VARIATION IN POLYPHENOL COMPOSITION WITHIN SPECIES OF EUCALYPTUS L'HERIT

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Abstract—The nature of the polyphenols in the leaves of thirty-two samples of Eucalyptus camaldulensis collected throughout Australia showed a close similarity except in cases suspected of hybridization. Several other species showed a similar constancy of composition. The ratio of the amount of leucoanthocyanins varies considerably in certain species. Stilbenes, which are considered to be taxonomically abnormal composads in the bases of the genus Eucalyptus, have been found in twenty-nine species and in some cases the stilbene-lacking form of the same species has also been found. A small number of other species possess two or more forms which yield different mixtures of polyphenols on acid hydrolysis. A nomenclatural system which incorporates chemical variation in composition into the botanical nomenclature, is suggested. Possible causes of variation are considered.

INTRODUCTION

AUSTRALIA is the habitat of almost all the 500 odd species, sub-species and varieties of the genue Eucalpptus¹ (Family Myrtaceae). Only seven species grow naturally in the neighbouring islands of New Britain, New Ireland, New Guinea and Timor, namely E. polycarpa (41),*
E. alba (257), E. confertiflora (19a), E. deglupta (437), E. papuana (17), E. tereticornis (178) and the species formerly named E. decaisneana.

Australia is an old land surface and possibly was formerly an undulating plain covered by a comparatively uniform vegetation. During climatic changes, the *Eucalyptus* genus arose (possibly in the Oligocene period) and was subject to further physiographic changes, particularly those occurring in the Upper Pliocene period. No doubt evolutionary adaptation has continued until very recent times and is still proceeding wherever communities survive in the face of extensive land clearance. It is probable that samples of almost all the species that existed before settlement can still be found, although some are almost extinct (only one specimen of *E. carnabyi* (597b) is known). There is therefore an almost unique opportunity to study the changes in the polyphenols resulting from evolutionary development. It is probable, of course, that some species have been lost during climatic changes. Thus the species—e.g. *E. microcorys* (314), *E. deglupta* (437), *E. curtisii* (9), *E. guilfoylei* (305) and *E. jacobsiana* (52a)—which have no near relatives could be remnants of evolutionary lines.

- The botanical nomenclature and number (given after each name) of species is that of Blakely² with the revisions by Johnston and Marryatt.³ For the sake of brevity, the authorities, which are readily available elsewhere,^{2,3} are not given in this paper. The antheral classification of Blakely² is followed as his comprehensive description of the genus is the most complete available.
- ¹ A detailed description of the genus is given in:
- (a) A. R. PENFOLD and J. L. WILLIS, The Eucalypts. Hill, London (1961).
- (b) M. R. JACOBS, Growth Habits of the Eucalypis. Forestry & Timber Bureau, Canberra (1955).
- ² W. F. Blakely. A Key to the Eucalypts (2nd Ed.). Forestry & Timber Bureau, Canberra (1955).
- ³ R. D. Johnston and R. Marryatt, Taxonomy and Nomenclature of Eucalypts. Forestry & Timber Bureau Leaflet No. 92 (1965).

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The south-western portion of Australia possesses a flora that is botanically peculiar to, and geographically isolated from, the rest of the continent. It is possible the ancestral forms of these species originally grew across the central area that is now a large desert region (part of which was formerly covered by the sea). The separation of plant populations apparently began before the Miocene period. Most of the sub-divisions of the genus must have been in existence before separation occurred, as representatives are found on both sides of the continent. However, the Eastern and Western representatives of a particular group may differ appreciably and have proceeded in time along different evolutionary lines. Excluding perhaps the mallees of the drier areas, none of the seventy-odd species found in the south-west corner of Australia is found in the east and vice versa.

On the other hand, there is little difference between the mainland and Tasmanian species. So far, isolation has had, at the most, minor effects botanically and apparently only some intermediate forms were lost when the island was separated from the mainland in geologically recent times.

Eucalypts grow under a wide range of climatic conditions. E. striaticalyx (149), E. kondininensis (151), E. flocktoniae (584), E. brockwayi (584a), E. salmonophloia (593) and other species are found in semi-arid regions with an annual rainfall of about 10 in. E. deglupta (437) grows in humid areas of New Britain which have an annual rainfall of 100-200 in. or more. Some parts of Tasmania have a rainfall of more than 50 in. and are covered with excellent stands of E. regnans (369) and E. delegatensis (370). E. grandis (58) grows in regions of New South Wales with an annual rainfall of 80 in. Although some areas of Northern Australia have good summer rains of about 40 in./yr, they are subject to long winter droughts, with relatively high temperatures and consequent high evaporation. By world standards, Australia's rainfall is erratic so that its effectiveness cannot be measured by its amount alone. In many parts of Australia, the maximum temperatures each summer are over 38° (100°F) and in Central Australia these temperatures can continue for many days. E. niphophila (397) is found at altitudes up to 6500 ft and can withstand temperatures of less than -7° (20°F) E. gunnii (239) can withstand -15° . In most cases, eucalypts are found on low fertility soils of various but usually acid types. The newer (in the pedogenic sense) soils have a fertility level that is generally higher than that of the old land surfaces.

In recent years, reappraisals of certain aspects of Blakely's² classification have been undertaken. To provide further data for these studies, and more particularly to find suitable material for the study of the biosynthesis of polyphenols found in wood, the leaves of more than 770 samples from over 330 Eucalyptus species have been examined. These results will be reported in subsequent papers. During this survey a number of examples of variation in polyphenol composition in the one species were encountered. A detailed study of this aspect in E. sideroxylon (541) has already been given,⁴ and a description of other species is presented in this paper with an attempt to correlate this variation with the influence of environment. A proposal for the adoption of suitable nomenclature to describe these variations is also made.

RESULTS

The Constancy of Components in Samples of One Species

E. camaldulensis (197) is probably the most widespread eucalypt in Australia, and is found in all States except Tasmania. Samples from most of the provenances have been examined (Table 1). Botanically it is a variable species but, with regard to the polyphenol

4 W. E. HILLIS and K. Isoi, Phytochem. 4, 541 (1965).

aglycones detected, a close similarity has been found between many samples collected from a widespread area. A few exceptions exist, such as the Queensland samples from Pentland (Fig. 1. Io), Mareeba (Go) and Hughenden (In) which contain significant amounts of kaempferol. Samples from these areas are suspected of being polycrosses with *E. tereticornis* (178) and possibly *E. alba* (207) (E. Larsen, personal communication) and the presence of kaempferol supports this view. The samples from Dubbo, New South Wales (Mp), and Fortescue River, Western Australia (Ic), also contain kaempferol and may be similar crosses, as well as the samples from the Blank, Lachlan and Macquarie Rivers, N.S.W. (No, Np, Mp), which contain aromadendrin (dihydrokaempferol). Botanical data on these latter samples have not yet been received.

Apart from the constancy of the composition of this species, other examples and their composition will be discussed in future papers. For the present purpose, the following examples can be given. Samples of *E. gummifera* (45) have been collected from localities 200 miles apart and *E. citriodora* (53), *E. longifolia* (81) and *E. wandoo* (120) 300 miles apart, but the composition of all samples of each species is identical. *E. rubida* (235), *E. regnans* (369) and *E. delegatensis* (370) are found in Tasmania and on the mainland; all the samples are identical in polyphenol composition although those from the first species have been collected from localities 600 miles apart and from the other two species 400 miles apart. In contrast, samples of *E. sieberi* (371) from Tasmania differ considerably from the mainland form. Six hundred miles separated the samples of *E. thozetiana* (470) and 500 miles the samples of *E. drummondii* (590) but the compositions are identical. Samples collected from other species and from localities that are not separated as much as the above also show a very close similarity in composition.

The Constancy of the Ratio of Components in Samples of the One Species

Although the polyphenol aglycones of *E. camaldulensis* (197) remain the same for most samples, the ratio of their amounts—such as quercetin relative to ellagic acid—differs widely (Table 1) but no influence of environment can be seen. It will be noticed that all of the large number of samples of this species lack leucoanthocyanins. However, in some others the amount of leucoanthocyanin varies from sample to sample of the one species.

Examples of the latter variation are given by the samples of *E. odorata* (455) and *E. obliqua* (362) (Tables 2A and 2B). Tests have shown that this variation is unlikely to be due to storage of, or the analytical methods used to examine, the samples. The relative amounts of leucoanthocyanins in different samples of the one species vary more than do other compounds.

The Occurrence of Taxonomically Abnormal Compounds in a Species

Stilbenes have been found in twenty-nine species and only one of these—E. guilfoylei (305)—has been placed in the Renantherae (Table 3), but this species is now considered to be wrongly classified. A few samples of the closely related Angophora genus have been examined and the sample of A. cordifolia found to contain stilbenes. Both stilbene-containing and stilbene-lacking forms of some species have been found (Nos. 17, 147, 151, 237, 274, 541, 550, 577, 578, 593 and the closely related pairs of species 19 and 19a and taxa 236 and 236a, Table 4). In these species there is no consistent qualitative difference in composition of the basic polyphenols of the two forms and, in some cases, this composition in the two

forms is the same. There are indications that the ratio of ellagic or gallic acids may be significantly less in the variant sample. Stilbenes are almost invariably associated with chlorogenic and p-coumarylquinic acids.

The species containing stilbenes in the leaves are distributed randomly in the non-renantherous sections and although there may be a few groups of related species, there is no over-all relationship. The stilbenes are considered to be taxonomically abnormal compounds.

The Variations in Occurrence of Components in a Species

Different samples of a relatively small number of the species examined yielded different mixtures of compounds on acid hydrolysis (Table 5). The taxonomically-representative composition cannot be determined without reference to the composition of a number of closely related species.

The nature of the flavonol glycosides varies among different samples of *E. camaldulensis* (197) (Table 1) and apparently such compounds have little significance at species level. A similar behaviour has been observed in samples collected in the different provenances of *E. sideroxylon*⁴ (541). The latter work indicated an association between composition of polyphenols in the alcohol extract and locality. Consequently, further work may show it to be possible from an examination of the aglycones and the glycosides, to ascertain the original provenances of certain *E. camaldulensis* which are growing vigorously in countries outside Australia.

DISCUSSION

Variation in Chemical Composition within a Species

The samples of *E. camaldulensis* (197) were collected from a very large area in which exist a wide range of environmental conditions. In view of this, the composition of the polyphenols in this species shows little variation. The composition of several other species is very similar even though the samples were collected from widely separated areas.

However, there is a significant number of eucalypt species showing appreciable variation in composition of the polyphenols (see Tables 4 and 5). The possibility that these variations in composition are due to undetected hybridism or to wrong identification is most unlikely. This possibility can be definitely discounted in the samples of *E. eugenioides* (318), *E. caliginosa* (324), the Papuan samples of *E. confertiflora* (19a) and *E. tereticornis* (178), and the various samples of *E. sideroxylon* (541).

A similar behaviour has been noticed before in the Eucalyptus genus as in 1924, Penfold and Morrison⁵ reported the existence of two varieties of E. piperita (427) the leaf oil of one containing 40-50 per cent piperitone whereas the oil of the other was low in piperitone but high in phellandrene, eudesmol and cineole. The eudesmol content of some samples of E. sparsifolia (352) can be less than 1 per cent, whereas other samples contain about 60 or about 90 per cent.⁶ The cineole content of the leaf oil of E. radiata (411) varies between 3 and 60 per cent, the citronellal content of E. citriodora (53) 5 and 85 per cent, and piperitone content of E. dives (417) 14 and 40 per cent. Yet samples from these species growing in some localities possess a relatively constant content of the major oil constituents with the amount being close to the upper limit given above.⁶ Five varieties of E. radiata (411), three of E.

⁵ A. R. PENFOLD and F. R. MORRISON, J. Proc. Roy. Soc. New South Wales 58, 124 (1924).

⁶ J. L. WILLIS, H. H. G. McKern and R. O. HELLYER, J. Proc. Roy. Soc. New South Wales 96, 59 (1963).

andreana (406), four of E. micrantha (434), four of E. dives (417) and four of E. citriodora (53) are already known. An examination of the individuals within a species population of E. dives revealed the large differences in oil composition of four variants which were sometimes growing within a few feet of each other.

The variation of chemical composition in a morphologically homogeneous species has received little attention in the past, but chemical variation is of widespread occurrence in the plant kingdom and has been found in samples from the Thallophyta, Bryophyta, Pteridophyta, 8 to the Gymnospermae and Angiospermae. Some of the variations are of a quantitative nature and the factors affecting such variations have been discussed by Flück. However the study of some species has revealed qualitative or widely different quantitative differences in chemical composition. Such variations have been found to occur in many types of plant tissues from several families and to involve several classes of organic compounds.

The linalool content of the leaves of *Thymus serrulatus* (Fam. Labiatae) grown in soils of adequate moisture content is four times, or more, greater than in the leaves of plants growing in arid conditions. ¹⁰ *Pycnanthemum lanceolatum* (Fam. Labiatae) growing in the United States contains carvacrol whereas in those growing in Russia this component is absent and replaced by menthone and pulegone. ¹¹ Differences in leaf oil composition within species and between species of *Backhousia*, *Melaleuca* and *Leptospermum* (Fam. Myrtaceae) have recently been discussed by McKern. ^{11a}

"Chemical races" have been found 12 in Cinnamomum camphora (Fam. Lauraceae) with the major component in the leaf oil being either camphor, cineole, borneol, safrole, linalool or a sesquiterpene. Three variants of C. camphora yield wood oil containing a high percentage of either cineole, linalool or camphor. The wood oil of Ocotea pretiosa (Fam. Lauraceae) usually contains principally safrole, but variants have been found yielding 1-nitro-2-phenylethane together with a large amount of eugenol methyl ether, and varying amounts of safrole. Some of the trees yielding mainly safrole also form small amounts of camphor. Oleoresin collected after boring holes into the tree, or by damaging the developing sapwood, can vary in composition when collected from chemical variants; whereas Pinus sylvestris (Fam. Pinaceae) usually yields Δ^3 -carene, one tree yielded instead α -pinene, camphene and β -pinene but one variety contained a high proportion of β -pinene. Several chemical varieties of P. ponderosa have also been recorded. Several chemical varieties

The seed of Crotolaria spectabilis (Fam. Leguminosae) growing naturally in the U.S.A. contains a large amount of monocrotaline which was the only alkaloid detected. The seed from C. spectabilis naturalized in Queensland, Australia, contains very large amounts of

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¹⁵ R. H. SMITH, Science 143, 1337 (1964); Forest Service Res. Pap. PSW-15 (1964).

¹⁶ R. Adams and E. F. Rogers, J. Am. Chem. Soc. 61, 2815 (1939).

monocrotaline and its O-acetyl derivative spectabiline (present in almost equal amounts).¹⁷ Seeds of the American plant were grown in Queensland and found to be botanically indistinguishable from the Australian specimens but quite different chemically owing to the absence of spectabiline (C. C. J. Culvenor, personal communication). C. retusa growing in some parts of Northern Australia are very similar to the American plants in that monocrotaline is the predominant alkaloid in the seeds, but samples from other areas contain a considerable proportion of its N-oxide.¹⁸

The alkaloids micrantherine, daphnoline and daphnandrine have been found in one sample of bark from Daphnandra micrantha (Fam. Monimiaceae) but the second or third or both the last two alkaloids are absent or undetected in other samples. The alkaloidal composition of the leaves and the bark of both Flindersia maculosa and F. dissosperma (Fam. Rutaceae) differs from sample to sample. Variation in the composition of alkaloids in the bark of Cinchona ledgeriana (Fam. Rubiaceae) has been reported. The Solanaceae of tropical origin form scopolamine, while representatives from the temperate regions produce hyoscyamine; the North Australian Duboisia myoporoides (Fam. Solanaceae) forms scopolamine throughout its growth but the South Australian forms synthesize hyoscyamine in the later stages. Variation in the later stages.

Few cases are known of variation in polyphenol composition. The existence of several varieties of Eucalyptus sideroxylon containing flavonoids or, in addition, relatively large amounts of stilbenes has been reported.⁴ Samples of Smilax glycyphylla (Fam. Liliaceae) from New South Wales contain the dihydrochalcone phloretin 2'-rhamnoside, from one part of Queensland the hydroxyxanthone mangiferin, and from another part of Queensland the leaves contain neither of these unrelated compounds and in fact very little phenolic material.²⁴ A variation in polyphenol composition has been recorded ²⁵ for Evodia micrococca var. micrococca (Fam. Rutaceae). The leaves from one area contained more than 2·9 per cent and from another 0·5 per cent pinoresinol dimethyl ether whereas E. micrococca var. pubescens yielded instead (+)-sesamin.²⁵

Variable compositions of the polyphenols of the heartwood of *Pterocarpus indicus* (Fam. Leguminosae) have been reported. In one case pterocarpin and homopterocarpin were found,²⁶ and in another, only angolensin,²⁷ in the third homopterocarpin ²⁸ and in the fourth pterocarpin, angolensin, small amounts of isoliquiritigenin and formononetin and traces of other compounds.²⁹ All the populations of *Galium molugo* (Fam. Rubiaceae) appear to contain two chemical varieties, one containing hesperidin and the other lacking this compound,^{30, 31} and the variation occurs independently of the geographic origin of the plants.

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31 R. HEGNAUER, Pharm. Ztg. ver. Apotheker-Ztg. 104, 382 (1959).

Other examples of chemical variation in morphologically homogeneous species are known to exist, so that the variations in polyphenol composition of the eucalypts given in Tables 4 and 5 are not unique. With the increasing activity in the field of chemotaxonomy and with the introduction of techniques permitting the rapid examination of samples collected in the search for "elite" specimens in plant populations, it is to be expected that increasing numbers of such chemical variants will be found. The diversity of individuals in a species must be considered in chemotaxonomic studies and attention to these deviations from normal metabolism should assist the proper evaluation of the importance of different chemical features in taxonomic studies. One deficiency, at the present time, is the absence of a suitable nomenclatural system which defines such deviations in a way which enables their recognition at any time.

Proposed Nomenclatural System to Define Variation in Chemical Composition

With the intensification of study of plant species, not only is it expected that further examples of chemical variation will be discovered but also that manifestations of other or associated genetic differences will be found in the morphological, physiological and other features of the plant. Thus, the establishment of a nomenclature for chemical variation must take cognizance of this situation.

Infra-specific chemical variation has recently been considered by several workers.³²⁻³⁴ Nomenclatural treatment has been discussed by taxonomists^{21,35-38} and, of the different proposals, the suggestion by Tétényi³⁸ that the term infra-specific chemical taxonomic units (taxa) should be used when a substantial chemical, but no morphological, difference exists, seems the most suitable from a phytochemical viewpoint. This system can supplement the gamut of features used in classical taxonomic nomenclature. He proposed the use of *chemovar* (chvar.) and *chemoforma* (chf.) for infra-specific categories of natural species, but in the light of the examples given above, his nomenclature needs to be expanded.

At this stage, the variations can be divided into four main types which represent different stages of the biosynthetic pathway. It is possible for samples of one species to contain representatives of different classes of:

- (a) Non-convertible compounds, e.g. flavonoids-stilbenes; dihydrochalcones-hydroxy-xanthones; phenylpropane (safrole)-monoterpenes (borneol).
- (b) Structurally related and possibly interconvertible compounds, e.g. phellandrene-piperitone and possibly also cineole; limonene-pinene; scopolamine-hyoscyamine; angolensin-homopterocarpin; dihydrokaempferol-kaempferol.
- (c) Compounds which differ only in the number of free hydroxyl groups etc., e.g. kaemp-ferol-quercetin-myricetin; safrole-eugenol-methyleugenol-myristicin-elemicin; resveratrol-rhapontigenin-astringenin; monocrotaline-spectabiline; sesamin-pinoresinol dimethyl ether.
- (d) Compounds which differ only in the position and type of sugar moiety or in their stereochemistry, e.g. quercitrin-isoquercitrin-rutin; catechin-epicatechin.

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32 E. SCHRATZ, Planta Med. 8, 282 (1960).
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³³ G. DILLEMAN, Planta Med. 8, 263 (1960).

³⁴ F. JAMINET, Planta Med. 8, 275 (1960).

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³⁶ R. Mansfeld, Taxon 7, 41 (1958).

³⁷ J. Lanjouw, *Taxon* 7, 43 (1958).

³⁸ P. Tétényi, *Taxon* 9, 241 (1960).

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In addition, the composition of samples of one species may differ only in a quantitative manner.

A series of nomenclatural sub-divisions is needed to clarify the variations. The term "subspecies" implies a divergence in the process of speciation and is used in the taxonomic studies which examine the totality of the organism's characters. At present, there is little evidence to indicate that change in composition leads to the development of new species, so that the lowest ranks of taxa appear to be the most suitable categories for classification. It is tentatively suggested that the following sub-divisions:

chemovarietas (chvar.)
chemosubvarietas (chsubvar.)
chemoforma (chf.)
chemosubforma (chsubf.)

be used to describe the classes (a)–(d) mentioned above.

The chvar. and chsubvar. can be appropriately named after the major class of compound or the major chemical component present. The composition of the minor classes—subdivisions (c) and (d)—of secondary chemical components in an extract is usually complex so that a description of all the various taxa would defy in most cases a systematic naming. It would appear that chf. and chsubf. should be given letters and numbers in the order of their discovery.

The above system can be applied when any number of classes of compounds are considered, e.g. polyphenols and essential oils.

Classification of plants is a severely practical activity and taxonomy will always be based primarily on morphological characters as these are the most readily assessed. Consequently, other features which can further delineate or subdivide the botanical units are best incorporated into the existing system. Thus, the following nomenclatural system may prove suitable:

- E. dalrympleana subsp. dalrympleana (236), chvar, stilbenoid;
- E. dalrympleana subsp. heptantha (236a), chvar. flavonoid; and in detail
 - (a) E. sideroxylon (541) chvar, stilbenoid, chf. S1, chsubf. qi.
 - (b) E. sideroxylon (541) chvar. stilbenoid, chsubvar. dihydrokaempferol, chf. S2.
 - (c) E. sideroxylon (541) chvar. stilbenoid, chf. S1, chsubf. qii.
 - (d) E. sideroxylon (541) chvar. flavonoid, chsubf. qii.

Where in example (a) the chemoforma S1 represents the entities from Baradine⁴ containing three stilbene glycosides and the chemosubforma qi containing quercetin 3-rhamnoside as the major flavonol glycoside. In example (b) chsubvar, dihydrokaempferol represents the entities from Gilgandra⁴ containing dihydrokaempferol, and S2 the entities containing two stilbenes piceid and astringin. Example (c) represents the entities from Heathcote⁴ containing three stilbene glycosides and quercetin 3-glucoside as the distinctive flavonol glycoside, and example (d) the normal form from Heathcote containing quercetin 3-glucoside.

In most situations, definition of the chemical nature of the sample would be unnecessary, but when so required the chemical sub-divisions may be incorporated in the present system irrespective of the sub-divisions based on other features. The samples of a species which show only quantitative variations in composition are much more difficult to delimit and define as the differences are continuous. In addition it would be necessary to determine how much such variations are due to morphogenetical, ontogenetical, seasonal and environmental factors.

The accepted botanical practice of designating as the type that specimen from which the species or form was first described, could be difficult in the treatment of chemo-taxa. For one thing, such a practice would involve much labour at the outset to compare the new chemotaxonomic units with the herbarium specimens of the taxa to which they belong. Therefore, we propose for expediency to describe chemovariation in morphologically homogeneous species in terms of "normal" and "aberrant" chemo-taxa and where conveniently possible to refer these forms back to the established morphological taxa. For example, those leaf samples from a eucalypt which contain stilbenes are considered as "aberrant" chemotaxa.

The Origin of Chemotaxa

Differences in chemical composition of samples of morphologically homogeneous species are troublesome at this stage of the development of chemotaxonomy, but they do provide invaluable material for the determination of many stages of the biosynthesis of secondary components. In addition they present an opportunity for selective breeding of plants. Accordingly if the origin of chemotaxa and the factors initiating deviation in metabolism were known, the discovery or development of the desired chemical variants would be assisted.

The variation in the nature of the flavonol glycosides in *E. sideroxylon*⁴—and to a lesser extent in *E. camaldulensis* (Table 1)—indicates an association between composition and locality. These variations could be reflections of change in metabolism and adaption to different environments and may represent biotypes and ecotypes (cf. Refs. 31 and 8).

The variants listed in Tables 4 and 5—for which the names chemovarietas and chemoforma are proposed—represent more profound changes. The stilbenoid chemovarietates showed a marked chemical difference with large amounts of stilbene being formed, and in addition the sensitivity of the method of detection shows the absence of these compounds in the normal variety. Several species of the non-renantherous sections of the Eucalyptus genus possess chemotaxa of the stilbenoid type. The number is sufficiently large to show the appearance of stilbenes to be more significant than a chance deviation of metabolism. The capacity to form stilbenes is a characteristic of many series of the Macrantherae, Terminales, Graciles and Platyantherae Sections. The presence of stilbenes in Angophora cordifolia could have phlyogenetic significance.

The examination of several seedlings during growth and the rate of incorporation of the ¹⁴C label into different polyphenols in different samples of the one species show there is no loss in selectivity of enzyme action when stilbenes are formed.³⁹

The variations could be ecologically directed as in recent times, the habitat of many Eucalyptus species has become increasingly dry and nineteen of the twenty-eight species possessing stilbenoid chemotaxa were collected in low rainfall regions or areas of high evaporation rates. E. melliodora (550) may also belong to this group as the two Victorian samples were normal and a peripheral sample from Mt. Lindesay, Queensland, contained stilbenes. E. angophoroides (224), E. dalrympleana ssp. dalrympleana (236), E. glaucescens (237), E. nitens (263), E. baeuerlenii (270) and E. smithii (274) were obtained from areas of moderate rainfall or from trees of unknown origin in botanic gardens. It may be significant that a sample of E. urnigera (240) collected from an area near Hobart with a relatively high rainfall was the only chemotaxon containing small amounts of stilbenes whereas the others contain large amounts.

³⁹ W. E. HILLIS, Unpublished data.

550 W. E. Hills

This association of water stress and stilbenes is further supported by an examination of the distribution of the chemotaxa. *E. papuana* (17) samples collected in high rainfall or high humidity areas of New Guinea lack stilbenes, whereas those from the arid and semi-arid regions of central Australia contain stilbenes. *E. confertiflora* (19a) samples from New Guinea do not possess stilbenes but the very closely related *E. clavigera* (19) from the Darwin area contains them. The coastal samples of *E. sideroxylon* (541) lack stilbenes but these compounds are strongly present in those collected from the low rainfall areas of central New South Wales.⁴

The strong association between environment and stilbene formation is weakened by the discovery of normal and aberrant chemotaxa of *E. sideroxylon* growing within 100 yd of each other in both a central and a coastal region of Victoria and in regions of 20–25 in. rainfall. Similarly, both forms of *E. salmonophloia* have been found growing close together in Western Australia. (These examples show that soil condition is not necessarily a factor in stilbene formation.) The variability may be due to a continuing adaption by the species to form a genotype best suited to the present environment. However it should be mentioned that *E. camaldulensis* (197) (of the Macrantherae) grows under a variety of conditions and several samples have been collected from arid regions. Although the possibility that the latter have access to subterranean water cannot be ruled out, no sign of stilbene formation has been detected in these samples.

EXPERIMENTAL

In most cases, the samples of the leaves were collected through the generosity of people who were familiar with the *Eucalyptus* species concerned. The location of the trees from which the samples were taken has been recorded (Tables 1-5) and can be determined by reference to Fig. 1. Each unit square of the grid is about 150×150 miles, so that in a few cases several samples of the one species were collected from different populations but within the limits of one square. Samples collected from botanic gardens or arboreta are designated by "Z".

The samples were collected from trees that had grown out of the juvenile stage, and the leaves examined were mature leaves that had just reached full size.

The leaves were heated with 2 N HCl and the amyl alcohol extract of the resultant liquor resolved on paper chromatograms with Forestal (hydrochloric acid-acetic acid-water, 3:30:10), 6% acetic acid or benzene-acetic acid-water (6:7:3) solvents. The chromatograms were examined under u.v. light (365 and 254 m μ) before and after exposure to ammonia vapour, and then sprayed with diazotized p-nitroaniline as described previously (Hillis and Hingston ⁴⁰).

In addition, the amyl alcohol extract was examined by two-dimensional paper chromatography using first, butanol-acetic acid-water (6:1:2) and then 6% acetic acid. This was necessary in order to form a more reliable conclusion concerning the identity of some of the unidentified compounds. This technique revealed the presence of compounds F and G, and separated H and J. Also it revealed ellagic acid when large amounts of delphinidin were present and separated ferulic and sinapic acids. Moreover, in many cases, it showed whether the abnormal colour of the flavonols, as seen in the chromatograms resolved with Forestal solvent, was due to an overlying impurity or to the presence of other compounds.

⁴⁰ W. E. HILLIS and F. J. HINGSTON, J. Sci. Food Agric. 14, 866 (1963).

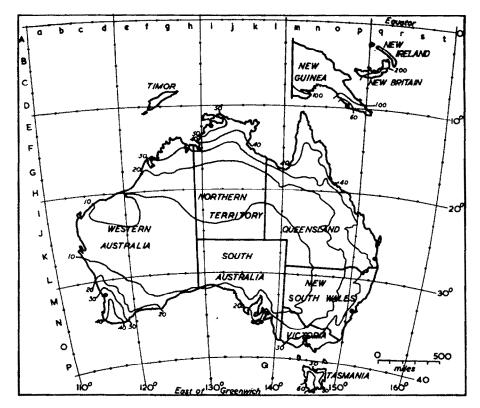


Fig. 1. The natural habitat of *Eucalyptus* species. The isohyets give the average annual rainfall in inches.

The scores of most of the polyphenols were assessed under long wave-length u.v. light. The remainder were assessed after exposure to ammonia vapour, or after spraying with diazotized p-nitroaniline and the score determined by relating the intensity of colour and size of spot to that of a spot with a known score.

The method of scoring used was to choose the compound or compounds with the largest area and strongest fluorescence when "Forestal" chromatograms were placed under long wave-length u.v. light, and to give it or them the score of 5. The size of the other spots was related to this spot and given an appropriate score. If a substance was clearly present but with a score of less than 1, it was given score T (trace). For reasons of clarity the absence of a compound has been represented by "—" in the Tables. A very small number of species, contained extra large amounts of a compound which did not give a "basic" polyphenol (i.e. quercetin, ellagic acid etc.) on hydrolysis. These compounds were given a score above 5 (see Blakely No. 134, 151, 167, 205, 575, 584, Table 4) so that the basic polyphenols could be compared with those of other species.

Caffeic, p-coumaric, ferulic, sinapic and gentisic acids were added to the chromatograms as markers to assist in identification of the different components. Delphinidin, cyanidin, myricetin, quercetin, kaempferol, ellagic, gallic and the above acids, aromadendrin, taxifolin have been previously identified in the acid hydrolysis products. 40 "Pelargonidin" has chromatographic properties and colour reactions similar to authentic pelargonidin but its identity has not yet been ascertained. In the alcohol extracts, chlorogenic and p-coumaryl quinic

Table 1. Composition of E. camaldulensis (197) leaves collected from different parts of Australia *

No. of samples examined	Location (see Fig. 1)	Leucodelphinidins	Leucocyanidins	"Leucopelargonidins"	Myricetin	Quercetin	Kaempferol	Ellagic acid	Unknown compd. A	Unknown compd. B	Gallic	Gentisic	Caffeic A 2:42	p-Coumaric Actions	Sinapic	Ferulic	Macrantherin	Renantherin	Unknown compd. C	Unknown compd. D	Taxifolin	Aromadendrin	Unknown compd. E	Astringin	Rhapontin	Piceid	Chlorogenic acid	p-Coumarylquinic acid	F)	o -	H Unknown compounds	I	1	K)	(V	20 (Glycosides	ı v	×
	Jj, Om	_	_		_	5	_	5	2	4	_	4	_	_	3	_	3	_	_	_	_	1	_	-	_	-	3	3	3	5	_	_	_	-		2	-	1 -	
	Fi	_		-	-	5	-	5	4	3	5	3	-	~	-	-	1	-	-	-	-	1	T	-	-	-	-	_	3	3	-	-	-	-	5	-			- -
4	Ol, Oo, Pm	_	-		-	5	_	5	2	2	5	2	_		-	1	~	_	-	-	-	1	Ţ	-	-	-	T	2	2	4	1	-	-	-	3	-	2	т]	l –
2	Lo, Ln	_	_	_	_	5	1	5	2	т	5	1	_	2	2	1	_	_	_	_	_	_	_	_	_	_	4	4	2	5	_	_	_	_	5	_	1 .	_ 1	1 2
	Pn Pn	т	т	_	_	5	_	5	2	2	5	3	_	_	_	_	_	_	_								•	•	~	-					-		•	•	
	Kn, Nk	_	_	_	_	4	_	5	3	3	5	2	-	_	2	1	_		_	_	_	т	_	_		_	3	T	5	5	_	_	_		5	_		T 7	r -
	Mm	_	_	_	_	4	_	5	3	2	5	2	2	-	2	1	3	_		_	_	Т	_	-	_	-	3	3	2	3	_	_	_		5	_		т 1	гτ
1	Fj		_	_		4	T	5	3	3	5	2	2	-	_	1	_		_	-	_	_	-	_	-		1	_	4	3	_	_	_	-	5	_			
1	Io		-	_	_	4	2	5	2	2	5	3	2		T	T	3	_	_	_	-	_	-	_	_	-	3		4	2		_		-	5	_			- T
1	Go		_	_	_	4	3	5	3	2	5	2	T	-	T	T	1	_	-	_	_	1		-	_	-	3	_	3	T	_	_	_	-	3	2	- :	1 -	- 2
1	No	~	_	_	_	3	-	5	5	5	5	2	_	-	1	1	T	_	_	_		2	T	_	_		1	1	5	5	_	_	_	-	3	-	3	1 -	- 1
1	Np		_	-	-	3		5	5	5	5	2	_	-	1	1	_	_	-		_	2	1	_	_	-	1	1	3	2	_	_	-	-	3	_	3	1 4	1 1
	Mp	~	-	-	-	3	-	5	5	3	5	2	1	-	2	1	_	-	-	_		3	-	_	-		1	1	2	2	_	-		-	3	-	3 2	2 т	Τĵ
	Hj	-	-	_	-	3		5	2	3	5	3	2	-	T	T	T	_	-	_	-	-	~	_	_	-	1	_	3	3	_	-	-	-	4	-			
2	Jj	-	-	-	-	3.		5	2	3	5	2	1	-	1	T	2		-	_		-		_	_	-	-	-	2	3	_	-	-	-					
1	Pn	~	_	-	_	3 .		5	4	3	5	2	_	_	2	T	_	_	_	_	-	_		_	-	-	1	_	2	2	_	-	_	_	5	_			- T
1	Mp	-		_	_	3	2	5	5	2	5	2	1	-	2	1	3	_		-	-	T	~		_	-	-	3	2	2	1	-	_	_	2	3		– T	Γ -
	Ic		-	_	-	3	4	5	2	-	5	2	1	_	-	-	-	-	-	-	-	_	-	-	_	-	3	3	2	2	_	-		_	2	1	2 ·		- T
2	Nn	-	-	_	- ,	2 ·	-	5	4	3	5	2	T	-	T	T	T	_	1	_	-	_	~	_	_	-	Ŧ	T	2	2	1	-	_		2	_	2	1 1	-
2	Oo, Pn		_	_	- :	2 ·	-	5	4	1	5	2	_	_	-	_	_	-	_	_	_	_		_	_	_	1	1							2	_			
2	In		_	_	- :	2	2	5	3	3	5	2	2	_	_	_	1	_	-	_	_	T	~-	-	_	_	4	1	3	3	_	~	_	_	2	_	1 :	r -	

^{*} Numbers (1-5) represent relative amounts (see Experimental); T=trace; -=not present.

TABLE 2A. COMPOSITION OF E. odorata (455) LEAVES COLLECTED FROM DIFFERENT LOCALITIES*

No. of samples examined	Location (see Fig. 1)	Leucodelphinidins	Leucocyanidins	"Leucopelargonidins"	Myricetin	Quercetin	Kaempferol	Ellagic acid	Unknown compd. A	Unknown compd. B	Gallic }	Gentisic	Caffeic	p-Coumaric Acids	Sinapic	Ferulic	Macrantherin	Renantherin	Unknown compd. C	Unknown compd. D	Taxifolin	Aromadendrin	Unknown compd. E	Astringin	Rhapontin	Piceid	Chlorogenic acid	p-Coumarylquinic acid	F)	O	H Tributa	I Conknown compounds		K J
1 1 1 5 1 1	Ol Ol Ol Ol Ol Ol	5 3 2 T -	- 1 - - -		1 T T T	5 3 2 4 3 3	1 - T - 3	5 5 5 5 5 5	1 - 1 2 2 3	- 1 - 2 3 3	5 5 5 5 5 5	2 2 2 2 2 2 3			- 1 - - 4	- 2 - 1 - 2	3 1 1 2 3 1	- - - - -	- - - - -	-		- - - T	1 - - 1 -	- - - - -				1 1 1 1 2 1	2 2 1 2 2	- - - 1	1 2 1 T	- - - -	1 - 1 1 T	

^{*} See footnote to Table 1.

TABLE 2B. COMPOSITION OF E. obliqua (362) LEAVES COLLECTED FROM DIFFERENT LOCALITIES*

No. of samples examined	Location (see Fig. 1)	Leucodelphinidins	Leucocyanidins	"Leucopelargonidins"	Myricetin	Quercetin	Kaempferol	Ellagic acid	Unknown compd. A	Unknown compd. B	Gallic	Gentisic	Caffeic	p-Coumaric Actus	Sinapic	Ferulic]	Macrantherin	Renantherin	Unknown compd. C	Unknown compd. D	Taxifolin	Aromadendrin	Unknown compd. E	Astringin	Rhapontin	Piceid	Chlorogenic acid	p-Coumarylquinic acid	Ę.	9	H	I Cuknown compounds	-	×
1	Pn Ol	3	1	- -	1	3	-	2	- 1	1	5	2	-	-	-	_	~ T	-	_	-	-	- т	-	-	_	_	-	-	2	1	_	-	-	-
î	Po	2	ī	_	2	3	_	3	_	3	5	3		_	_	_	_	_		_	_	_	_	_	_	_	_	_	2	1	_	_	_	_
1	Pn	2	1	_	2	4	_	3	_	-	5	2	_	_	_	_	_	_	_	_	_	_	_	_	_	-	_	_	2	1	_	-	_	_
1	Pn	1	T	-	5	4	_	1	_	_	5	2	-	_	-	_	-	_	-	-	_	_	_	-	-	_	-	-	2	1	_	-	_	-
1	Op	-	T	-	4	2	_	1	2	_	5	2	-	-	-	-	-	-	_	-	-	-		-	-	_	_		2	1	-	-	-	
1	Rp	T	T	-	3	5	-	3	-	2	5	2	-	-	-	-	-	_	1	1	-	-	-		-	-	-	-	1	-	-	-	-	-

Nature of flavonol glycosides remain the same in all samples.

* See footnote to Table 1.

TABLE 3. Eucalyptus and Angophora species containing stilbenes in the leaves

Blakely No.	Species	Section	Series (+sub-series)
17	Е. рариапа	Macrantherae	Corymbosae
18	E. grandifolia	Macrantherae	Corymbosae
19	E. clavigera	Macrantherae	Corymbosas
90	E. campaspe	Macrantherae	Obliquae
92	E. griffithsii	Macrantherae	Obliquae
108	E, macrandra	Macrantherae	Cornutae
134	E. dundasii	Macrantherae	Dumosae
147	E. rugosa	Macrantherae	Dumosae
151	E. kondininensis	Macrantherae	Dumosae
155	E. corrugata	Macrantherae	Dumosae
166	E. doratoxylon	Macrantherae	Decurvae
167	E. decurva	Macrantherae	Decurvae
205	E. melanoxylon	Macrantherae	Exsertae
224	E. angophoroides	Macrantherae	Globulares
236	E. dalrympleana ssp. dalrympleana	Macrantherae	Globulares
237	E. glaucescens	Macrantherae	Globulares
240	E. urnigera	Macrantherae	Globulares
263	E. nitens	Macrantherae	Globulares
270	E. baeuerlenii	Macrantherae	Viminales
274	E. smithii	Macrantherae	Viminales
305	E. guilfoylei	Renantherae	Ochroxylor
541	E. sideroxylon	Terminales	Rhodoxyla
550	E. melliodora	Terminales	Melliodora
564	E. gracilis	Graciles	Aridae
575	E. umbrawarrensis	Platyantherae	Subulatae
577	E. oleosa var, longicornis	Platyantherae	Subulatae
578	E. oleosa	Platyantherae	Subulatae
584a	E. brockwayi	Platyantherae	Subulatae
593	E. salmonophloia	Platyantherae	Leptopodae
	Angophora cordifolia	-	· <u>-</u>

TABLE 4. RELATIVE STRENGTHS OF THE MAIN COMPONENTS IN THE LEAVES OF EUCALYPTS AND AN ANGOPHORA POSSESSING STILBENOID CHEMOTAXA

			N		A	Acid-	hydro	olyse	d pro	duc	ts		In	alco	hol e	extra	ct¶
Blakely No.†	Type‡	Origin§	Number examined	D	С	P	M	Q	K	Е	G	Ā	As	Rh	Pi	Ch	C
17	Norm.	Do	3	_	2	_	5	4	1	3	5	_	_	_	_	2	_
	Var.	Jj	1	-	2	3	5	3	T	5	1	1	2	-	3	3	-
	Var.	Jj	1	-	T	5	5	3	-	4	5	-	2	-	1	3	-
	Var.	Hj	1	-	-	_	1	5	2	5	5	-	_	2	_	3	-
18	Var.	Ei	1	_	1	2	3	5	2	4	3	1	5	5	5	3	3
19	Var.	Ei	1	T	T	-	T	1	-	5	5	2	-	-	5	-	3
19a	Norm.	Do	3	2	2	_	5	3	2	3	5	2	_	_	_	2	2
	Norm.	Do	2	_	_	_	_	4	2	5	5	2	_	-	_	2	2
90	Var.	Me	1	1	1	-	2*	5*	2*	2	4	5	T	2	T	_	1
92	Var.	Me	1	_	_	_	_	5*	_	5	4	_	1	2	_	3	3
108	Var.	Lb	1	_	_	-	T*	5*	2	4	5		5	-	3	1	3
134	Var.	Me	1	_	T	_	_	3*	_	5	5	_	8	T	T	-	3
147	Norm.	Z	1	_	2	_	_	5	_	4	2	_	_	_	_	_	_
	Var.	Ok	1	_	1	_	_	3	_	5	3	_	5	2	1	4	4
151	Norm.	Me	1	_	_	_	_	3*	3	5	3	9		_	_	4	_
	Var.	Me	1	_	1	_	_	4*	T	5	5	9	1	4	3	Т	_
155	Var.	Z	1	_	_	_	2	5*	_	4	, 5	_	5	2	3	T	7
166	Var.	Ne	1	_	т	T	1	5	_	1	1	_	5	_	1	2	3
167	Var.	Nd	1	_		_	1	5	3	3	4	_	8	_	î	_	2
205	Var.	Z	î	_	т	_	_	3	_	5	2	_	8	1	ì	~	2
224	Var.	Õo	î	_	i	т	5	1	_	4	5	_	5	_	2	3	2
2,2,4	Var.	ž	î	_	_	_	_	ŝ	_	5	5	_	2	5	5	_	•
236	Var.	Op, Z	5	_	_	3	_	5	T	5	5	_	4	5	2	3	3
236a	Norm.	Z Z	ĭ	_	_	_	_	5	ŝ	2	3	_	-	_	~	4	3
237	Norm.	ž	î	_	_	_	_	3	_	5	3	_	-	_	_	3	ĭ
231	Var.	Ž	i	_	_	3	T	5	_	5	3	_	5	_	2	ĭ	3
240	Var.	Rp	2	_	т	_	•	5		5	4	_	ĭ	_	_	4	2
263	Var.	Ž	ĩ	_	Ť	2		5	_	4	5		5	_	3	2	2
270	Var.	ž	î	_	•	_	_	4	_	5	2	_	5	5	4	2	2
274	Norm.	Oq	6	3	2	_	2	2	_	5	5	_	_	_	_	_	-
214	Var.	Z	1	2	ī	_	2	5	-	5	5	_	5	1	3	3	2
305	Var.	Nc, Z	3	_	2	2	_	3	_	5	4	_	5	_	1	5	3
541	Norm.	140, 2	19	_	3	T	_	3	_	5	3	_	3	_	1	2	2
341	Var.	**	25	_	1	1	_	5	_	4	2	-	- Va	riabl	~ ~		
660				_	1	1	_	5	1	5	4	1	¥2	Tiabi	e, se		
550	Norm.	Oo, Op	1	1	1	2		2		5	4	3	5	_	_	2	2
	Var.	Lq	3	_	2	_	T 1	2 5*	_	4		2		3	T		2
564	Var.	Md	-	_	_	-	_	5*	T		5		2		T	2	
575	Var.	Fi	1	_	_	1	_		T	3	2	-	-	9	T	3	-
577	Norm.	Nc	2	-	-	_	T	2*	_	5	5	-	_	-	-	1	3
	Var.	Nc	3	_	_	-	T	3*	-	5	5	-	3	-	_	1	4
578	Norm.	Nm, Ol		-	-	-	-	2*	_	5	2	_	-	-	-	3	1
	Norm.	On	2	-	_	-	2	4*		5	3	2	_	_	-	1	3
50.	Var.	Ok	1	-	-	-	_	5*	-	5	2	_	2	1	T		1
584a	Var.	Me	1	-	-	-	_	1	-	5	2	~	8	-	T	T	3
593	Norm.	Nd	1	_	-	_	2	4*	4	5	5	7	_	_	-	1	2
	Var.	Nd	3	-	-	-	-	4*	-	5	2	-	5	4	1	1	2
	Var.	Nd	3		-	-	-	4*	-	5	2	-	2	5	_	1	2
	Var.	Nd	2	-	-	-	-	4*	-	5	2	-	5	_	3	1	2
	Var.	Nd	1	-	_	-	_	4*	_	5	2	_	1	5	3	1	2
A. cora	tifolia	Ор	1	-	1	_	2	5	-	4	5	-	5	-	T	3	-

[†] For species names see corresponding Blakely No., Table 3. In addition, 19a = E. confertiflora and 236a = E. dalrympleana ssp. heptantha. § Origin, see Fig. 1; ** see Ref. 4. ‡ Norm. = normal; Var. = variant. || D = delphinidin; C = cyanidin; P = "pelargonidin"; M = myricetin; Q = quercetin; K = kaempferol; *= abnormally coloured flavonol. E = ellagic acid; G = gallic acid; A = aromadendrin; ¶ As = astringin; Rh = rhapontin; Pi = piceid; Ch = chlorogenic acid; Cq = p-coumarylquinic acid. See also footnote to Table 1.

Table 5. Relative strengths of the main components in the leaves of eucalypts with variable flavonoid composition

Diale-1			Nineshaa			Acie	i-hyd	roly	sed p	rodi	icts‡		
Blakely No.	Species	Origin†	Number examined	D	С	P	M	Q	ĸ	E	A	В	G
19a	E. confertiflora	Do	3 2	2	2	_	5	3 4	2	3 5	T		
22	E. setosa	Do Jj	2 1	-	T	_	-	3	2	5	3 2 -	5 2	
52	E. watsoniana	In Kq	3 1	T T	2	-	2 2 5	3 2 4	T -	5 5 4	T	- 1	;
96	E. cornuta	Lq Z Z	1 1	T -	1	_	- 2	2 5	_	5	- 2 2	2	
98	E. gomphocephala	Mc, Z Z	2	- -	-	- -	4	5* 2*	-	5 5	3 2	1 3	
120	E. wandoo	Mc, Md Nc, Md	6	-	_	_	- -	4	- 2	5 5	1 2	3	
178	E. tereticornis	Do Do	3	3	2 2	- 2	5	3 2	т 2	2 5	5	- -	
210	E. ovata	Nq, Op On, Pn Z	2 4	1	1	-	4	4 5	3	5 2	1 -	2 T	
212	E. camphora	Ro Nq, Op Nq	1 4 1	- - 4	3 T 2	-	- - T	5 4 5	- 3 5	3 5 4	1	1	
277	E. viminalis	Oo, Po Ol, Pn, Z	8	-	T	-	-	3	-	5	2	2	
286a	E. apodophylla	Pn, Ro Fi Id	4 1 1	2	т 1	- -	- - T*	5 4 4*	1 -	5 5 5	3 2 -	1 - 5	
291	E. intertexta	Jj Kd	1 1	- 3 1	2 T	_	2 1*	3 4*	=	5 5	-	2	
318	E. eugenioides	Nq Lq Z	1 1 1	2	3	_	_	5 5 5	- -	2 3 4	- - 5	- 4	
324	E. caliginosa	Mq, Z Mq	2 1	1	2 1	=	2	5 5	- -	3	- -	- 3	
325	E. phaeotricha	Kq Lr	î 1	5 1	T T	-	- 1	2 2	_ 2	5	-	2	
371	E. sieberi	Pp, Oq Po	4	2	i	-	5	3	-	3	1	3	
420	E. risdonii	Ro Z Ro	2 2 1	- 4 1	- -	-	т 5 5	3 T 3	-	5 2 3	2 - -	4	
422	E. andrewsii	Z Lr	i 1	<u>-</u>	- 3	<u>-</u>	2 2	5 5	_	3	3	3	
484	E. moluccana	Oo Lq	2 2	- 1	1	_	- Т	4	1	5 5	3	3	
486	E. albens	Op Op	2	3	T -	- -	1	5 5	1	5 5	3	2	
541	E. sideroxylon	§ Lq	19 1	<u>-</u>	3 1	T _	<u> </u>	3 5	- 1	5 5	_	-	
542	E. leucoxylon	Pn Po, Op	1 2	1 1	2 1	_	- -	5 2	_	5 5	1	1 2	
561	E. calycogona	Md, Om Om	3 1	3	1 1	-	-	5* 4*	_	3 5	-	2	

 $[\]dagger$ Origin, see Fig. 1. \ddagger D=delphinidin; C=cyanidin; P="pelargonidin"; M=myricetin; Q=quercetin; K=kaempferol; \S See Ref. 4. E=ellagic acid; A and B=unknown compounds; G=gallic acid. * Represents the score of M or Q with an abnormal colour. See also footnote to Table 1.

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acids have been identified by chromatographic comparison and piceid and rhapontin by isolation.⁴¹ Recent work indicates that astringin contains 3,4,3',5'-tetrahydroxystilbene glucoside and perhaps other stilbenes.⁴²

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⁴¹ W. E. HILLIS and M. HASEGAWA, Biochem. J. 83, 503 (1962).

⁴² M. HASEGAWA and W. E. HILLIS, Unpublished data.