Phytochemistry, 1973, Vol. 12, pp. 2969 to 2977. Pergamon Press. Printed in England.

WOOD POLYPHENOLS OF *EUCALYPTUS POLYANTHEMOS*

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(*Received* 17 *May* 1973. *Accepted* 2 *July* 1973)

Key Word Index—*Eucalyptus polyanthemos* Myrtaceae; red box; wood ellagitannins; methylellagic acids and glucosides; heart-wood formation; taxonomy.

Abstract—Sixteen major components have been detected in woody tissues of *Eucalyptus polyanthemos*. The components identified include 2,3- and 4,6-(hexahydroxydiphenoyl)-glucose, a di-(hexahydroxydiphenoyl)-glucose known as pedunculagin, an ellagitannin which appears to be a cyclic di-(hexahydroxydiphenoyl)-diglucose, 3,4,3′-tri-*O*-methylellagic acid and its 4′-glucoside, 3,4,3′,4′-tetra-*O*- and 3,3′-di-*O*-methylellagic acids. A 3,3′-di-*O*-methylellagic acid glucoside and 2 gallotannins are possibly present in addition to the unidentified ellagitannin D-13. The distribution of these components in the woody tissues is discussed in relation to heartwood formation. The trimethylellagic acid glucoside was also present in the heartwood of other members of the red-box group of eucalypts.

INTRODUCTION

Eucalyptus polyanthemos Schau (Myrtaceae 'Red Box') is a small tree which may attain 15–24 m in height and up to 1 m diameter. It occurs throughout south-eastern Australia and with 5 other species belongs to the distinctive red box group which extend from South Australia to southern Queensland. The polyphenols in the leaves of these species are generally similar.[1]

The heartwood is red, dense and moderately durable, surrounded by a distinctive white intermediate zone 2–3 mm wide and then a pale coloured sapwood. A chromatographic examination showed the extractives included a distinctive mauve fluorescent component and appreciable amounts of compounds D-6 and D-13[2] which are among the most chromogenic ellagitannins in alkaline pulping liquors[3] and difficult to separate from extracts of other sources because of large amounts of closely related compounds. This species was studied to gain more information on extractives of commercial significance and their relationship to heartwood formation.

RESULTS

Characterization of Components

Chromatographic examination revealed at least 26 polyphenols excluding polymers. Some of these had been observed previously in *E. delegatensis*[2–4] and were given the prefix D. Those components present in greater than trace amounts are recorded in Table 1, which gives the average composition of monomeric components in cross-sections from several trees.

[1] HILLIS, W. E. (1967) *Phytochemistry* **6,** 373.
[2] SEIKEL, M. K. and HILLIS, W. E. (1970) *Phytochemistry* **9,** 1115.
[3] HILLIS, W. E. (1969) *Appita* **23,** 89.
[4] HILLIS, W. E. and SEIKEL, M. K., unpublished.

TABLE 1. THE AMOUNT OF EXTRACTIVES AND CHARACTERISTICS OF COMPONENTS IN DIFFERENT TISSUES IN *Eucalyptus polyanthemos*

	R_f* × 100		Colours‡			Extractives in Tissue %‖				
				with sprays		O.S.	M.S.	I.Z.		H.W.
	BAW	6HA	UV_{254}†	NSSC	FeCN	(3·0)	(2·7)	M. (4·7)	Or. (16·6)	(20·0)
Components										
D-1	30	76	pu.	slow r.-b.	+	+	+	T	+	+
D-2§	30	57	pu.	slow pi.-or.	+	+++	+	+	++	+
D-4§	25	74	f.bu.	slow pi.-or.	+	T	+	0	T	T
D-5	14	65	f.bu.	slow pi.-v.	+	0	0	0	+	+
D-6§	18	49	pu.	pi. → v → b.	+	+	++	++	+++	+++
D-13§	12	62	pu.	pi. → v → b.	+	+	++	++	+++	+++
Ellagic acid	46	2	m.	y.	(+)	T	T	+	++	+++
Gallic acid§	69	42	bu.	slow pi.-or.	+	+	+++	+++	+++	++
P-4§	44	49	bu.	—	+	0	+	+	+	T
P-7	59	43	w.bu.	—	—	+	+	++	+++	+++
P-9§	51	38	pu.	pi.-or.	+	+	++	++	++	T
P-10	67	63	f.bu.	slow or.	+	+	++	+	T	0
P-11	83	11	bu.m.	—	—	0	0	0	T	++
P-13	58	36	pi.	—	—	T	+	+	+	+
P-14	5	45	pu.	v.-b.	+	0	0	0	0	+
P-15	21	34	—	—	+	0	0	0	0	+

* R_fs were measured from leading edge of spot and can be variable. 6HA = 6% HOAc.

† With the exception of ellagic acid P-7, P-11, P-13 the compounds are absorbing in UV (254 nm), under other conditions the fluorescence listed can be seen. With strong NH_3, ellagic acid and parts of P-11 become yellow.

‡ b.—brown; bu.—blue; f.—faint; m.—mauve; or.—orange; pi.—pink; pu.—purple; r.—red; v.—violet; w.—white; y.—yellow. After 24 hr all NSSC colours became brown. O.S.—outer sapwood; M.S—middle sapwood; I.Z.—intermediate zone; M.—mauve band; Or.—inner orange band; H.W.—heartwood.

§ Gibb's reagent oversprayed with basic lead acetate gave a violet-grey with D-2, D-4, D-6, D-13 but a green-blue characteristic of gallotannins with gallic acid, P-4, P-9.

‖ The relative amounts shown in brackets were assessed from chromatograms of each tissue.

Ellagitannins D-1, D-2, D-4, D-6 and D-13 and ellagic and gallic acids were identified by co-chromatography with authentic compounds using previously described methods[2] and chromogenic reagents, particularly 'NSSC'[3] (see Experimental). D-1 is 2,3- and D-4 is 4,6-hexahydroxydiphenoyl ('HHDP')—glucose and D-2 is pedunculagin.[2] D-5 in chromatograms was provisionally identified by comparison with concentrates from *E. delegatensis*[2] containing this compound. Colours given by the above compounds with NSSC are slightly but distinctly different and so is their rate of change of colour. These differences are difficult to specify quantitatively but readily distinguished when authentic compounds are examined simultaneously. Ellagic acid was further identified by UV and IR spectra, and gallic acid chromatographically and by distinctive colour reactions.

TABLE 2. RATIO OF PRDOUCTS FORMED ON HEATING D-6 WITH N H_2SO_4 FOR DIFFERENT TIMES

	Time (hr)										
Product†	0·5	1	2	3	4–6	7	8,9	10	11	12–15	16
D-6	++*	++	++	+	+	+	T	T	0	0	0
D-6-2	+	+	++	++	++	++	+	+	T	0	0
D-6-3	0	T	T	T	T	T	T	T	T	0	0
D-6-4	0	T	T	T	+	+	+	T	T	T	0
D-6-5	0	0	0	T	+	++	+	+	+	+	T
Ellagic acid	+	+	+	+	++	++	+++	+++	+++	+++	+++

* Ratio of amounts: +++ large; ++ medium; + small; T—trace; 0—absent.

† For chromatographic properties see Table 3.

Ellagitannin D-6

Ellagic acid and glucose are the only recognizable acid and alkaline hydrolysis products of D-6 and are present in about equal molar proportions. Alkaline hydrolysis of D-6 that had been exhaustively methylated with diazomethane produced hexamethoxydiphenic acid. No indication of MW or other useful data could be obtained on mass spectrographic examination. D-6 does not react with aniline phthalate indicating substitution of the carbon 1 hydroxyl of glucose. A study of the stages of acid hydrolysis of D-6 (Table 2) shows that D-6-2 and D-6-5 are the significant intermediates and that they do not correspond to any known ellagitannins; insufficient supplies of pure D-6 prevented the preparation and separation of these intermediates. However, incubation with *Penicillium waksmanii* Zaleski yielded D-6-2 (probably through the transient D-6-1) and ellagic acid. This fungus has a strong α-glucosidase and weak β-glucosidase activity and whereas it does not affect 2,3- or 4,6-HHDP-glucose it hydrolyses corilagin (1-galloyl-3,6-HHDP-β-glucose) very slowly and pedunculagin ($\overline{2,3}$; $\overline{4,6}$-di-(HHDP)-glucose or $\overline{1,6}$; $\overline{2,3}$-di-(HHDP)-glucose)^2 relatively quickly. The latter compound yielded 2,3-HHDP-glucose and another main component which may be a HHDP-monolactone-glucose. The effect of *P. waksmanii* on D-6 indicates that it is an aryl ester involving the hydroxyl of carbon 1 on α-glucose. Furthermore both D-6 and D-6-2 have a band at 830 cm^{-1} in their IR spectra indicative of α-glucose (and a weaker band at 890 cm^{-1} indicative of β-glucose) and β-glucosidase is without effect on them.

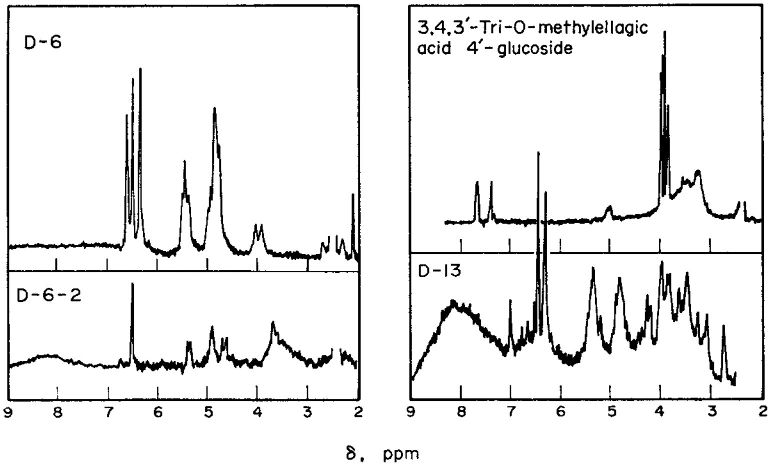

Fig. 1. NMR spectra of ellagitannins and trimethylellagic acid glucoside (TMS δ = 0). Compounds dissolved in dimethylsulphoxide-d_6.

The NMR spectrum of D-6 shows three sharp peaks (δ 6·36, 6·50 and 6·60 ppm) in the aromatic region (Fig. 1). The spectrum of D-6-2 has only one sharp peak (δ 6·50 ppm) in this region and on acid hydrolysis D-6-2 gives ellagic acid and glucose. The one peak probably represents the 2 protons on a HHDP moiety that is symmetrical to enable the protons to be in magnetically equivalent environments. In this regard it is similar to hexamethoxydiphenic acid which has a single aromatic proton peak at 7·52 ppm. There is no evidence of free carboxyl groups in the IR spectra of D-6 or D-6-2 and the bands at 1740 and 1735 cm^{-1} are assigned to the ester groupings of HHDP with two glucose moieties.

Because D-6-2 reacts weakly with aniline phthalate it is postulated the esters involve HHDP and the carbon 2 hydroxyls[5] of the two glucose moieties. D-6-2 is formed from D-6 with the liberation of ellagic acid. If it is assumed that D-6 contains 2 HHDP and 2 glucose moieties and D-6-2 only 1 HHDP moiety then the yield of D-6-2 obtained after hydrolysis with *P. waksmanii* was 94·6% theoretical. The evidence given above supports the view that the sharp 6·50 ppm singlet in D-6-2 represent two protons. In D-6 the 3 singlets at δ 6·36, 6·50 and 6·60 are of the same intensity and it follows that each of them represents two protons. Accordingly, in view of the known shifts for protons on HHDP moieties the signal δ 6·60 would represent the protons on the second HHDP moiety in D-6 and these protons would be in magnetically equivalent environments. In view of the sharpness of the peaks in D-6 the HHDP and glucose moieties must be in the same conformation and possibly the glucose moieties also.

The sharp singlet at δ 6·36 is in the region occupied by 'acylated H-1' signals and there are at least 10 compounds with this substitution in the glucose moiety and a singlet or doublet in this region.[2,6,7]

D-6 had 3 groups of shifts in an envelope spectrum similar to D-3[2] and D-6-2 had 4 groups. Integration was not accurate and signal to noise ratio was high owing to limited solubility in solvents. Decoupling did not yield further information and as other work on D-2 showed that an increase in temperature did not simplify the spectrum it was not attempted with these samples. The ratio of aromatic peaks (δ 6·60 and 6·50) to glucose peaks (δ 6·36–3·0) was close to 4:14 for D-6. On the basis of one HHDP to one glucose moiety the ratio would be 4:14. In the case of D-6-2 the singlet at δ 6.36 is absent and on the assumption that this can be assigned to an 'acylated H-1' signal, the ester linkage between the 1 and 1′ of the two glucose moieties has been ruptured to release HHDP. Enzymic hydrolysis (above) indicates that D-6-2 contains 1 HHDP and 2 glucose moieties and the ratio of the protons in these moieties in the NMR spectrum has been estimated to be close to 2:14 which is the expected value. D-6-2 reacts weakly with aniline phthalate indicating that the HHDP is esterified with the carbon 2 hydroxyls of the two glucose moieties.

If D-6 is a bimolecular compound the spectrum is notably sharp in the overlapping of double signals in the aromatic region. Certainly, in the glucose region, the peaks are of the envelope type which could cover several minor signals. Attempts to assign the shifts to specific glucose protons on the basis of earlier studies[2] did not result in definite conclusions.

A molecule which satisfies the above requirements has the constitution of the two HHDP moieties esterified with the 1,1′ and 2,2′ hydroxyls of two α-glucose moieties. Attempts to build several models were made and when glucose is in the 1C conformation (see formula VI[8]) a model with little strain was obtained. No other evidence concerning this or other glucose conformations could be obtained with existing techniques so that the proposed constitution of D-6 requires confirmation.

Ellagitannin D-13

This compound was isolated in a chromatographically pure form but little information could be obtained on its nature. All evidence indicates it has a higher molecular weight than

[5] SCHMIDT, O. T. and SCHMADEL, H. (1961) *Annalen* **649**, 157.

[6] JOCHIMS, J. C., TAGIEL, G. and SCHMIDT, O. T. (1968) *Annalen* **717**, 169.

[7] SCHMIDT, O. T., EBERT, W. and KOPP, M. (1969) *Annalen* **729**, 251.

[8] HILLIS, W. E. (1972) *Phytochemistry* **11**, 1207.

the other ellagitannins. A chromatographic study of the products formed at intervals during 7 hr of acid hydrolysis revealed the formation of glucose and ellagic acid and a number of materials which appeared to be ellagitannins but had not been previously encountered.

Component P-4 and P-9

R_f values and chromogenic sprays indicate these components are gallotannins.

Component P-7

Purification of Component P-7 was monitored by PC and TLC and considerable difficulty was experienced in the removal of the last traces of tetra-*O*-methylellagic acid which necessitated repeated recrystallization from 70% ethanol. Acid hydrolysis revealed the presence of 3,4,3′-tri-*O*-methylellagic acid and glucose in equimolar proportions so that component P-7 is the 3,4,3′-tri-*O*-methylellagic acid 4′-glucoside which has not been previously isolated from plant material. Its acetate gave a satisfactory analysis. Hydrolysis by β-glucosidase and the presence of a 890 cm^{-1} band in the IR spectrum show that it is a β-glucoside. The differences in properties of our material (colourless needles, m.p. 266–267°) and those of a synthesized material (pale yellow prisms,[9] 205–207°) may be due to the difficulties in purification. The difficulties in obtaining pure di- and tri-*O*-methylellagic acids and satisfactory analyses have previously been observed.[10,11]

Component P-11

This component could not be purified adequately and chromatographic examination of concentrates showed it to be a mixture of 3,3′-di-,3,4,3′-tri- and 3,4,3′,4′-tetra-*O*-methylellagic acids. Comparisons were made with materials prepared synthetically (see Experimental).

Component P-13

This component was present in very small amounts, but in comparison with chromatograms of mixtures of P-7 and authentic 3,3′-dimethylellagic acid 4′-glucoside it is probably the latter.

Both the extracted heartwood and its extractives contained polymerized leucocyanidin which, after heating with butanol–HCl, formed cyanidin that was identified chromatographically.

DISCUSSION

General

When a fresh log is cut, the narrow white intermediate zone (IZ; about 3 mm wide) can be distinguished from the surrounding creamy coloured sapwood (about 2 cm wide). Under UV the IZ has a strong mauve fluorescence. After the surface of the cross-section has dried in air, the boundary between the sapwood and IZ becomes dark brown and the portion of the zone adjacent to the heartwood becomes orange-coloured. Chromatographic examination (Table 1) showed there was little difference in the relative amounts of resolvable components in the IZ and heartwood. The 2-D PCs showed there were greater amounts of

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[9] Row, L. R. and Rao, G. R. S. (1962) *Tetrahedron* **18,** 357.

[10] Cain, B. F. (1963) *N.Z. J. Sci.* **6,** 264.

[11] Briggs, L. H., Cambie, R. C., Lowry, J. B. and Seelye, P. N. (1961) *J. Chem. Soc.* 642.

leucoanthocyanin polymer forming an unresolved streak on the BAW axis in the heartwood extract than in the IZ or sapwood extracts. This observation was supported by that of the anthocyanin-red colour given by sections of the wood when heated with BuOH–HCl. The leucoanthocyanin polymer could be largely extracted from that part of the IZ which turned orange, but, even after prolonged methanol extraction, the heartwood remained red and gave a strong leucoanthocyanin reaction. The leucoanthocyanin possibly masks the appearance given by the strongly mauve fluorescent P-7 that is evident in the outer IZ.

Differences in Composition of Different Tissues

Whereas the pedunculagin is the major component in the outer sapwood extractives, other ellagitannins (D-6, D-13), gallic acid and gallotannin P-9 are more important in the middle sapwood. 3,4,3′-Tri-*O*-methylellagic acid 4′-glucoside is formed in the large amounts in the outer IZ and in greater amounts in the heartwood. Large amounts of leucoanthocyanins are first formed in the growth rings at the IZ–heartwood boundary.

This sequence of formation of components in the IZ indicates the change in the metabolic processes across the zone. In the outer IZ, the polyphenols are derived mainly, if not entirely, from the pentose phosphate cycle and shikimic acid. In the inner IZ, in addition to the increased formation of these polyphenols there is also the formation in a few rows of cells of leucoanthocyanins which involve not only the pentose phosphate cycle but also acetate units from the tricarboxylic acid cycle.

An increase in peroxidase activity at the heartwood boundary has been observed by other workers[12,13] and was found also in the IZ of cross-sections of red box. The appearance of the thin brown band at the outer periphery of the IZ indicates there may be a further localized increased activity of peroxidase where it oxidizes the trihydroxy phenolic compounds present.

The above evidence of different metabolic and enzymic activity at different parts of the IZ indicates the complex nature of the processes forming heartwood. It is notable that increased amounts of the 3,4,3′-tri-*O*-methylellagic acid 4′-glucoside are formed in the IZ and heartwood. The ellagitannins and gallotannins also contain a glucose core. The heartwood extractives of many genera do not contain compounds with sugar moieties, and in those cases the sugar has presumably been metabolized during the period of energy requirements at the heartwood boundary.

Composition of the Extractives of other Red Boxes

The heartwood extractives of *E. bauerana* Schau., *E. fasciculosa* F. Muell., *E. conica* Deane et Maiden and *E. rudderi* Maiden were examined. (Samples of these other members of the distinctive red box groups[14,15] were obtained from the Standard Wood Collection of this Laboratory.) In all the extractives the trimethylellagic acid glucoside was a distinctive component and with the exception of *E. fasciculosa* the component which is possibly the 3,3′-di-*O*-methylellagic acid glucoside was present also in trace amounts.

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[12] WARDROP, A. B. and CRONSHAW, J. (1962) *Nature* **193**, 90.

[13] LAIRAND, D. B. (1963) *Drevarsky Vyskum* **1**, 1.

[14] BLAKELY, W. F. (1965) *A Key to the Eucalypts*, 3rd Edn (Com. Australias), For Timber Bur., Canberra.

[15] PRYOR, L. D. and JOHNSON, L. A. S. (1971) *A Classification of the Eucalypts*, Australian National University Press.

TABLE 3. PC PROPERTIES OF D-6 BY-PRODUCTS

Product	$R_f(\times 100)$ BAW	$R_f(\times 100)$ 6HOAc	Product	$R_f(\times 100)$ BAW	$R_f(\times 100)$ 6HOAc
D-6	17	51	D-6-4	31	30
D-6-1	11	64	D-6-5	49	29
D-6-2	21	74	Ellagic acid	45	4
D-6-3	30	47			

EXPERIMENTAL

General methods. Most of the methods used have been described previously.[2] In addition to BAW and 6HOAc the other chromatographic solvents used were conc. HCl–HOAc–H_2O (3:30:10; 'Forestal'); top phase of *n*-BuOH–EtOH–1·5 N NH_4OH (4:1:3; 'BEN') and the sugars were examined with BAW, *n*-BuOH–pyridine–H_2O (10:3:3, 6:3:1) and EtOAc–pyridine–H_2O (12:5:4). The same amount (2 mg) of sample was used in the preparation of all the chromatograms used for the comparison of relative amounts. The chromatograms were viewed under short-wave UV light (254 nm) before or after exposure to NH_3 vapour and in addition to the chromogenic sprays previously used[2] the following were also used: Na_2SO_3–Na_2CO_3–H_2O (15:3·5:350, 'NSSC') for ellagitannins, vanillin–HCl for flavans. The leucoanthocyanins were detected by heating in *n*-BuOH–HCl (19:1) at 100° for 30 min and the anthocyanidins identified with Forestal solvent. Chromatoplates of silica gel G.F. 254 with a thickness of 250 μ were prepared in a constant temperature room at 20° using EtOAc–$CHCl_3$–HCOOH (2:10:1) for purification. The NMR spectra were determined with a Varian HA-100 spectrometer, with acetone-d_6 or dimethylsulphoxide-d_6 as solvents.

Material examined. A log (40 cm diam.) of *E. polyanthemos* was collected at Broadford, Victoria and within 3 days peeled into veneers (5 mm thick). Sample strips from the various zones, were vacuum dried, ground and extracted in a Soxhlet with MeOH for 15 hr. Fresh veneer from the outer heartwood was split into strips, soaked in EtOH and later dried, ground and extracted (×6) at room temp. with EtOH. The ground material was dried and weighed and the fractions of the extracted materials were expressed on this basis. The EtOH extract was concentrated *in vacuo* and slowly poured into boiling H_2O, filtered hot, the precipitate washed several times with hot H_2O and then extracted in a Soxhlet with Et_2O to leave Insoluble A. The aqueous soluble portion was extracted in a liquid–liquid extractor with Et_2O (4 × 6 hr), then EtOAc (8 × 6 hr with daily change of solvent) and the remainder freeze-dried. The Insoluble A was refluxed with 70% EtOH (6×) to leave ellagic acid (1·8% yield). The Et_2O extract (0·9% yield) contained yellow oily material, component P-11 and gallic acid. The oily material was removed with light petrol. and the remaining material examined chromatographically. The first 4 batches of EtOAc extract left crystalline material on the side of the flask and this was removed with hot EtOH and crude P-7 (0·5%) settled on cooling. The EtOAc extracts (7·2% yield) contained mainly ellagitannins which were separated on Sephadex columns as previously described. Other methods of separation such as polyamide columns and borate-butanol extractions were much less efficient. The extracted aqueous material contained polymeric material with appreciable amounts of D-6 and D-13 and was freeze-dried. Anhydrous acetone removed D-6 and D-13 along with small amounts of polymer.

Column chromatography on Sephadex. The methods previously reported[2] were used on the EtOAc extract or acetone extract of the extracted aqueous material. The adsorbent was prepared essentially as recommended by the supplier. About 25 mg of sample/ml of swollen adsorbent gave the best results.

Ellagitannin D-6. The accompanying ellagitannins in the concentrate were removed by repeated passage through G25 (fine) Sephadex columns (0·9 × 33 cm), collecting the appropriate fractions which were immediately freeze-dried. The final product was a colourless, amorphous powder homogeneous by 2-D PC examination using BAW and 6HOAc and different sprays and gave only faintly yellow solutions. It turned increasingly dark on heating and did not melt. Its IR spectrum (KBr disc) showed peaks at 3400s, 1740s, 1615s, 1500w, 1445s, 1355s, 1310s, 1175s, 1095s, 1040s, 1000m, 915w, 895w, 860w, 830w, 800w, 790w, 775w, 750m, 685m, 660m cm^{-1}. Attempts to obtain the MS of D-6 and its silylated derivatives were unsuccessful. As the purity of methylated D-6 could not be ascertained its MS was not obtained. NMR: δ (DMSO-d_6) 6·60 (*s*); 6·50 (*s*); 6·36 (*s*); 5·47, 5·43, 5·36 (envelope); 4·96, 4·90, 4·82, 4·74 (envelope); 4·00, 3·88 (*bd*) ppm.

D-6 (5 mg) in N H_2SO_4 was heated at 90–95° for 16 hr, 2-D PCs were prepared after 0·5 and 1 hr and then hourly for 16 hr. Chromatograms which showed differences are described in Table 2. D-6 was no longer visible after 11 hr and D-6-5 after 15 hr. (Compound D-6-1 (Table 3) was observed in trace amounts only on fungal hydrolysis.) When heated at 100° with H_2O, D-6 gave a trace of ellagic acid after 1 hr and this became strong in 4 hr. Complete acid hydrolysis yielded glucose (identified chromatographically) and ellagic acid *ca.* 1:1 ratio. Alkaline hydrolysis with NaOH in MeOH under N_2 for 66 hr yielded glucose and ellagic acid which were identified chromatographically. There was no change when D-6 was incubated (29°) with β-glucosidase or α-amylase. When D-6 (90 mg in 9 ml H_2O) was incubated (29°) for 27 days with *Penicillium waksmanii* and filtered through a Millipore filter (0·5 μ pores) and freeze-dried, D-6-2 (61·8 mg) and insoluble ellagic acid were obtained. When *P. waksmanii* was incubated with maltose, trehalose and cellobiose, glucose was obtained, 2,3,- and 4,6-HHDP glucose were unaffected and corilagin was hydrolysed very slowly but the liberated gallic acid appears to be destroyed by the fungus.

A MeOH solution of D-6 (100 mg) was methylated with CH_2N_2 in Et_2O, evaporated, redissolved in MeOH and the methylation procedure repeated 8× to yield 127 mg of methylated product which was not pure. Other methylation procedures were tried with no greater success. The above product was hydrolysed with N H_2SO_4 for 18 hr at 100° but the product could not be crystallized. It was chromatographically (TLC using EtOAc–$CHCl_3$–HCOOH, 2:10:1) identical with authentic hexamethoxydiphenic acid prepared synthetically or obtained from methylated 2,3-HHDP-glucose. Saponification with N NaOH in MeOH for 5 hr under reflux yielded on acidification and recrystallization a few crystals m.p. about 260°. The mother liquor gave one spot (TLC) identical with hexamethoxydiphenic acid.

D-6-2. This compound has R_fs very close to those of D-4 but was distinguished by the colour reactions with NSSC and gives a slow pink with a mauve tinge changing to dull mauve after 15 min. It is a colourless compound which does not melt and an IR spectrum (KBr disc) with peaks at 3400s, 1735s, 1615s, 1500m, 1445s, 1365s, 1300s, 1200s, 1115s, 1085s, 1035s, 985m, 955w, 945w, 915w, 910w, 890w, 870w, 865w, 855w, 850w, 830m, 820w, 810w, 800w, 785w, 765w, 750m, 680m, 660m cm^{-1}. NMR δ (DMSO-d_6): 6·49 (*s*); 5·36, 5·32 (*d*); 4·90 (envelope); 4·89, 4·85 (*d*); 4·83, 4·66, 4·58, 3·69, 3·62, 3·54 (envelope) ppm.

Acid hydrolysis with N H_2SO_4 gave glucose and ellagic acid. Repeated methylation with diazomethane in Et_2O gave a mixture containing one main component which could not be crystallized from different aq. ethanol mixtures.

Hexamethoxydiphenic acid. Tetramethylellagic acid (1 g) was refluxed in 20% NaOH solution for 45 min, cooled, dimethyl sulphate (5 ml) added, and refluxed for 1 hr. The H_2O-washed product was saponified with 15% NaOH (3·5 ml) for 1 hr, acidified with HCl, refluxed 50% EtOH 10 min, filtered, and the filtrate evaporated and suspended in Et_2O which was extracted with 5% Na_2CO_3. The extract was acidified extracted with Et_2O, and the washed extract evaporated and the residue recrystallized from 50% EtOH (×5) m.p. 258° (lit.[16] 240°). (Found: C, 56·4; H, 5·3; OMe, 42·7. Calc. for $C_{20}H_{22}O_{10}$: C, 56·9; H, 5·2; OMe, 44·6%.) Hydrolysed methylated ellagitannin D-1 gave as the main product a compound with the same R_f on TLC using EtOAc–$CHCl_3$–HCOOH (2:10:1). NMR δ (acetone-d_6): 7·37 (*s*), 3·86 (*s*) 3·81 (*s*), 3·53 (*s*) ppm.

Ellagitannin D-13. The appropriate fractions were purified in the same manner as D-6. Ellagitannin D-13 was chromatographically identical with D-6-1 produced during hydrolysis of D-6 with *P. waksmanii.* However, solutions of D-13 were unaffected by this fungus or by β-glucosidase or α-amylase. NMR δ (DMSO-d_6): 7·02 (*s*); 6·48 (*s*); 6·36, 6·33 (*d*); 5·38, 5·36, 5·23 (envelope); 4·92, 4·84 (envelope); 4·30, 4·22 (envelope); 4·01 (*s*); 3·88, 3·85 (*d*); 3·65, 3·48, 3·26, 3·09 (envelope); 2·74 (*s*) ppm. The ratio of aromatic protons to sugar protons is about 1:7. Solutions of the compound were hydrolysed as previously described[2] but although a number of degradation products including ellagitannins were produced only ellagic acid and glucose were recognized.

P-7; 3,4,3′-*Tri-O-methylellagic acid* 4′-*glucoside*. The crude compound was recrystallized repeatedly (more than 9 times) from 70% EtOH to obtain colourless needles m.p. 266–267° (pale yellow prisms,[9] 205–207°). (Found: C, 52·6; H, 4·6; OMe, 17·5. $C_{23}H_{22}O_{13}$ requires: C, 54·5; H, 4·3; OMe, 18·4%.) λ_{max} (nm), in MeOH; 220, 247, 350, 365; in MeOH + Na acetate, 219, 247, 350, 365. The fluorescence of the compound was strong white blue in UV light which did not change in NH_3 vapour. It did not react with $AlCl_3$, $FeCl_3$, $FeCl_3$ (1%)–K_3 Fe(CN_6) (0·3%) (1:3), fresh 0·1% stabilized diazotized *p*-nitroaniline in 20% Na acetate. The solid gave a yellow colour with HNO_2. ν_{max} (KBr disc) 3430s, 2930s, 2930w, 2860w, 1750s, 1610s, 1570w, 1485s, 1460w, 1450w, 1440w, 1410m, 1355s, 1325m, 1255m, 1200w, 1170w, 1150w, 1100s, 1080s, 1035m, 990m, 915w, 890w, 870w, 860w, 790w, 755w, 740w, 680w. NMR δ (DMSO-d_6): 7·71 (*s*); 7·44 (*s*); 5·08 (*d*), 4·01 (*s*), 3·88 (*s*); 3·68, 3·66, 3·58, 3·50, 3·46, 3·33, 3·31, 3·27, 3·25 (envelope) ppm. P-7 (20 mg), pyridine (1·0 ml), Ac_2O (0·5 ml) were heated at 100° for 1 hr, and the acetate recrystallized from 70% ethanol, yield (14·7 mg, m.p. 253–254°. (Found: C, 55·3; H, 4·6; OMe, 12·1; CH_3CO 23·2. $C_{31}H_{30}O_{17}$ requires: C, 55·2; H, 4·4; OMe, 13·7; CH_3CO 25·5%). P-7 (2 mg) was incubated with β-glucosidase (2 mg) in H_2O (2 ml) at room temp. for 2 days and TLC examination EtOAc–$CHCl_3$–HCOOH (2:10:1) showed the only compound present was 3,4,3′-tri-*O*-methylellagic acid. P-7 (50 mg), 2 N HCl (10 ml), in 70% EtOH (10 ml) were refluxed for 6 hr, evaporated in vacuum, dissolved in hot H_2O (25 ml), cooled, crystals separated (33·2 mg, theory 34·0 mg), and recrystallized from aqueous acetone, or dimethylformamide m.p. 294–295°, m.m.p. 294–295° (lit.[17] 283° for 3,4,3′-tri-*O*-methylellagic acid). (*Anal.* Found: C, 58·4; H, 3·4; OMe, 23·05. Calc. for $C_{17}H_{12}O_8$: C, 59·3; H, 3·5; OMe, 27·0%.) λ_{max} (nm), in MeOH;

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246, 370; MeOH + Na acetate; 218, 252, 405. The IR spectrum was identical with that of authentic 3,4,3′-tri-*O*-methylellagic acid. The aglycone (14 mg), pyridine (1·0 ml), Ac_2O (0·5 ml) were heated at 100° for 1 hr and the acetate recrystallized from 70% EtOH, dried in vacuum at 45°, one spot TLC EtOAc–$CHCl_3$–HCOOH (2:5:1) *ca.* R_f 0·9, yield 10·7 mg, m.p. 270° (lit.[11] 264–265°). (Found: C, 59·2; H, 3·7; OMe, 22·6; MeCO, 11·6. Calc. for $C_{19}H_{14}O_9$: C, 59·1; H, 3·7; OMe, 24·1; MeCO, 11·2%). λ_{max} (nm) in MeOH, 246, 345, 358; in MeOH + Na acetate, 217, 246, 345, 358. The mother liquor from the above crystals was passed through columns of Amberlite 120 and Duolite A4, dried over solid NaOH pellets (yield 15·0 mg, theory 16·0 mg). It was chromatographed concurrently with other sugars and had the same R_f as glucose in BAW (R_f 0·14); *n*-BuOH–pyridine–H_2O (6:3:1, R_f 0·23), EtOAc–pyridine–H_2O (12:5:4, R_f0·40). The sugars were visualized by silver nitrate or aniline oxalate.

P-11; *Methyl-*O*-ellagic acids.* The methylellagic acids were prepared by suspending ellagic acid in MeOH, adding excess diazomethane in Et_2O and keeping at 0° for 3 days. The hot acetone extract of the evaporated material was separated into its components on a silica gel column (2 × 40 cm) using chloroform as eluting solvent. Tetra-*O*-methylellagic acid was eluted first, followed by tri- and finally di-*O*-methylellagic acids, and the three components were present in about equal amounts. Separation was almost complete and recovery was good. The di-*O*-methylellagic acid partly crystallized in some of the tubes of the relevant fraction. The tri- and di-*O*-methylellagic acids can also be removed in a smaller volume of eluting solvent by replacing $CHCl_3$, after the tetra-*O*-derivative was removed, with EtOAc–$CHCl_3$–HCOOH (2:10:1). Tetra-*O*-methylellagic acid was also separated by extracting a $CHCl_3$ solution with 5% Na_3CO_2 solution to remove the other ethers. The acids were recrystallized from dimethylformamide until chromatographically homogeneous. The tetra- and tri-methyl ethers were also sublimed. The tetra-*O*-methylellagic acid had m.p. and m.m.p. 355° (decomp.) (lit.[18] 355°). (*Anal.* Found: C, 60·5; H, 4·1; OMe, 34·4. Calc. for $C_{18}H_{14}O_8$: C, 60·3; H, 3·9; OMe, 34·6%). The tri-*O*-methylellagic acid had m.p. and m.m.p. 294–295° (lit.[17] 283°). (*Anal.* Found: C, 59·3; H, 3·6; OMe 26·3. Calc. for $C_{17}H_{12}O_8$: C, 59·3; H, 3·5; OMe, 27·0%.) The 3,3′-di-*O*-methylellagic acid had m.p. and m.m.p. 339–340° (lit.[18] 336–338°). (*Anal.* Found: C, 58·1; H, 3·1; OMe, 19·4. Calc. for $C_{16}H_{10}O_8$: C, 58·2; H, 3·1; OMe 18·8%.) The IR spectra of the ethers were identical with authentic compounds. The R_fs with BAW and BEN on PCs and EtOAc–$CHCl_3$–HCOOH (2:10:1) on TLC were identical with those of components of the concentrate of P-11. These components were separated on thick silica gel plates, the appropriate bands extracted with dimethylformamide and the UV spectra determined and found to be identical with the appropriate authentic compound.

Acknowledgements—We thank Mr. R. I. Willing for the NMR spectra, Professor K. Hayashi and Dr. J. A. Wunderlich for mass spectral examinations, Dr. B. F. Cain for samples of authentic *O*-methylellagic acids; Dr. H. Greaves for the identification of *Penicillium waksmanii* Zaleski and Donald Lang and Andrew Rozsa for help with some of the experimental work. Analyses were done by the Australian Microanalytical Service.

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