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VARIATION IN THE CHEMICAL COMPOSITION OF *EUCALYPTUS SIDEROXYLON*

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Abstract—*Eucalyptus sideroxylon* leaves collected throughout the habitat of this tree were of two classes. The polyphenol aglycones in the leaves collected from different samples of the normal form of the tree were very similar in nature and amount but the composition of the glycosides was generally characteristic of a geographical region. The latter compounds included the 3-glucoside, 3-rhamnoside, 3-rutinoside of quercetin, the 3-rhamnoside and 3-rutinoside of kaempferol; catechin, epicatechin, shikimic, chlorogenic and *p*-coumarylquinic acids and some unidentified products were also present. Leaves of the second class were characterized by large amounts of stilbenes, chlorogenic and *p*-coumarylquinic acids and in some cases dihydrokaempferol 3-rhamnoside; several of the polyphenols in the first class were also present. The leaves of the second class were collected from trees recognized as variant forms and in two areas trees of both forms have been found growing close together. Both forms contain leucocyanidin and esters of gallic, ellagic and gentisic acids. A new flavone, 5,4′-dihydroxy-7-methoxy-6,8-dimethylflavone was isolated from a variant sample and the 4′-methoxy derivative was detected in both forms. The wood extractives of both forms were identical and contained resveratrol and another hydroxystilbene and their glucosides.

While the composition of the flavonoids in the leaves of different variant forms remains relatively constant the major stilbene in leaves of the second class varies; it can be the 3-glucoside of either 3,4′,5-trihydroxy-, or 3,3′,5-trihydroxy-4′-methoxy- or another hydroxy-stilbene.

INTRODUCTION

Eucalyptus sideroxylon A. Cunn. ex Woolls[1] (Family Myrtaceae) is found in relatively small areas throughout a region of about 800 miles (North to South) by 500 miles in eastern Australia. The main locations are inland Victoria and inland New South Wales but separated from these are isolated occurrences along the coast of these two states. The tree sometimes grows in small almost pure stands, but usually is scattered. It is typically found on poor, shallow soils. The inland plains of New South Wales have a low annual rainfall (12–15 in.), whereas most of the other areas have up to double this amount.

The existence of a different physiological form or chemical variety of *E. sideroxylon*, which contains stilbenes in the leaves, has been previously reported.[2] An examination of the polyphenols in the leaves of more than two-thirds of the species of the genus *Eucalyptus*[3] and most of those of the *Angophora*[3] (Family Myrtaceae) has shown that the presence of stilbenes has little taxonomic significance. The composition of the polyphenols in the *E. sideroxylon* leaves which do not contain stilbenes are similar to those of closely related species so that accordingly these leaves are recognized as belonging to the normal form of the tree.

The trees originally examined[2] were grown from seed of unknown origin. At that time, collection of leaves from different localities in Victoria failed to produce samples containing

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[1] L. A. S. JOHNSON, *Contr. N.S.W. Natn. Herb.*, 3, 103 (1962).
[2] W. E. HILLIS and M. HASEGAWA, *Biochem. J.* 83, 503 (1962).
[3] W. E. HILLIS, In preparation.

stilbenes. In order to ascertain the possible origin of the above seed, further samples have been collected from trees growing throughout its natural habitat, and variant forms were found.

Leaves of the normal and variant forms were examined to establish the identity of unidentified components so as to permit subsequent biosynthetic studies. Wood and kino samples were also compared chromatographically to determine if the effects of variation were evident in other parts of the tree.

RESULTS

Leaf Extractives

The compounds isolated from *E. sideroxylon* leaves are listed in Table 1 together with some of their properties and their identity is given in Table 2. Polyphenols 6 and 7 were present in larger quantities in normal than in variant leaves and the normal form yielded sufficient material for identification.

TABLE 1. PROPERTIES OF ISOLATED POLYPHENOLS

	R_f value in			Spectral properties λ_{max} in 95% EtOH (mμ)				
Polyphenol	BAW	6HOAc	PhOH	Alone	+ 5% $AlCl_3$	NaOEt	+ Satd with NaOAc	+ Satd. with NaOAc H_3BO_3
1	0·92	0·00	0·92	280, 293*, 332	351, 400†	277, 337, 400	280, 293*	—
2	0·79	0·52	0·72	294	318, 388	332	333	—
3	0·73	0·30	0·50	259, 266*, 353	357, 401*	402	269	375
4	0·58	0·18	0·50	261, 303, 364	360*, 410	414	269	387
5	0·80	0·33	0·68	267, 348	346, 390*	399	275	270, 352†
6	0·44	0·40	0·40	260, 302†, 364	363, 398	415	270	—
7	0·50	0·40	0·62	269, 302†, 353	348, 400	400	275	356
8	0·67	0·42	0·63	288	311, 350†	300	289, 327	290, 329*
8A	0·91	0·07		289	309	325	292, 322	290, 330*
9	0·44	0·09		309, 324	—		309, 324	—
10	0·29	0·07		307, 325			307, 322	—
11	0·20	0·05		260, 305, 324	—	—	260, 324	260, 310, 345

* Inflection. † Broad band.

Polyphenol 1 has been identified as a new compound 4',5-dihydroxy-7-methoxy-6,8-dimethyl flavone (*sideroxylin*). Eucalyptin (5-hydroxy-4',7-dimethoxy-6,8-dimethylflavone)[4] was identified by thin-layer chromatography, and found to be present in small amounts in the leaf wax of both normal and variant forms. Sideroxylin could not be detected in the wax of either. Chromatographic examination of a fresh leaf extract showed that a compound with the properties of polyphenol 5 (tentatively identified as kaempferol 3-rhamnoside) was present. Consequently this compound did not arise by dehydrogenation of polyphenol 2

[4] D. H. S. HORN and J. A. LAMBERTON, *Chem. & Ind. (London)* 691 (1963).

TABLE 2. IDENTIFICATION OF ISOLATED POLYPHENOLS

Polyphenol	Identified as	M.p. and mixed m.p. (°C)	Yield (%)
1	4',5-Dihydroxy-7-methoxy-6,8-dimethyl flavone (sideroxylin)	286–7	0·01*
2	Dihydrokaempferol 3-rhamnoside (engelitin)	172–5	0·44*
3	Quercetin 3-rhamnoside	182–5	0·17*
4	Quercetin 3-glucoside	221–2	0·08*
5	Kaempferol 3-rhamnoside	—	0·002*
6	Quercetin 3-rhamnosylglucoside	186–195	0·40†
7	Kaempferol 3-rhamnosylglucoside	187–190	0·07†
9	3,4',5-Trihydroxystilbene 3-glucoside (piceid)	—	—
10	3,3',5-Trihydroxy-4'-methoxy stilbene 3-glucoside (rhapontin)	—	—
11‡	Hydroxy stilbene 3-glucoside (astringin)	—	—

* Calculated yield from dried leaves of variant sample "Gilgandra No. 1".
† Yield from dried leaves of normal sample "Heathcote".
‡ See text for details of the characteristics of this compound.

(dihydrokaempferol 3-rhamnoside; engelitin) during isolation. Polyphenol 8 was also present in trace amounts and appears to be an isoflavanone glucoside.

Piceid (3,4',5-trihydroxystilbene 3-β-D-glucoside) and rhapontin (3,3',5-trihydroxy 4'-methoxystilbene 3-β-D-glucoside) have been previously isolated from this source.[2] The instability of polyphenol 11, (astringin) prevented adequate purification and complete characterization, but most of the evidence indicates that it is the 3,3',4',5,5'-pentahydroxy-stilbene 3-β-D-glucoside. However, the melting point and R_f values of the acetate of the aglucone differ from those of authentic acetate and the examination of astringin is being continued.

In addition to the compounds listed in Tables 1 and 2, catechin, epicatechin, chlorogenic, *p*-coumarylquinic and shikimic acids were identified by co-chromatography using two-dimensional paper chromatograms. Other polyphenols were also present but in amounts too small to permit identification.

Two-dimensional chromatograms showed that though there was much variation in composition of the glycosides and other polyphenols in different samples, there was a certain degree of uniformity in those from the Gilgandra area (Table 3).

Leaf Aglycones

In almost all cases, the major aglycones detected in the acid-hydrolysates from leaves of the normal form from different localities were the same and were present in about the same amount. There was some variation in the minor components in samples from different areas. The amounts of phenolics relative to the main component (ellagic acid = 5) on a chromatogram developed with Forestal solvent in the leaves from the coastal regions of eastern Australia was close to: cyanidin (from leucocyanidins, 3), quercetin (3), kaempferol (1), ellagic acid (5), ferulic acid (1), gentisic acid (2) and gallic acid (5); for unidentified components, *A* (see Experimental) was always present (3), the component of provisionally named *macrantherin* was usually present (2), and the related substance *C* was also usually present (1). The samples from Lorne (Victoria) and the near-by area of Airey's Inlet differed in that the quercetin had a score of 1–2. The samples of leaves from Queensland differed from the others

TABLE 3. RELATIVE AMOUNTS OF COMPONENTS DETECTABLE IN CHROMATOGRAMS OF *E. sideroxylon* LEAVES

Origin	Type	No. of samples	Component*†										
			Stilbenes			Other components							
			9	10	11	2	3	4	6	p.Cq	Chl.	Cat.	eC
Baradine	Variant	4	3	5	3	0¶	2	0	0	5††	4**	2	0
		4	1	5	3								
Gilgandra	Variant	1	5	0	2								
		3	3	0	5								
		2	1	0	5								
		2	5	5	2	5	5	2‡	1	5	4	0§	0
		2	4	5	3								
		2	3	5	5								
Heathcote	Variant	2	3	5	3								
		2	5	5	2	0	0	2	2	5	5	5	0
	Normal	2	0	0	0	0	0	4	4	4	3	5	0
Airey's Inlet	Variant	1	1	5	4	0	2	0	0	1	1	1	3
	Normal	3	0	0	0	0	2	0	0	0	0	3	5
Lorne	Normal	1	0	0	0	0	2	0	0	0	0	3	5
Stanthorpe	Normal	1	0	0	0	0	3††	4	4	1	1	5	0
"Coastal"‡‡	Normal	12	0	0	0	0	2	0	0	0	0	3	5
Garden variety *rosa*	Normal	1	0	0	0	5	3	2	0	2	3	3	0

* The component with the largest spot area and intensity of fluorescence was given a score of 5 and the others related to it. † See Table 2 for identity of numbers; p.Cq = *p*-coumarylquinic acid; Chl. = Chlorogenic acid; Cat. = Catechin; eC = epiCatechin. ‡ Absent in 6 samples. § Score 2 in 4 samples. ¶ Score 2 in 3 samples. ¶ Score 4 in 2 samples. ** Absent in 2 samples. †† Other flavonol glycosides also present. ‡‡ See Experimental for location.

in that the anthocyanidins detected were delphinidin (3) and cyanidin (1), and also that myricetin (1) was present.

The presence of stilbenes in the variant leaves hindered the assessment of the relative amounts of several of the above aglycones. The score for cyanidin was 1, quercetin (5), ellagic acid (4), gentisic acid (2), and gallic acid (2) and component A was present in trace amounts. A component similar in properties to pelargonidin was present with a score 1.

Heartwood Extractives and Kino

Two-dimensional chromatograms of heartwood extractives from sixteen variant forms and five normal forms were almost identical. Large amounts of resveratrol (3,4',5-trihydroxystilbene) piceid and ellagic acid, moderate amounts of gallic acid, catechin and ellagitannins of unknown constitution and traces of astringin aglucone were present. An ellagic acid-like compound of unknown constitution was also present but rhapontigenin (3,3',5-trihydroxy 4'-methoxystilbene) could not be detected.

Similar chromatograms were obtained on the examination of heartwood extractives of *E. accedens*, *E. decorticans*, *E. le souefii*, *E. foecunda*, *E. salubris*, *E. torquata*. The extractives of *E. dundasi*, *E. anceps* and *E. incrassata* contained in addition small amounts of astringin (polyphenol 11).

Chromatographic examination failed to resolve the kinos from both the normal and physiological forms.

DISCUSSION

The composition of the polyphenols in *E. sideroxylon* leaf samples varied in two ways (*a*) some samples contained stilbenes in large amounts (*b*) the composition of the flavonoid glycosides differed significantly in samples from different localities.

Leaf Stilbenes

The ratio of the different stilbenes varied considerably in the samples from one area, and there was no composition that could be considered as characteristic of a locality. Seeds were collected from each of the sample trees in the Gilgandra district (a low rainfall area), and from them seedlings were grown in a glass-house under moist conditions. After two years, the composition of the leaves was the same as the parent tree.

All the samples from the inland plains of New South Wales contain appreciable quantities of stilbenes. Similar samples were found at Heathcote and Airey's Inlet, Victoria, but in these cases both normal and variant forms were growing within 100 yd of each other. The proximity of the normal and variant forms indicates that the formation of stilbene is not entirely due to a semi-arid environment as suggested by the above and other[3] evidence. Two sub-species of *E. sideroxylon* have been recognized[1] and while the variants appear to belong to ssp. *sideroxylon*, not all the samples of the latter contained stilbenes. Variation in leaf and fruit size is not related to the occurrence of stilbenes.

Examples of physiological forms or chemical varieties of eucalypts having greatly differing compositions of essential oils have been known for sometime.[5] Up to four different forms of some species exist with no significant variation in morphological characteristics. In at least two cases different forms have been found growing within a few feet of each other.

Leaf Flavonoids

With the exception of the Queensland (Stanthorpe) sample, the ratio of the aglycones quercetin and kaempferol, ellagic, gallic and gentisic acids to each other was relatively constant in samples of the normal form. However, there was a big difference in the relative amounts of the different quercetin glycosides, engelitin (dihydrokaempferol 3-rhamnoside), catechin and epicatechin in samples from different areas, and the composition of these polyphenols appeared to be characteristic of a region.

Although samples were collected from a large area in the Gilgandra district, normal forms were not found, and all samples contained engelitin. (Its presence in *E. sideroxylon* var. *rosa* may not be significant as this was a garden specimen and the extent of hybridization unknown.) Most of the other species in the section Terminales (to which *E. sideroxylon* belongs) have been examined but none contain engelitin in the leaves. Other work[6] has shown that engelitin is a characteristic component of many species of the section Platyantherae.

Heartwood Extractives

The uniformity in composition of heartwood extractives of both forms of *E. sideroxylon* further supports Erdtman's view of the conservative nature of this tissue. The composition of heartwood extractives of several botanically dissimilar *Eucalyptus* species is very similar and these extractives have a restricted taxonomic value. In an examination of the heartwood

[5] A. R. Penfold and F. R. Morrison, *J. Roy. Soc. N.S.W.*, **61**, 54 (1927).
[6] W. E. Hillis, Unpublished.

extractives of Blakely's sub-section Longiores (based on an antheral classification), a separation of the species was made on the basis of presence or absence of stilbenes.[7] However, Blakely's series in this sub-section are much more clearly defined and are based on additional characters and many of the differences noted with regard to stilbenes are not unexpected. It should be noted that stilbenes are not limited to this part of the genus and have been found in the heartwood of species belonging to most sections except the Renantherae. Stilbenes have been found in section Macrantherae (*E. accedens*, *E. anceps*, *E. dundasi*, *E. foecunda*, *E. incrassata*, *E. le souefii* in the series Dumosae), section Platyantherae (*E. salmonophloia*, series Leptopodae and *E. salubris* series Contortae), section Terminales (*E. sideroxylon*, series Ironbark) and in section Porantheroideae (*E. decorticans*, series Siderophloiae).

EXPERIMENTAL

Plant Material

Adult foliage from mature trees was collected from several localities and chromatographic examination showed that the leaves collected at or near the following localities were the normal form. In Victoria: Ballarat, Bendigo, Stawell, Heathcote and the coastal or near-coastal regions of Lorne, Airey's Inlet, You Yangs, Red Knob near Bruthen, Lakes Entrance, Nowa Nowa. In New South Wales: the coastal region of Merimbula, Tathra, Bermagui, Narooma. In Queensland: Stanthorpe. Samples of the variant form were collected in large areas around Gilgandra and Baradine in central N.S.W. Normal and variant forms were found within 50 yards of each other at Heathcote and at Airey's Inlet, Victoria. The sample "variety *rosa*" was collected from a garden specimen specially grown because of its red flowers.

Leaf and wood samples were collected from the same trees growing in Gilgandra and Heathcote and the same area in Bendigo and Airey's Inlet. Kino samples were collected from normal forms growing near Lakes Entrance, Victoria and from variant forms at Heathcote, Victoria and those growing under cultivation.

Wood samples of other eucalypt species were from the Standard Collection of the Division of Forest Products.

Chromatographic Examination

Two-dimensional chromatograms were prepared using first, *n*-butanol:acetic acid:water (6:1:2, "BAW") then 6% acetic acid ("6HOAc"). One-dimensional chromatograms were also prepared using these solvents and also phenol:water (upper phase), hydrochloric acid:acetic acid:water (3:30:10, "Forestal") and benzene:acetic acid:water (6:7:3, "BeAW") in the examination of polyphenols and phenolic acids. The location of sugar units in the relevant polyphenols was ascertained by the microdegradative techniques of Chandler and Harper[8] and the type of sugar identified chromatographically with BAW, butyl acetate:pyridine:ethanol:water (8:2·2:1) and ethyl acetate:pyridine:water (12·5:4) alongside authentic sugars.

The chromatograms were examined under u.v. light before and after exposure to ammonia vapour. The chromogenic sprays used were: diazotized *p*-nitroaniline in 20% sodium acetate,[9] vanillin–hydrochloric acid[10] and ferric chloride–potassium ferricyanide[11] for

[7] D. E. HATHWAY, *Biochem. J.* **83**, 80 (1962).
[8] B. V. CHANDLER and K. A. HARPER, *Aust. J. Chem.* **14**, 586 (1961).
[9] T. SWAIN, *Biochem. J.* **53**, 200 (1952).
[10] E. C. BATE-SMITH and T. SWAIN, *Chem. & Ind. (London)* 377 (1953).
[11] G. M. BARTON, R. S. EVANS and J. A. F. GARDNER, *Nature* **170**, 249 (1952).

polyphenols, aniline phosphate[12] and ammonia–silver nitrate for sugars and periodate–aniline[13] for shikimic acid.

Chromatoplates of Silica Gel G.F.254 (E. Merck, A.G. Darmstadt) with a thickness of 250μ were prepared in a constant temperature room at 20°. The following solvents were used: I, chloroform:acetic acid (9:1); II, chloroform:ethyl acetate:formic acid (5:4:1); III, toluene:ethyl formate:formic acid (5:4:1); IV, toluene:dioxane:water (1:1:1 upper phase).

Leaf Hydrolysis Products

These products were prepared and examined by the previously reported procedures.[14] The unidentified components encountered in this work were: *A*, which possessed under u.v. light a strong yellow-white fluorescence that faded in ammonia vapour R_f 0·50/0·10 (BAW/6HOAc); B, *Macrantherin*, which gave a characteristic canary yellow with diazotized *p*-nitroaniline; R_f 0·88 (Forestal), 0·58 (BeAW) 0·40 (6 HOAc) 0·90 (BAW); and *C*, with the same properties as Macrantherin but with R_f 0·80 (Forestal), 0·13 (BeAW).

Isolation of Polyphenols

(*a*) Dried, crushed leaves from "Variant sample Gilgandra No. 1" (500 g) were exhaustively extracted with methanol in a soxhlet-type apparatus, the extract concentrated under vacuum and then slowly poured into vigorously stirred water. The waxy material was removed, re-dissolved in methanol and reprecipitated as before until chromatographic examination showed that polyphenols were absent. The turbid aqueous liquor was extracted with petrol (40–60°) to remove oily materials.

Portion of the aqueous liquor was extracted in a liquid–liquid extractor with ether and then ethyl acetate for several daily periods. During the night periods, small amounts of a yellow deposit (polyphenol 1) formed. The aqueous liquor was finally extracted several times with *n*-butanol.

The evaporated ethyl acetate extract was most successfully separated by dissolving in water and adding to a polyamide (Grisamid, Type TPU, Knapsack Griesheim) column and eluting first with water and then with ethanol–water mixtures of increasing ethanol concentration. Crystals (polyphenol 2) separated from the concentrated aqueous and 20% ethanol eluates. The mother liquor from these eluates and the 30–70% ethanol eluates were evaporated to dryness and the ethanol soluble extract of the residue streaked onto washed No. 3 Whatman papers and developed with 6HOAc. The different bands were separately extracted with methanol and the extracts further purified in the same way but developed with BAW to obtain the Bands 3, 4 and 6. Band 3 was further separated into polyphenols 3 and 5, and Band 6 separated into polyphenols 6 and 7 by streaking onto borate-impregnated No. 3 Whatman papers and developing with water-saturated *n*-butanol. Polyphenol 4 was obtained from Band 4.

The concentrated butanol extracts were extracted five times with borate buffer (pH 8·2). When polyphenol 11 was removed it left polyphenols 9 and 10 and impurities in the butanol.

(*b*) Dried, crushed leaves from "Normal sample Heathcote" (260 g) were extracted with methanol and the waxy and oily materials removed as above. The aqueous liquor was extracted several times with *n*-butanol and the extracts evaporated *in vacuo*. The extracts were

[12] A. S. F. Ash and T. M. Reynolds, *Aust. J. Biol. Sci.* 7, 435 (1954).
[13] S. Yoshida and M. Hasegawa, *Arch. Biochem. Biophys.* 70, 377 (1957).
[14] W. E. Hillis and F. J. Hingston. *J. Sci. Food Agr.* 14, 866 (1963).

dissolved in a minimum amount of ethanol, added to a polyamide column and the monomeric polyphenols eluted with ethanol. The eluates were evaporated then streaked onto washed No. 3 Whatman papers and developed with 6HOAc. The desired bands were cut out, extracted with methanol and the extracts streaked onto paper and developed with BAW. The band containing polyphenol 6 was extracted with methanol and the evaporated extract recrystallized from water. The extract of the band containing catechin and polyphenol 7 was evaporated, dissolved in butanol and extracted seven times with borate buffer (pH 8·2) to remove catechin. The butanol solution was streaked onto paper and developed with BAW and the appropriate band extracted to yield polyphenol 7 which was recrystallized from water. The band containing polyphenol 8 was also dissolved in butanol and extracted several times with borate buffer (pH 8·2) to remove catechin and the butanol solution purified as before

Identification of Polyphenols

Polyphenol 1. Sideroxylin—The yellow deposit was recrystallized from ethanol as light yellow needles m.p. 286–7 (Found: C, 68·2; H, 5·2. Calc. for $C_{18}H_{16}O_5$, $\frac{1}{4}H_2O$: C, 68·2; H, 5·2%); u.v. absorption max. (Table 1); i.r. absorption max. (KCl disc) 2·95 (OH); 6·08 (C=O); 3·23, 6·28, 6·40 (aromatic); 3·40, 6·95, 7·24 (-CH_3); 3·55, 9·67 (-OCH_3); 12·00 μ (1,4 disubstituted benzene); NMR spectra (T.M.S., $\delta = 0$) in deuterochloroform and pyridine: δ 2·22 (3H), 2·33 (3H) for aromatic methyl 3·85 (3H) for methyl of aromatic methoxyl, 6·70 (1H) p.p.m. for 3-position proton; in deuterochloroform and dimethylsulphoxide: centred at δ 7·01 and 7·91 p.p.m. (J = 8·30 c/s) for H on benzene ring.

When spotted on paper the compound was opaque in u.v. light and yellow after exposure to ammonia, it gave a yellow colour with diazotized *p*-nitroaniline and no reaction with Gibbs reagent. Caustic degradation yielded *p*-hydroxybenzoic acid recognized by its R_f values and colour reactions.

The compound obtained after methylation with diazomethane melted at 196–198 (mixed m.p. with eucalpytin[4] 197–198), u.v. absorption max. in ethanol 283, 325 and in ethanol and sodium acetate 325 mμ. When spotted on paper, it was opaque in u.v. light and cream after exposure to ammonia, and did not react with diazotized *p*-nitroaniline. It behaved as eucalpytin when examined by thin-layer chromatography. Sideroxylin is thus 5,4'-dihydroxy-7-methoxy-6,8-dimethylflavone.

Polyphenols 2–7. These compounds, the properties of which are recorded in Tables 1 and 2, were degraded as previously described and their identity (Table 2) confirmed (except for polyphenol 5) by direct chromatographic comparison with authentic samples. Attempts to dehydrogenate[15] engelitin (polyphenol 2) to kaempferol 3-rhamnoside (polyphenol 5) were unsuccessful. Polyphenol 5 was chromatographically different from kaempferol 3-glucoside (astragalin). The amounts of polyphenols 6 and 7 isolated from the variant leaf sample were insufficient for complete identification and larger amounts were isolated from a normal leaf sample from Heathcote.

Polyphenol 8. This compound (5 mg) was obtained as a colourless powder when an aqueous solution was concentrated. It was colourless on paper under short and long wavelength u.v. light in the absence or presence of ammonia. It gave an orange colour with diazotized *p*-nitroaniline, but did not react with vanillin-hydrochloric acid or magnesium-hydrochloric acid. It slowly turned pink with hydrochloric acid vapour after reduction with sodium

[15] J. M. GULDER, T. H. SIMPSON and D. B. THOMAS, *J. Chem. Soc.* 170 (1955); H. L. HERGERT and O. GOLDSCHMID, *J. Org. Chem.* 23, 700 (1958)

borohydride[16] and treatment with sodium amalgam followed by acidification gave a pink colour. The compound was not extracted from butanol solution with sodium borate (pH 8). Spectral data (Table 1) indicates the presence of free C5 and C7 hydroxyl groups although the existence of two peaks of equal intensity after the addition of sodium acetate is unusual. The chromatographic (Table 1), the absorption max. in ethanol[17] and the above properties could be possessed by an isoflavanone glycoside. Hydrolyses with 2N hydrochloric acid gave glucose and the aglucone polyphenol 8A (Table 1) with the above colour reactions. The properties of the aglucone are consistent with the view that it is a 5,7-dihydroxy-isoflavanone possessing a further hydroxyl group.

Polyphenol 11. Astringin. The borate soluble extract was acidified and extracted with butanol. The dark brown powder obtained from the extract possessed a m.p. range of 75–125° (decomp.). It possessed the R_f values given in Table 1 and under u.v. light had an intense blue-white fluorescence which changed to green-white in ammonia vapour. A purple colour was obtained on spraying with diazotized *p*-nitroaniline. On hydrolysis with 2N HCl it yielded glucose identified chromatographically and a stilbene identical in R_f values and colour reactions with a stilbene obtained from authentic 3,3′,4′,5,5′-penta-acetoxystilbene.

A portion of the brown powder was methylated with diazomethane and twice recrystallized from ethanol m.p. 79–89° (decomp. 100°). After 5 days at room temperatures in the presence of β-glucosidase, most of the compound was hydrolysed. The aglucone was acetylated, oxidized with permanganate in acetone for 30 min and the products acidified. Various attempts to separate the oxidation products were unsuccessful. After hydrolysis with 2% methanolic KOH, examination by T.L.C. using solvents I, II, and III showed that there were components with R_f values of 0·77 and 0·81; 0·63 and 0·69; 0·09 and 0·19 respectively. The compound with the highest R_f values was identical with 3,4,5-trimethoxy benzoic acid examined simultaneously and the other with 3-hydroxy 5-methoxy benzoic acid.

Acetylation of the brown powder with acetic anhydride and pyridine yielded a product which after recrystallization from 50% methanol had m.p. 100–115°.

The brown powder was dissolved in water and hydrolysed with β-glucosidase at room temperature for 5 days. The aglycone was recovered from the incompletely hydrolysed solution by extraction with ether, acetylated with acetic anhydride and pyridine and the acetate recrystallized from methanol m.p. 113°. (3,3′,4′,5,5′-penta-acetoxy stilbene, 173–174°[18]; 3,3′,4′,5-tetra-acetoxy stilbene, 114[18]; 115, 125[19]). Ultra-violet absorption max. of both acetates in 95% EtOH:— $\lambda_{max.}$ 300, 313, shoulder 325 mμ. When the acetate and authentic penta-acetoxystilbene were examined simultaneously by T.L.C. the respective R_f values with different solvents were as follows:— I, 0·81, 0·81; II, 0·75, 0·69; III, 0·75, 0·69 and IV, 0·53 and 0·43.

Polyphenols 9, 10. Chromatographic properties of polyphenols 9 (piceid) and 10 (rhapontin) are given in Table 1. They are most easily distinguished from each other (and from polyphenol 11) by the change in their fluorescent colour under u.v. light when the paper is exposed to strong ammonia vapour. Piceid changed from light blue to intense duck-egg blue and rhapontin from intense blue to a light magenta. The colour changes for the aglucones were identical with those of the glucosides.

[16] E. Eigen, M. Blitz and E. Gunsberg, *Arch. Biochem. Biophys.* **68**, 501 (1957).

[17] S. Balakrishna, J. D. Ramanathan, T. R. Seshadri and B. Venkatarami, *Proc. Roy. Soc.* **268A**, 1 (1962); I. A. M. Cruickshank and D. R. Perrin, *Life Sciences* **9**, 680 (1963); D. R. Perrin and W. Bottomley, *J. Am. Chem. Soc.* **84**, 1919 (1962).

[18] F. E. King, T. J. King, D. H. Goodson and L. C. Manning, *J. Chem. Soc.* 4477 (1956).

[19] J. Cunningham, E. Haslam and R. D. Haworth, *J. Chem. Soc.* 2875 (1963).

Methylflavones in leaf wax

Dried uncrushed leaves of both forms were immersed in boiling petrol (b.p. 50–70) for 5–20 min and the bright yellow concentrated extracts examined by the T.L.C. technique using Silica Gel G.F. 254 and solvents I. II and III. Sideroxylin and eucalyptin were run at the same time and had in solvent I R_f values of 0·45, 0·68; in II, 0·91, 0·94; in III, 0·35, 0·64 respectively. A component in the leaf wax of both forms behaved identically with eucalyptin.

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