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POLYPHENOLS OF *EUCALYPTUS SIDEROXYLON* WOOD

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Abstract—Twenty-three major components were detected in the methanol extractives of the heartwood of *Eucalyptus sideroxylon*. The components identified include resveratrol, resveratrol-β-glucoside, 3,3'-di- and 3,3',4-tri-*o*-methylellagic acids and their glucosides. The 3,3'-di-*o*-methylellagic acid 4'-glucoside isolated had properties significantly different from those previously reported for this compound. Also present were gallic acid, catechin, ellagic acid, an unidentified stilbene, the ellagitannins D-6 and D-13, polymerized leucocyanidin and an oily material. The sapwood contained gallic acid, small amounts of ellagitannins and ellagic acids and traces of other components. The heartwood extractives of related eucalypt species were also examined.

INTRODUCTION

THE HEARTWOOD of *Eucalyptus sideroxylon* is rated as highly durable in soil burial tests and is widely used in south-eastern Australia because of these properties. The polyphenols of this dense timber with thick cell walls were isolated and identified to enable subsequent testing of their contribution to the durability of the wood.

RESULTS AND DISCUSSION

Composition of extracts

The various extractives obtained from the heartwood are given in Table 1. The methanol extractives amounted to 13·2% (oven dry basis); the heptane fraction did not contain phenolic compounds detectable by chromatography.

Over 22 individual compounds were detected by paper chromatography of the MeOH extract (Table 2). The most prominent components showed an intense blue fluorescence in UV, some of which became more intensely blue or blue–white on exposure to NH_3 vapor (the stilbenes S-1, S-3, S-5, S-8, S-13, S-14) whilst others turned yellow (ellagic acid S-15, and derivatives S-2, S-4, S-19). The ellagic acid derivative S-11 did not change color with NH_3 vapor.

Compound S-1 (Table 2) was further identified as resveratrol (3,4',5-trihydroxy stilbene) after isolation from the L subfraction of the ether extract (Table 1). Compound S-8 (from subfraction G) was identified as resveratrol β-glucoside. Compound S-3 is probably the *cis*-isomer of resveratrol and S-5 is possibly the *cis*-isomer of S-8.

Compound S-13 had the chromatographic and spectral properties of a stilbene. It appeared very similar to the unidentified stilbene in *E. wandoo*.[1] Several attempts using

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[1] HATHWAY, D. E. (1959) *Biochem. J.* **72**, 369.

different techniques were made to isolate the compound but without success owing to its instability particularly in solution. An examination of a concentrate showed a UV spectrum very similar to that of resveratrol but there were significant spectral differences after the addition of sodium ethoxide and particularly after the addition of sodium acetate–boric acid However, the presence of vicinal hydroxyl groups that were indicated from these observations could not be confirmed by chromatography on borate-impregnated paper.

TABLE 1 FRACTIONS OBTAINED FROM THE METHANOL EXTRACT OF *Eucalyptus salubris* sbm

Fraction	Sub-fraction	Appearance	Components (in approximate order of concn)	Amount Heart-wood (%)	Amount Sub-fraction (%)
Ether soluble	Heptane soluble	Red oil		0·95	
	Heptane insoluble	Pink powder		0·85	
	H_2O soluble "F"		S-6 S-13, S-14		22
	$NaHCO_3$ soluble "G"		S-2, S-4, S-14, S-8, S-13		32
	Na_2CO_3 soluble "K"		S-1 S-13, S-2, S-4 S-8 S-3, S-5		4
	Ether soluble "L"		S-1, S-3, S-13, S-5, S-15		42
Ethyl acetate soluble		Amber powder	S-10, S-11, S-15 S-13, S-14 S-9, S-16, S-12 S-17, S-18, S-23	0·95	
H_2O soluble		Red brown gum	S-17 S-18, S-23, S-22 S-10 S-11 S-21 S-20, S-19	7·5	
Insoluble		Tan powder	S-15, S-16, S-23	1·15	

Compounds S-2, S-4 and S-10 were readily distinguished from the other purple-blue fluorescent components because in NH_3 vapor they turned a characteristic yellow color, very similar to the change shown by S-15. Compounds S-2 and S-4 were extracted from the ether extract with sodium bicarbonate They were identified as 3,3′,4-tri-*o*-methyl- and 3,3′-*o*-methyl-ellagic acids respectively by direct chromatographic comparison with authentic samples

Compounds S-10 and S-11 were obtained from the dried ethyl acetate extract by filtering a hot 70% ethanol solution to remove ellagic acid (S-15) and allowing the filtrate to stand at room temperature for several days. The precipitate contained a mixture of 3,3′-di-*o*-methylellagic acid-4′-glucoside and 3,3′,4-tri-*o*-methylellagic acid-4′-glucoside identified by chromatography. When a portion of the mixture was dissolved in water and incubated overnight with β-glucosidase and then chromatographed, these two compounds had disappeared and the precipitate which had formed contained the aglucones in about equal proportions The crude precipitate was also mixed with 0·1 M sodium bicarbonate and the insoluble material (S-11) removed and identified by direct comparison with authentic 3,3′-4-tri-*o*-methylellagic acid-4′-glucoside S-10 was removed from the sodium bicarbonate solution after acidification and identified as 3,3′-di-*o*-methylellagic acid-4′-glucoside

Attention is drawn to the different properties of the 3,3′-di-*o*-methylellagic acid-4′-glucoside we have isolated and those reported by earlier workers [2] We found that only on repeated crystallization from 70% ethanol could our compound be obtained chromatographically pure as colorless crystals m.p brown 275° and infusible 355° (light brown prisms,

m.p. 214–215°).[2] We were unable to hydrolyse the compound with "4% methanolic H_2SO_4" in 6 hr[2] and have found that 4% H_2SO_4 in 70% ethanol for 2 hr was necessary. We have also been able to isolate the components quantitatively in equimolar proportions whereas in the other work no ratio is given. These workers do not report the preparation of an acetate but the acetate we obtained gave the correct analysis and had a sharp m.p. Methylation of the di-*o*-methylellagic glucoside from *Terminalia paniculata* yielded pale yellow prisms of a tri-*o*-methyl glucoside with m.p. 205–207°. We had considerable difficulty[3] in purifying this compound to obtain colorless needles m.p. 266–267°. The difficulties in obtaining pure compounds of this series and satisfactory analyses have previously been observed.[4,5] The UV, IR and NMR spectral data of our 3,3′-dimethylellagic acid-4′-glucoside are given in a previous paper[6] and the data are consistent with other members of this series of compounds.[6]

TABLE 2 MAJOR COMPONENTS IN THE EXTRACTIVES OF THE HEARTWOOD OF *Eucalyptus sideroxylon*

Component	Color* on chromatogram SUV†	Other‡ A	B	C	Method of characterization	Identity
S-1	pu-bu→w bu	pa-bu	pa-t	—	R_f, UV, GLC	Resveratol
S-2	pu-bu→y	bu	pa-t	—	R_f, UV, IR, NMR	3,3′,4-Tri-*o*-methylellagic acid
S-3	pu-bu→w bu	pa-bu	pa-t	—	R_f, GLC	*cis*-Isomer of resveratrol
S-4	pu-bu→y	bu	pa-t	—	R_f, UV, IR, NMR	3,3′-Di-*o*-methylellagic acid
S-5	pu-bu→w bu	bu	pa-t	—	—	Possibly *cis*-isomer of S-8
S-6	m→m	bu	t-br	—	R_f R_f, red with vanillin-HCl	Gallic acid Catechin
S-7	pu-bu	—	—	—	—	—
S-8	pu-bu→w bu	pa-bu	—	—	R_f, GLC	Resveratrol β-glucoside
S-9	y	—	—	—	—	—
S-10	pu-bu→y	bu	pa-t	—	R_f, UV, IR, NMR	3,3′-Di-*o*-methylellagic acid-4′-glucoside
S-11	w-bu	—	—	—	R_f, UV, IR, NMR	3,3′,4-Tri-*o*-methylellagic acid-4′-glucoside
S-12	pu-bu	—	—	—	—	—
S-13	pu-bu	bu	pa-t	—	R_f, UV	Stilbene
S-14	pu-bu	—	—	—	—	—
S-15	bu-bu→y	bu	pa-t	—	R_f, UV, IR, NMR	Ellagic acid
S-16	pu	—	—	—	—	—
S-17	dark bu	bu	t	red	R_f	Ellagitannin§
S-18	dark bu	bu	t	pu	R_f	Ellagitannin
S-19	dark bu	bu	t	t	—	Possibly ellagitannin
S-20	dark bu	bu	t	t	—	Possibly ellagitannin
S-21	dark bu	bu	t	t	—	Possibly ellagitannin
S-22	dark bu	bu	t	t	—	Possibly ellagitannin
S-23	br	bu	t	—	Red with vanillin-HCl	Contains polymerized leucocyanidin

* Bu = blue, br = brown, m = mauve, pa = pale, pi = pink, pu = purple, r = red, t = tan, w = white, y = yellow

† SUV = short wave length, UV, change in SUV after exposure to NH_3 vapor

‡ After spraying with (A) $FeCl_3$–$K_3Fe(CN)_6$, (B) pNA[3], (C) NSSC[3]

§ S-17 and S-18 are chemically identical to *Eucalyptus* ellagitannins D-6 and D-13[7,8] and to *Quercus alba* ellagitannins H-1 and H-3[9] respectively

[2] RAO, L R and SUBBA RAO, G S R (1962) *Tetrahedron* **18,** 357
[3] HILLIS, W E and YAZAKI, Y (1974) *Phytochemistry* **12,** 2969
[4] CAIN, B F (1963) *New Zealand J Sci* **6,** 264
[5] BRIGGS, L H, CAMBIE, R C, LOWRY, J B and SEELYE, P N (1961) *J Chem Soc* 642
[6] HILLIS, W E and YAZAKI, Y (1974) *Phytochemistry* **12,** 2963

Compounds S-17 and S-18 in the ethyl acetate extract and the water soluble residue were chromatographically identical with authentic ellagitannins D-6 and D-13 respectively from *E. delegatensis*,[7,8] which in turn are identical with ellagitannins H-1 and H-3 in *Quercus alba*.[9]

Compound S-23 gave a flavan reaction with the vanillin-hydrochloric reagent and is probably the polymerized leucocyanidin which gave rise to cyanidin when the MeOH extract was heated for 30 min at 100° with butanol–HCl and chromatographed in Forestal solvent.

The sapwood contained 3·7% (oven dry basis) of MeOH-soluble extractives Chromatographic examination for polyphenols revealed appreciable amounts of gallic acid, polymerized material which was chromatographically irresolvable and contained leucoanthocyanins, small amounts of ellagitannins and ellagic acid and very faint traces of materials which could be stilbenes or methylellagic acids

Taxonomic aspects

In view of the value of stilbenes in heartwood extractives as taxonomic markers for the Blakely subsection Longiores[1] a chromatographic examination was also made of heartwood extracts of ironbarks and other species grouped with *E sideroxylon*.[10,11] All extracts were found to contain ellagic acid, small and variable amounts of gallic acid and in almost all cases the ellagitannins D-6 and D-13. The composition of *E. paniculata* Sm extractives closely resembled that of *E. sideroxylon* and the timbers have similar durability, density and other properties.

Other species probably containing small amounts of resveratrol and its glucoside were *E. decorticans* Maiden, *E. pruinosa* Schau, and *E. melanophloia* F. Muell. This group of species differs from the others in that the extractives contain a higher proportion of 3,3′-di-*o*- and 3,3′,4-tri-*o*-methylellagic acids and their glucosides Mono-*o*-methylellagic acid and its rhamnoside appeared to be present also in *E pruinosa* and *E. melanophloia* and tetra-*o*-methylellagic acid in the former. *E crebra* F. Muell appeared to contain traces of resveratrol but no methylellagic acid derivatives.

E. drepanophylla F Muell ex Benth, *E fibrosa* F. Muell subsp. *fibrosa*, *E. siderophloia*, *E. jensenii* Maiden contained 3,3′-di-*o*- and 3,3′,4-tri-*o*-methylellagic acids and their glucosides and small amounts of 3-mono-*o*-methylellagic acid. *E cullenii* contained larger amounts of 3-mono-*o*- and in addition 3,3′,3,4′-tetra-*o*-methylellagic acid The composition of the extractives of *E. leucoxylon* F Muell showed most resemblance to this species.

The use of stilbenes as taxonomic markers for the group of eucalypts known as ironbarks may have some value However, as it has been found that *E. sideroxylon* variants containing stilbenes in the leaves are common,[12] it would be necessary to examine samples from different areas before conclusions could be made. Also the suggestions that there may be some relationship between eucalypt ironbarks and eucalypt boxes is supported by the presence of the 3,3′,4-tri-*o*-methylellagic acid-4′-glucoside in both groups [13]

[7] SEIKEL, M K and HILLIS, W E (1970) *Phytochemistry* **9**, 1115
[8] HILLIS, W E (1969) *Appita* **23**, 89
[9] HART, J H and HILLIS, W E (1972) *Phytopathology* **62**, 620
[10] BLAKELY, W F (1965) *A Key to the Eucalypts*, 3rd Edn, Com Australia, For Timber Bureau Canberra
[11] PRYOR, L D and JOHNSON, L A S (1971) *A Classification of the Eucalypts*, Australian National University Press
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[13] HILLIS, W E and YAZAKI, Y (1974) *Phytochemistry* (in press)

EXPERIMENTAL

The general methods used have been previously reported [3] The UV absorption spectra of different components were determined in EtOH soln before and after the addition of dilute aqu solutions of NaOH or NaOAc.

Plant material and fractionation The log of red ironbark (*Eucalyptus sideroxylon* A Cunn ex Woolls) collected at Rushworth, Victoria, Australia, had a diameter inside bark of 38 cm with a sapwood thickness of about 2 5 cm Fresh sapwood and heartwood were converted to thin longitudinal shavings, extracted with MeOH (at 22–24°) for 3 days, the extract concn under vacuum at less than 40°, finally freeze-dried and stored at −10° The extracted sapwood was cream colored, the extracted heartwood was brown–red The heartwood MeOH extract was redissolved in a minimum amount of MeOH, allowed to stand for 2 weeks and compound S-15 separated by centrifugation The clear liquor was slowly added to constantly stirred H_2O to produce a suspension which was extracted with Et_2O in a liquid–liquid extractor for 24 hr The dried Et_2O extract was redissolved in MeOH and washed 5–6 times with heptane The aqu material that had been extracted with Et_2O was partly concentrated *in vacuo* and extracted with EtOAc (six 4-hr extractions) in a liquid–liquid extractor The insoluble material which appeared in the aqu layer was separated All fractions were vacuum- or freeze-dried

A red oil extracted with heptane from the Et_2O extract was not examined further The Et_2O soluble fraction was redissolved in Et_2O–MeOH (9 1), and extracted (each 4 times) in turn with H_2O saturated with $NaHCO_3$ or with Na_2CO_3 The aqu extracts were washed with Et_2O to remove entrained material and the washings returned to the main solution The Na_2CO_3 or $NaHCO_3$ extracts were neutralized, extracted with EtOAc, and the extracted components, which were recovered in 92% yield, were identified chromatographically The EtOAc soluble material from the original methanol extract was refluxed with 25 times its weight of 70% EtOH for 10 min, filtered while hot, and the filtrate allowed to stand 20 weeks

Characterization of compounds S-1, S-3, S-5, S-8, S-13 The L subfraction of the Et_2O extract (Table 1) was crystallized from 70% EtOH Further recrystallization yielded a product which by m p and m m p (262–264°) was identical with authentic resveratrol The fraction containing a concentrate of S-1 was silylated with hexamethyldisilazane-trimethylchlorosilane-*N,o*-bis (trimethylsilyl) acetamide in pyridine (2 1 1 10) and examined by GLC using SE-30 (3 0%) and Apiezon L (3·8%) columns (programming from 200° to 240° at 1°/min) The presence of resveratrol was confirmed by co-injection of an authentic silylated sample The fraction also gave a smaller peak which was probably that of the *cis*-isomer of resveratrol (probably compound S-3) Compound S-8 (from subfraction G) corresponded with authentic resveratrol β-glucoside by GLC (programming from 240–300° at 1°/min) Compound S-5 is possibly the *cis*-isomer of S-8 [14] When chromatograms of heartwood polyphenols of *E sideroxylon* and *E wandoo* were compared, S-13 had R_f values very similar to those of the unidentified stilbene in *E. wandoo* [1] The following comparative R_f values were obtained PC using *n*-BuOH–EtOH–H_2O (4 1 5) resveratrol glucoside 0 52, resveratrol 0 82, oxyresveratrol 0 71, S-13 0 61, TLC on silica gel using $CHCl_3$–EtOAc–HCOOH (5 4 1), resveratrol 0 52, oxyresveratrol 0·45, S-13 0 38 S-13 was chromatographically different from rhapontin and is dissimilar to other known stilbenes and is so unstable as to disappear from aqueous solutions overnight

Characterization of compounds S-10, S-11 and S-15 These compounds were obtained from the dried EtOAc extract by refluxing with 25 times its weight of 70% EtOH for 10 min, filtering hot to remove ellagic acid (S-15) and allowing the filtrate to stand at room temp for several days The crude ppt (50 mg), drained from the stored filtrate, contained S-10 and S-11, and was mixed with 0 1 M $NaHCO_3$ (25 ml) and filtered to remove the insoluble tri-*o*-methylellagic acid glucoside (S-11) The filtrate was extracted in a liquid–liquid extractor with EtOAc to completely remove the tri-*o*-methylellagic acid glucoside which still remained The extracted layers were acidified with 5 N HCl, cooled to 0° for 15 min and the insolubles separated, washed with H_2O, dissolved in hot 70% EtOH (10 ml) and stood overnight to yield chromatographically pure colorless crystals (25 mg) which turned brown at 275° and did not melt at 355° (lit [2] light brown prisms m p 214–215°) *Anal* Found C, 53 2, H, 4 0, –OMe, 11 9. Calc for $C_{22}H_{20}O_{13}$ C, 53 6, H, 4 0, –OMe, 12 6% The crystals (10 mg), were hydrolysed with 4% H_2SO_4 in 70% EtOH (10 ml) under reflux (2 hr), cooled, added H_2O (7 ml), concn to half vol and the ppt (6 7 mg) identified by TLC (EtOAc–$CHCl_3$–HCOOH, 2 10 1), m p and m m p (340–341°) and IR in KBr as 3,3′-di-*o*-methylellagic acid The wt of ppt was 6 7 mg and consequently the ratio of aglycone to sugar is 1 1 The aqueous filtrate was passed through an Amberlite IR 48 (–OH) column, evaporated *in vacuo* at 45°, compared on PC with authentic sugars using BAW, 6% HOAc, and EtOAc–pyridine–H_2O (25 5 4) after spraying with $AgNO_3$ in alkali The sugar was identified as glucose The glucoside was acetylated with Ac_2O and pyridine and recrystallized as colorless needles from 70% EtOH m p 251–252° *Anal* Found C, 54 7, H, 4 1, –OMe, 7 9, –COMe, 30 0, calc for $C_{32}H_{30}O_{18}$ C, 54 7, H, 4 3, –OMe, 8 8, –COMe, 30 6% Compound S-10 was 3,3′-di-*o*-methylellagic acid-4′-glucoside By chromatographic and spectral comparison with authentic material S-11 was 3,3′,4-tri-*o*-methylellagic acid-4′-glucoside

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