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THE DISTRIBUTION AND POTENTIAL TAXONOMIC VALUE OF ALKYLATED ELLAGIC ACIDS

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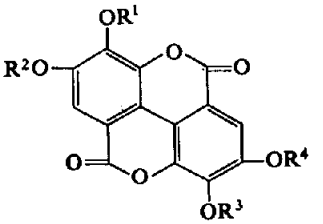
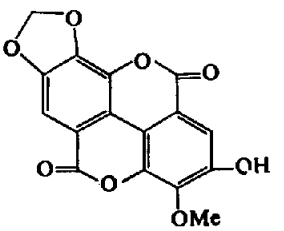
Abstract—Four alkyl ethers of ellagic acid have been shown to occur frequently in three families of the order Myrtales. The relative amounts of these compounds, as estimated from chromatograms, appear to constitute a definite taxonomic character. Other systematic implications are discussed.

INTRODUCTION

ELLAGIC acid (I) is a widespread constituent of dicotyledonous plants, both as the free compound and, as ellagitannins, in more or less complex combination with gallate and sugar residues. Numerous individual workers have isolated ellagic acid, and a chromatographic survey showed that it occurred in fifteen out of forty orders of dicotyledons.[1] It is particularly abundant in the Fagales and in the nine families comprising the group Myrtineae[2] of the Myrtiflorae, often co-occurring with myricetin and leucodelphinidin. There has therefore been some interest in this compound for its systematic significance,[3,4] both because of its distribution and also because of its relationship to flavonoid biosynthesis. Ellagic acid may be formed at the shikimic acid stage, or later, by a loss of two carbon atoms from a trihydroxycinnamic acid type precursor; the final step in its synthesis, oxidative linking of two gallate residues followed by lactonization, is known to take place easily under physiological conditions.[5] It occurs frequently in those plants where the trihydroxycinnamic acid related to myricetin might be expected to be present but has not been detected.[3]



(I) $R^1=R^2=R^3=R^4=H$
(II) $R^1=Me$; $R^2=R^3=R^4=H$
(III) $R^1=R^3=Me$; $R^2=R^4=H$
(IV) $R^1=R^2=R^3=Me$; $R^4=H$



(V)

[1] E. C. BATE-SMITH, *Chem. & Ind.* B.I.F. Rev, R.32 (1956).
[2] J. C. WILLIS, *A Dictionary of the Flowering Plants and Ferns*, 6th edition, University Press, Cambridge (1931).
[3] E. C. BATE-SMITH, *J. Linn. Soc.* (*Botany*) **58**, 95 (1962).
[4] R. E. ALSTON and B. L. TURNER, *Biochemical Systematics*, Prentice-Hall, Englewood Cliffs, N.J. (1963).
[5] D. E. HATHWAY, *Biochem. J.* **67**, 445 (1957).

In contrast to the known widespread distribution of ellagic acid, simple alkyl ethers of this compound have been isolated only rarely. Those known from plant sources are 3-*O*-methylellagic acid (II), 3,3′-di-*O*-methylellagic acid (III) and 3,3′,4-tri-*O*-methylellagic acid (IV). Reported cases of the isolation of these compounds are listed chronologically in Table 1. These show an apparent correlation with those plant taxa in which ellagic acid is abundant, most of the instances being from the Myrtiflorae. The relationship need not be inevitable since large-scale surveys of polyphenols have usually been carried out with acid-hydrolysed extracts. In the plant ellagic acid may actually be present combined with glucose and as esterified hexahydroxydiphenic acid.[3]

The apparently very restricted occurrence of II, III and IV suggests that these compounds may have some advantages over ellagic acid from the chemotaxonomic point of view. They are secondary metabolites, reasonably stable and so insoluble that they are unlikely to be involved to any extent in metabolic processes. This is supported also by their isolation from non-living tissue; i.e. bark and heartwood rather than leaves. Compounds II, III and IV therefore appear to fulfil the criteria of Erdtman[15] for compounds useful for

TABLE 1. PUBLISHED REFERENCES TO ISOLATION OF ELLAGIC ACID METHYL ETHERS

Species	Family	Compounds	Location	Reference
Euphorbia formosana	Euphorbiaceae	III	Roots	6
Eugenia maire	Myrtaceae	IV	Bark	7
E. maire	Myrtaceae	III, IV	Bark	8
Sonneratia apetala	Sonneratiaceae	III, IV	Bark	9
Leptospermum scoparium	Myrtaceae	II, III, IV	Bark	10
Terminalia paniculata	Combretaceae	III*	Wood	11
Tamarix gallica	Tamaricaceae	III	Roots	12
Anogeissus latifolia	Combretaceae	IV	Bark, wood	13
Nothofagus fusca	Fagaceae	III	Wood	14

* Isolated as the 4-glucoside.

chemotaxonomic purposes. Furthermore, although they are very difficult to separate by classical methods, they are readily separated on paper chromatograms and identified by characteristic fluorescence colours and R_f values.[8]

In order to investigate the potential taxonomic value of these compounds we have examined members of all families in Hutchinson's order Myrtales,[16] concentrating particularly on the most widely separated families, Melastomataceae and Myrtaceae. The distribution of II, III and IV within the plant and consistency of occurrence in a species were also investigated, the latter particularly in view of the report that ellagic acid content was very

[6] J. SHINODA and C. P. KUN, *J. Pharm. Soc. Japan* **51**, 502 (1931).
[7] L. H. BRIGGS, R. C. CAMBIE, J. B. LOWRY and R. N. SEELYE, *J. Chem. Soc.* 642 (1961).
[8] B. F. CAIN, *New Zealand J. Sci.* **5**, 390 (1962).
[9] S. N. SRIVASTAVA, D. S. BHAKUNI, V. N. SHARMA and K. N. KAUL, *J. Sci. Ind. Res.* **21B**, 549 (1962).
[10] B. F. CAIN, *New Zealand J. Sci.* **6**, 264 (1963).
[11] L. RAMACHANDRA ROW and G. S. R. SUBBA RAO, *Tetrahedron* **18**, 357 (1962).
[12] A. H. ISRAILI, R. C. SHARMA and A. R. KIDWAI, *Indian J. Chem.* **3**(1), 48 (1965).
[13] K. K. REDDY, S. RAJADURAI and Y. NAYUDAMMA, *Indian J. Chem.* **3**(7), 308 (1965).
[14] W. E. HILLIS and T. INOUE, *Phytochem.* **6**, 59 (1967).
[15] H. ERDTMAN, in *Chemical Plant Taxonomy* (edited by T. SWAIN), p. 89, Academic Press, London (1963).
[16] J. HUTCHINSON, *The Families of Flowering Plants*, Vol. 1, 2nd edition, Clarendon Press, Oxford (1959).

variable in *Nothofagus* heartwood.[17] The presence of III as the 4-glucoside in *Terminalia paniculata* heartwood[11] is somewhat surprising since most polyphenols in heartwood or bark occur as the free compounds.[18] In order to determine the frequency of such combination extracts were examined before and after acid hydrolysis.

In the course of this work a new compound with chromatographic and spectral properties very similar to those of IV was detected on chromatograms of many extracts. This was isolated and shown to be 3′-*O*-methyl-3,4-methylenedioxyellagic acid (V)[19] and its distribution was studied along with I, II, III and IV.

RESULTS AND DISCUSSION

General

All compounds I–V were readily detected by chromatography of crude extracts. In leaf extracts chlorophyll or waxy constituents sometimes interfered with IV and V, while delphinidin in acid-treated extracts sometimes tended to obscure I. Radial paper chromato-

TABLE 2. R_f VALUES OF ELLAGIC ACID AND ITS NATURALLY OCCURRING ETHERS

	Chromatographic system					
	(1) Radial paper*		(2) One-dimensional paper		(3) Cellulose thin-layer	
Compound	A	B	A	B	A	B
I	0·49	0·0	0·35	0·0	0·44	0·0
II	0·64	0·14	0·60	0·04	0·72	0·15
III	0·86	0·37	0·83	0·27	0·90	0·35
IV	—	—	0·92	0·71	0·95	0·71
V	0·93	0·6	0·92	0·79	0·95	0·79

* Results according to Cain.[8]

A. Forestal (conc. HCl–HOAc–H_2O; 3:30:10). B. Top phase of *n*-BuOH–EtOH–1·5 N NH_4OH; 4:1:3).

graphy[8] failed to resolve IV and V and was unsuitable for detecting small amounts of these compounds. One-dimensional paper chromatography gave satisfactory results but cellulose thin-layer chromatography was adopted throughout because of faster developing time, better resolution and the wider concentration range applicable. However R_fs were very variable and all extracts were co-chromatographed with reference samples. R_f values for compounds I–V using these three chromatographic techniques are presented in Table 2.

The amount of each compound present on a chromatogram was estimated visually and a score assigned from + (trace) to + + + + (very strong, yellow colour clearly visible in the ammoniacal solvent, trailing spot). The relative amounts of compounds present do not correspond to the observed scores because of differing fluorescence responses. Under 250 nm radiation III, IV and V appear bright blue to blue–purple while I and II have a softer deeper colour (and are almost invisible under 350 nm radiation). The absolute amount of each compound to give a score of + on thin-layer chromatograms were as follows: (I)

[17] W. E. HILLIS and H. R. ORMAN, *J. Linn. Soc.* (*Botany*) **58**, 175 (1962).
[18] J. B. HARBORNE, *Biochemistry of Phenolic Compounds*, p. 162, Academic Press, London (1964).
[19] J. B. LOWRY, to be published.

0·5 μg, (II) 0·2 μg, (III) 0·02 μg, (IV) 0·05 μg, (V) 0·05 μg. No attempt was made to "correct" the observed scores since it was the appearance of the chromatograms that was being considered as a possible taxonomic factor.

In most cases these polyphenols, if present, were found throughout the plant. This has been noted earlier for *Leptospermum scoparium*, but in *L. flavescens* they occurred only in old bark and heartwood. In *Clidemia hirta* and several other plants a characteristic pattern appeared when the stem section exceeded 8 mm dia.; in thinner stems, only I was detected. This consideration limits the use of herbarium material, a negative result perhaps being due to not testing sufficiently old tissue. However there was no indication that misleading results concerning the relative amounts of II, III and IV would be obtained by studying lighter woody parts, and cautious use was made of herbarium specimens. Other comparisons showed that there was little change in the amounts of these compounds in dried material on standing, at least for several years.

Investigation of specimens from various sources showed that in general the pattern of ellagic acid derivatives was a stable species characteristic. Variable results were obtained from *Eugenia malaccensis* and *E. javanica*. These are common Malayan village trees in which selection may have given rise to distinct varieties (cf. variation in chromones of *E. aromatica*[20]).

Systematic results. The data for *ca.* 160 species are presented in Table 3. Where the pattern for acid-treated extract differed significantly from the untreated extract the results for both are indicated, otherwise the table refers to untreated extracts. The first conclusion is that II, III, and IV are much more common, at least in this plant order, than the literature would indicate. Thus 3-*O*-methylellagic acid recorded only once previously, has now been detected in *ca.* 64 species. The symmetrical dimethyl ether III is the most common of all, in accordance with previous records (Table 1). Secondly, the amount of the alkylated compounds is of the same order as ellagic acid. Hence it would appear that ellagic acid is formed by the same process, rather than by additional pathways such as breakdown of tannins. However in many cases I did occur by itself. The relative amounts of II, III, IV and V are clearly related so that, for example, large quantities of II and III may co-occur but not II and IV. This is in accordance with the known difference in chemical behaviour between those hydroxyl groups in ellagic acid *meta* to carbonyl and those *para*.[21] Methylation of tetra-*O*-acetylellagic acid with dimethyl sulphate in acetone gives the acetates of II, III and IV and a similar difference was observed in the methylation of methyl gallate. The actual patterns observed tend to fall into two classes; those in which II and III preponderate over IV and V ("less alkylated"), and the inverse case ("highly alkylated"). The occurrence of these patterns will now be considered at the family level.

Myrtaceae. The "less alkylated" pattern predominated but there was considerable variation. This is not surprising for we are considering the expression of a minor character in a rather large family. There appears to be little distinction between the tribes and both patterns were expressed within some genera. *Eucalyptus* and *Eugenia*, both very large "difficult" genera, showed much variation. It is of interest to compare these with the apparent consistency of *Tristania*, a small genus of forest trees resembling both *Eucalyptus* and *Eugenia*. The recently separated genera *Lophomyrtus* and *Neomyrtus* are here further distinguished by their polyphenols. In only a few cases are the compounds present as glycosides.

All New Zealand members of this family, and all Malayan members with the exception of most of *Eugenia* were studied. The rest were collected mainly in Western Australia. It is

[20] TH. MEYER, *Rec. Trav. Chim.* **65**, 843 (1946).
[21] L. JURD, *J. Am. Chem. Soc.* **81**, 4606 (1959).

TABLE 3. DISTRIBUTION OF ELLAGIC ACID ETHERS IN THE ORDER MYRTALES

Family, tribe, genus and species‡	Natural habitat§	Compounds* I	II	III	V	IV	Location†
MYRTACEAE							
I. Myrtoideae							
1. Myrteae							
Acmena smithii Poir.	A	+++	−	+++	−	−	b
Decaspermum fruticosum Forst.	M	+	−	+	−	+	sh
Eugenia aquea Burm.	M	++++	++++	+++	−	−	b
E. aromatica Baill.	M	++++	−	+	+	+	b
E. densiflora var. *angustifolia* Ridl.	M	+	+	−	−	−	b
E. grandis Wight	M	+++	++++	+++	−	−	b
E. jambos L.	M, T.A.	++	++	+	−	−	b
E. javanica Lam.	M	+++	++++	+	−	−	b
E. longifolia DC.	M	+++	+	−	−	−	b
E. maire A.Cunn.	N.Z.	++	−	++	−	++++	b
E. malaccensis L.	M	++	+++	++++	−	−	b
E. pachyphylla Kurz	M	++	+	−	−	−	w
E. palembanica Merr.	M	++	++	+	−	−	b
E. polita King	M	+++	−	−	−	−	b
E. subdecussatata var. *montana* Duthie	M	+++	−	++	−	−	b
E. wrayi King	M	++	−	−	−	−	b
E. xanthocarpa Thw.	M	−	−	++++	−	++++	b
Lophomyrtus bullata (Sol. ex A.Cunn.) Burret.	N.Z.	+++	+++	++	+	+	b
L. obcordata (Raoul) Burret.	N.Z.	++	+++	++++	+	+	b
Neomyrtus pedunculata (Hook f.) Allan	N.Z.	+	+	+++	++++	++++	b
Psidium cattleyanum Weinw.	Af	++++	++	+	+	+	b
P. guajava L.	T.A.	+	++	+++	−	−	b
Rhodamnia cinerea Jack.	M	++	−	−	−	−	w
R. trinervia Bl.	M	++	+	+	−	−	w
Rhodomyrtus tomentosa Wight (hydrolysate)	M	++	−	−	−	−	w
		+++	−	−	++	+++	
II. Leptospermoideae							
1. Leptospermeae							
Agonis flexulosa (Spreng.) Schau.	A	−	−	+	−	−	b
(hydrolysate)		++	+	+	−	−	
Astartea fascicularis (Labill.) DC.	A	+++	−	+++	−	−	b
Baekea camphorosmae Endl.	A	++	+	+	−	−	b
B. frutescens L.	M	+++	−	++	−	+	b

* Listed in order of increasing R_f.
§ A = Australia, Af = Africa, M = Malaysia, N.Z. = New Zealand, T.A. = Tropical America.
† b = bark, w = wood, s = stem, sh = stem from herbarium specimen.
‡ Families subdivided according to Willis.[2]

TABLE 3—*continued*

Family, tribe, genus and species‡	Natural habitat§	Compounds* I	II	III	V	IV	Location†
Callistemon phoeniceus Lindl.	A	+++	+	+++	–	–	b
C. lanceolatus Sweet	A	++	–	++++	–	+	b
Calothamnus quadrificus R.Br.	A	+++	–	–	–	–	b
Eucalyptus botyrioides Sm.	A	–	–	–	–	–	b
E. calophylla R.Br.	A	+++	–	–	–	–	b
E. citriodora Hook.	A	++	+	++++	–	–	b
E. deglupta Bl.	M	++++	–	+	–	–	b
E. ficifolia F. Muell.	A	++	–	–	–	–	b
E. redunca Schau. var. *elata* Benth.	A, M	+	++	–	–	–	b
E. saligna Sm.	A	++++	–	++	+++	++	b
E. torquata Luehm.	A	+	–	–	–	–	b
E. triantha Link	A	++++	++	–	–	–	b
E. youngiana F. Muell.	A	++	–	+	–	+	b
Leptospermum ericoides Rich.	N.Z.	++	+	+++	++++	++++	b
L. flavescens Sm.	M	++	+	++	+++	++	b
L. laevigatum F. Muell.	A	+++	+	++	–	–	b
L. scoparium Forst.	N.Z.	+++	+	+++	++++	+++	b
Melaleuca huegelii Endl.	A	+++	–	++	–	–	b
(hydrolysate)		+++	+	+++	–	++	
M. lanceolata Otto	M	++	–	+++	–	+	b
M. leucodendron L.	M	+++	+	–	–	–	b
(hydrolysate)		+++	+	–	–	++	
M. styphelioides Sm.		++	++	++	–	–	b
Meterosideros carminea Oliv.	N.Z.	+++	–	++++	+	+++	b
M. colensoi Hook. f.	N.Z.	+	–	++	+	–	b
M. excelsa Sol.	N.Z.	+++	++	++	–	–	b
M. fulgens Soland.	N.Z.	+++	+++	++++	+	+	b
M. perforata J.R.	N.Z.	+++	+++	++	–	–	b
M. robusta A.Cunn	N.Z.	+++	–	++++	–	+	b
M. umbellata Cav.	N.Z.	+	–	++	–	–	b
Myrtella bennigseniana (Volkens) Diels	M	+++	+	+++	–	+	sh
Tristania conferta R.Br.	A	–	+	+++	–	+	b
T. merguenensis Griff.	M	++	++	+++	–	–	b
T. obovata R.Br.	M	++	–	–	–	–	b
(hydrolysate)		++	++	++	–	+	
T. whiteana Griff.	M	+++	++	++++	–	–	b
2. Chamaelaucieae							
Chamaelaucium uncinatum Schau.	A	++	–	+	+	+	b
Verticordia densiflora Lindl.	A	+++	–	–	–	–	b
LECYTHIDACEAE							
Barringtonia acutangular Gaertn.	M	++	++	+++	–	–	b
B. macrostachya Kurz.	M	++++	++	+	–	–	sh
B. racemosa Roxb.	M	+++	+++	++++	–	+	b

TABLE 3—*continued*

Family, tribe, genus and species‡	Natural habitat§	Compounds* I	II	III	V	IV	Location†
B. schortechinii King	M	+++	+++	++	–	–	b
B. sumatrana Miq.	M	++	–	+	–	–	sh
Bertholettia excelsa Berg.	T.A.	++++	+++	++	–	–	b
Chydenanthus excelsus Bl. (Miers)	M	+	+	++++	–	–	b
Couropita guianensis Hook.	T.A.	++++	+++	–	–	–	b
Gustavia augusta DC.	T.A.	–	–	–	–	–	b
G. gracilliana Miers	T.A.	–	–	–	–	–	b
Lecythis ollaria L.	T.A.	++++	–	++	–	–	b
Planchonia valida (Bl.) Bl.	M	+	–	+++	–	–	b
RHIZOPHORACEAE							
Anisophyllea disticha Baill.	M	+	–	–	–	+	sh
Bruguiera gymnorhiza Lam.	M	+	–	+	–	–	w
Rhizophora stylosa Griff.	M	+	–	++	–	–	b
SONNERATIACEAE							
Sonneratia alba Sm.	M	++	+	++	–	++++	sh
S. caseolaris (L.)Engl.	M	+	–	–	–	–	sh
S. griffithii Kurz.	M	+++	++++	++++	–	++	b
PUNICACEAE							
Punica granatum St.Lag.	M, T.A.	+	–	–	–	–	b
(hydrolysate)		+	–	+	+++	+++	
COMBRETACEAE							
Lumnitzera littoralis Voigt	M	++	++	–	–	–	b
Terminalia catappa L.	M	+	–	+	–	+	b
T. phellocarpa King	M	–	–	–	–	–	w
MELASTOMATACEAE							
I. Melastomatoideae							
Osbeckiae							
Melastoma malabathricum L.	M	++	–	–	–	–	b
M. molle Wallich	M	–	–	+	+	–	sh
M. muticum Ridl.	M	+	–	–	–	–	w
M. sanguineum Sims	M	++	++	–	+	–	w
M. shizocarpa Ridl.	M	++	–	–	–	–	w
Osbeckia cupularis D.Don ex Wight & Arn.	T.A.	++++	–	–	–	+	sh
O. octandra DC.	T.A.	–	–	+	–	–	sh
(hydrolysate)		–	–	+	–	+++	
O. whiteana Benth. ex Wight & Arn.	T.A.	++++	–	–	–	+	sh
Oxysporeae							
Allomorphia malaccensis Ridl.	M	++	++	–	–	–	w
(hydrolysate)		++	++	–	+++	++	

TABLE 3—*continued*

Family, tribe, genus and species‡	Natural habitat§	Compounds* I	II	III	V	IV	Location†
Anerincleistus pauciflora Ridl.	M	+	+	–	–	–	sh
A. sublepidotus King	M	+++	–	++	–	–	sh
Ochthocharis sylvestris Ridl.	M	+++	–	–	–	–	sh
(hydrolysate)		+++	++	–	–	–	
Oritrephes grandiflora (Ridl.) Ridl.	M	–	–	–	–	+	w
(hydrolysate)		+++	+	–	–	+++	
Oxyspora hispida Ridl.	M	++++	–	+++	–	–	w
Sonerileae							
Phyllagathis griffithii King	M	–	–	–	–	–	sh
P. hispida King	M	+++	+	+	–	–	s
(hydrolysate)		+++	+	++	–	+	
P. rotundifolia Bl.	M	+	+	++	++++	+++	s
P. tuberculata King	M	+++	–	+	–	+	sh
Sarcopyramis nepalensis Wall.	M	++	–	–	–	–	s
Sonerila albiflora Stapf	M	–	–	–	–	–	s
S. begoniaefolia Bl.	M	+	–	–	–	–	sh
S. erecta Jack.	M	+++	–	–	–	–	s
S. heterostemon Naud.	M	+	–	–	–	–	s
S. nidularia Stapf	M	+	–	–	–	–	s
S. prostrata Ridl.	M	++++	–	–	–	–	s
S. rudis Stapf	M	+	–	–	–	–	s
Medinilleae							
Anplectrum divaricatum Triana	M	+	–	+	–	+	w
A. glaucum Triana	M	+	–	–	–	++	w
Dissochaeta annulata Hook. f.	M	+	–	–	–	++	w
D. bracteata Bl.	M	–	–	–	++	++	w
D. celebica Bl.	M	–	–	–	–	–	sh
D. gracilis Bl.	M	–	–	–	–	–	sh
D. sagittata Bl.	M	+++	–	–	–	+	w
Marumia muscosa (Bl.) Bakh. f.	M	+++	–	–	+	+	w
M. nemorosa (Bl.) Flor.	M	++	–	–	–	++	w
M. rhodocarpa Cogn.	M	–	–	–	+	+	sh
Medinilla clarkei King	M	+++	–	–	–	–	sh
M. crassinervia Bl.	M	+++	–	–	–	–	w
(hydrolysate)		+++	–	–	–	++	
M. heteroanthera King	M	+++	–	–	+	+	w
M. maingayi Clarke	M	–	–	+	++	+++	w
M. schortechinii King	M	+++	+	+++	–	++	w
Pachycentria tuberculata Korthals	M	+	–	–	–	–	sh
(hydrolysate)		+	–	+	–	+	
Pogonanthera pulverulenta Bl.	M	+++	+	+	+++	+++	w

TABLE 3—*continued*

Family, tribe, genus and species‡	Natural habitat§	Compounds* I	II	III	V	IV	Location†
Miconieae							
Clidemia hirta Don	T.A.	++	+	++	+++	+++	w
C. neglecta D.Don	T.A.	+	−	+	−	−	sh
C. rubra (Aubl.) Mart.	T.A.	+	−	−	+++	++	sh
Miconia albicans (Sw) Triana	T.A.	++++	++	+	+++	+++	sh
M. attenuatus DC.	T.A.	+++	+	+	+++	+++	sh
M. cilliata (Rich.) DC.	T.A.	+	+	+	−	+++	sh
M. laevigata (L.) DC.	T.A.	++	−	+	+++	+++	sh
M. macrothyrsa Benth.	T.A.	++	−	−	++	−	sh
M. prasina (Sw.) DC.	T.A.	++	+	−	+++	+++	sh
M. tamonea (Sw) Proctor	T.A.	++++	−	−	+++	+++	sh
Lasiandreae							
Acisanthera quadrata Guss. ex. Poir.	T.A.	++	−	−	−	−	sh
A. recurva (L.C.Rich.) Griseb.	T.A.	—	—	—	—	—	sh
Comolia lythrioides Naud. J. C.	T.A.	−	−	+	−	−	sh
C. vernicosa Triana	T.A.	−	−	−	−	−	sh
C. veronicaefolia Benth.	T.A.	++++	−	+	−	−	sh
C. vilosa (Aubl.) Triana	T.A.	+	−	−	−	−	sh
Nepsera aquatica (Aubl.) Naud.	T.A.	++++	++	−	−	−	sh
Tibouchina semidecandra Cogn.	T.A.	+++	−	+	−	−	w
Other species							
Creaghiella purpurea Stapf	M	++	++	−	++	+	sh
Dissotis rotundifolia (J. E. Smith) Triana	Af	+	−	−	−	++	s
Pterolepis glomerata (Rottb.) Miq.	T.A.	+	−	−	−	−	sh
(hydrolysate)		+	−	−	−	+	
II. Astronioideae							
Astronia smilacifolia Triana	M	+	+++	+	++	++	w
Kibessia hirtella Cogn.	M	+	+	++	+++	+++	sh
Pternandra coerulescens Jack.	M	+	−	+	++	++	sh
P. echinata Jack.	M	++	+	+	+++	+++	b
III. Memecyloideae							
Memecylon garcinioides Bl.	M	+++	++	+	+++	+++	w

interesting to note that almost all plants showing the highly alkylated pattern, here considered unusual in the Myrtaceae, came from New Zealand, or the Indo–Malaysian region. These areas are fringe habitats for the Myrtaceae, most members being found in the warm semi-arid regions bordering the tropics, and particularly in Australia (cf. the correlation between geography and flavonoid chemistry in the genus *Eucryphia*[22]).

Lecythidaceae. This is a small family which Bentham and Hooker included in the Myrtaceae, but most subsequent taxonomists have regarded as separate. The pattern of ellagic acid derivatives is very uniform in the species examined. In only one case was a trace of a 4-substituted compound observed, yet II and III were usually present in substantial amounts. All were absent from *Gustavia* including ellagic acid itself, even after acid hydrolysis.

Rhizophoraceae to *Combretaceae.* Only a few species were collected in these four families. The results suggest that ellagic acid ethers occur in the majority of species, and that III is the most common.

Melastomataceae. This is a large family with a pan-tropical distribution and with species adopting a wide variety of habits and specialized forms. In most of the herbaceous species ellagic acid derivatives were not present in significant amounts. Nevertheless results for *ca.* 70 species examined showed an overall pattern clearly distinct from those for the preceding families. The "highly alkylated" pattern was typical. Compounds IV and V usually showed up more strongly than III and if not were almost always present with it. Only in two cases did III attain a score of +++, compared with twenty-two cases in the Myrtaceae. This relative lowering of the incidence of III contrasts also with the preceding results in Table 1, and with those for other species of the remaining families of the Myrtales. There appears to be some correlation between occurrence of these compounds and sub-divisions of the family. The Astroniodiae and the Miconieae and Medinilleae within the Melastomatoideae showed consistently the "highly alkylated" pattern. In the Osbeckiae and the Lasiandreae they occurred only in traces and formed no pattern. Rather more compounds were detected after acid hydrolysis than in the Myrtaceae.

Taxonomic value. When it is possible to detect and measure relative amounts of related compounds the chemotaxonomic approach may yield valuable results as, for example, with the homologous hydrocarbons of leaf waxes.[23] In the case of polyphenols most studies have used compounds with substantial differences in chemical structure. The underlying characters looked for were such features as the presence or absence of the vicinal trihydroxy system.[3] However, recent work has shown in the case of anthocyanins that the patterns of glycosidic combination may be of more interest than the distribution of the aglycones.[24] It may be that similar interest will obtain with alkyl ethers of familiar polyphenols but so far there appears to have been little use of alkylation patterns as a taxonomic character, although highly *O*-alkylated flavonoids are characteristic of certain families, e.g. Rutaceae. The difficulty is probably that in the flavonoids each hydroxyl group is in a distinct chemical environment; there are a variety of possible monomethyl ethers for each compound and no simple chemical relationship links them. In the case of ellagic acid ethers it is easier to interpret the pattern of occurrence of I, II, III and IV, since there are only two classes of hydroxyl groups. The ratios of IV:III:II:I should indicate either the effectiveness of the methylating system if it operates off I itself; or the balance between methylation of the gallate

[22] E. C. BATE-SMITH, S. MARGARET DAVENPORT and J. B. HARBORNE, *Phytochem.* 6, 1407 (1967).
[23] G. EGLINTON and R. J. HAMILTON, in *Chemical Plant Taxonomy* (edited by T. Swain), Academic Press, London (1963).
[24] J. B. HARBORNE, in *Chemical Plant Taxonomy* (edited by T. SWAIN), Academic Press, London (1963).

precursor and the oxidative coupling reaction. In either case it represents a characteristic biosynthetic attribute of the plant, one which is minor in a taxonomic sense, but which can be estimated independently of other taxonomic features. The phylogenetic significance remains to be seen. Some workers have regarded more elaborate compounds as being more "advanced", and this would then be in accord with Hutchinson's ranking of the Melastomataceae and Myrtaceae.

3'-O-*Methyl*-3,4-*Methylenedioxyellagic acid.* The distribution of this compound is of some interest because it is so closely related to that of IV. Although the latter compound was frequently detected in substantial amounts without any V being present, the converse was never observed. When V occurred unaccompanied by IV it was only in trace amounts. This strongly suggests that V is formed from IV. However the only biosynthetic mechanism known at present for formation of methylenedioxy groups involves oxidative cyclization of the corresponding *o*-methoxyphenol.[25] Therefore IV and V should each be formed independently from III, IV by methylation of the less reactive 4-hydroxyl group and V by oxidative cyclization. If so it is hard to account for the close correlation between the distribution of IV and V, and it may be that an alternative biosynthetic pathway is available in the Myrtales for the formation of methylenedioxy groups.

EXPERIMENTAL

Collection of Material. Where possible bark or wood from mature trees was used. In those species which were slender climbers or had very thin bark the oldest accessible woody tissue was used. With herbaceous species all parts were examined.

Preparation of extracts. (1) Dried milled bark (5–10 g) was extracted with methanol (soxhlet) for 20 hr. After removal of solvent the residue was dissolved in dimethylformamide (5–10 ml). Insoluble matter (usually waxy and insoluble in aqueous sodium hydroxide) was neglected.

Hydrolysis. A small portion of the residue after removal of methanol was treated with 2 N HCl at 100° for 2 hr, the acid removed under vacuum and the residue dissolved in a few drops of dimethylformamide.

(2) Plant material from herbarium specimens, usually a fragment (*ca.* 200 mg) of stem, was broken up manually and heated with methanol (150 ml) under reflux for 20 hr. The methanol solution was decanted, evaporated to dryness, and the residue heated with a few drops of dioxan. After chromatograms had been obtained from this solution it was acid treated as in (1).

Chromatography. Glass plates (20 × 20 cm) were spread with cellulose powder (MN300, Machery Nagel and Co.) at a thickness of 250 μ. Solvent mixtures employed were those described by Cain (see Table 2). Tank equilibration was not necessary using Forestal but had some effect on R_f in the ammoniacal solvent. In each case several spots containing 0·5–2·0 μl of solution were applied, at least one spot containing appropriate reference compounds. Authentic samples of I were obtained commercially (Koch-Light and Co.). Published methods[10,16] were used for preparing II, and III and IV. A sample of the latter compound was also obtained from a previous investigation.[5]

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[25] D. H. R. Barton, G. W. Kirby and J. B. Taylor, *Proc. Chem. Soc.* 340 (1962).