZEALAND ARALIACEAE

COMPOUND SPECIES	NO. 1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	LA
<i>Stilbocarpa</i>	+	+					+	+																					
<i>robusta</i>	+	+					+	+																					
<i>S. polaris</i>	+	+				+	+	+																					
<i>Meryta</i>																													
<i>sinclairii</i>	+++	++	+	++	++	++	+++			+	+	+	+																
<i>Schefflera</i>																													
<i>digitata</i>	+++	++	++			+	++	+++				+																	
<b>Group I</b>																													
<i>P. arboreum</i>	+++	++	++	+	+		+++			++		+	+																
<i>P. colensoi</i>	++	++		+	+	+	++	+++				+	+	+															+++
<i>P. mackintyi</i>	+++			+		++	++	++				+	+																+
<i>P. laetum</i>	+++					+		+	+++			+	+	+	+	+	+	+	+										+
<b>Group IIA</b>																													
<i>P. lessoni</i>	+++	++			+	++	++	+++				+																	
<i>P. discolor</i>	+++	++				+	++	+++																					
<b>Group IIB</b>																													
<i>P. crassifolium</i>	+++				+	+		+				+																	
<i>P. ferox</i>	+++	++		++	++		+++																						+
<i>P. chathamicum</i>	+++			+			+++		+++																				+
<i>P. lineare</i>	+++	++		+			+++	+++				+	+																+
<i>P. simplicare</i>	+++	+	+		++		+++		+++			+	+																++
<b>Group III</b>																													
<i>P. anomalum</i>									+++															++	++	+++	++	+++	++

Key: Relative concentration, judged visually:

- +++ = high
- ++ = moderate
- + = low
- ± = trace
- IA = Leucanthocyjanidin

TABLE 2. RELATIVE LEVELS OF LEUCOANTHOCYANIDINS  
IN NEW ZEALAND ARALIADS

Species	Absorbance <sup>1</sup> (550 nm filter) reading	
Araliaceae		
<i>Stilbocarpa robusta</i>	1.5	
<i>Stilbocarpa polaris</i>	1.2	
<i>Meryta sinclairii</i>	1.4	
<i>Pseudopanax arboreum</i>	1.5	
<i>P. colensoi</i>	6.6	(high)
<i>P. mackintyreii</i>	1.1	
<i>P. laetum</i>	1.4	
<i>P. lessonii</i>	1.0	
<i>P. discolor</i>	5.0	(high)
<i>P. crassifolium</i>	1.9	
<i>P. ferox</i>	1.3	
<i>P. chathamicum</i>	1.0	
<i>P. lineare</i>	3.2	(moderate)
<i>P. simplex</i> (adult)	1.2	
<i>P. simplex</i> (juvenile)	3.1	(moderate)
<i>P. anomalum</i>	1.3	
Umbelliferae <sup>2</sup>		
<i>Daucus carota</i> (carrot)	1.9	
<i>Petroselinum crispum</i> (parsley)	1.1	
<i>Foeniculum vulgare</i> (fennel)	2.0	

Note 1. Leucoanthocyanidin standards were not available therefore only relative levels are given.

Note 2. Specimens of non-woody Umbelliferae are included to provide a base-line level.

commonly present in the Araliaceae.

Comparison of *Schefflera digitata* and *Meryta sinclairii* with the genus *Pseudopanax* shows that all possess an essentially similar range of phenolic compounds. The similarity of *S. digitata* to *Pseudopanax* Group I is in agreement with the observed similarities of shoot morphology. However, a greater difference might have been expected between *Pseudopanax* Group I and *M. sinclairii* in view of their differences in morphology. It seems therefore, that the family as a whole, with the notable exception of *P. anomalum*, exhibit a basic similarity in their phenolic constituents.

When this investigation was begun it was hoped that chemical evidence might help resolve the problem of the classification of the aberrant genus *Stilbocarpa* which is herbaceous whereas most other araliads are woody in nature. It was considered that the LA levels in the various plants might shed light on this problem since Harborne (1967) has suggested that there may be a correlation between the presence of LA's and woodiness. Many authors have considered the Araliaceae and Umbelliferae to have the status of an order, the Umbellales or Umbelliflorae (Cronquist 1968; Hegnauer 1964) within which Rodriguex (1971) also included the family Cornales. LA's have been reported to be absent from the Umbelliferae (Bate-Smith 1958; Harborne 1967), but present in some Cornales (Hegnauer 1964) and absent from the Araliaceae (Bate-Smith 1958). The present results (Table 2) show that LA's are present in four of the sixteen species examined. However, if the presence of LA's is associated with woodiness than they would be expected to be present in the woody araliads but not in *Stilbocarpa* which is herbaceous. One interesting point that needs further research is the finding of appreciable amounts of LA's in the juvenile form of *P. simplex* but only trace amounts in the adult form. It is therefore necessary to determine if this loss of LA's with maturity is a common feature of all araliads and this may help clarify the question of the anomalous distribution of LA's referred to above; in particular it could help resolve the problem of *Stilbocarpa*.

This study is only a beginning; it yet remains to examine the distribution of other groups of compounds such as proteins and allozymes, non-protein amino acids and other secondary metabolites. For example, Murray and Stanley (1952) have reported the occurrence of terpenoid compounds in the essential oil from leaves of *P. simplex* and this should be a worthwhile area for future chemosystematic investigations.

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