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KINOS OF *EUCALYPTUS* SPECIES AND THEIR ACID DEGRADATION PRODUCTS*

W. EDWIN HILLIS and YOSHIKAZU YAZAKI
Forest Products Laboratory, Division of Applied Chemistry, CSIRO, South Melbourne, Australia 3205

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Key Word Index—*Eucalyptus astringens*; *E. lehmannii*; *E. platypus*; Myrtaceae; kino; dihydrokaempferol 3-rhamnoside; acid degradation products.

Abstract—The kinos of *Eucalyptus astringens*, *E. lehmannii* and *E. platypus* contain mainly polymerized leucocyanidin, appreciable amounts of dihydrokaempferol 3-rhamnoside and trace amounts of other components. The acid degradation products of different kinos contained components not present in the products from wood and bark. These include phloroglucinol and two other components whose properties are described.

INTRODUCTION

INCREASING quantities of eucalypts are being planted throughout the world. One defect in some young, fast-growing *Eucalyptus* is that of kino formation and this can be more common in certain geographic regions. Kino is also formed when the active cambium of trees older than about 5 years is injured. The amount of kino formed vary considerably and although small amounts may not cause mechanical damage to the timber, they can increase alkali consumption in pulp manufacture.

In studying kino formation, it would be desirable to be able to estimate the amount present in small samples of wood. Earlier studies[1] have shown that kino forms degradation products with acid which are not produced by natural wood extractives. Identification of these products was undertaken to reveal whether one or more could serve as a measure of the amount of kino present and to indicate its composition.

During a study concerned with the role of ethylene in the initiation of polyphenol formation in eucalypts,[2] it was desirable to have an experimental species that gave a kino containing compounds readily resolvable by chromatography. An examination of the composition of the kino resulting from ethylene treatment might indicate whether ethylene initiated changes in the early or later stages of biosynthesis of polyphenols. The kino of *E. calophylla* R.Br. ex Lindl., studied earlier,[3] contains mainly polymerized leucopelargonidin and leucocyanidin. We found likewise that the kinos of *E. sideroxylon* A. Cunn. ex Woolls, *E. sieberi* L. Johnson, *E. macrorhyncha* F. Muell., *E. baxteri* (Benth.) Maiden et Blakely, *E. regnans* F. Muell., *E. camaldulensis* Dehnh., *E. marginata* Donn ex Sm. and others contained little, if any, monomers, the major portion being polymerized leucoanthocyanin. However, while the major portion of the kinos of *E. astringens* Maiden,

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* Part VI in the series "The Chemistry of Eucalypt Kinos". For Part V see HILLIS, W. E. and HORN, D. H. S. (1965) *Australian J. Chem.* **18,** 531.

[1] HILLIS, W. E. (1960) *Nature* **186,** 635.
[2] HILLIS, W. E. and SHAIN, L. in preparation.
[3] HILLIS, W. E. and CARLE, A. (1960) *Australian J. Chem.* **13,** 390.

E. lehmannii (Preiss ex Schau.) Benth., and *E. platypus* Hook. is polymerized flavans, small amounts of some monomeric compounds are also present. The nature of the components was determined in order to be able to detect changes, should they exist, in the kinos induced by administration of ethylene.

RESULTS AND DISCUSSION

Composition of Eucalyptus astringens, E. lehmannii *and* E. platypus *Kinos*

The main component resolved chromatographically appeared to be a flavonoid. It reacted with ferric chloride and coupled with diazotized *p*-nitroaniline. Its UV spectra with aluminium chloride[4] and with sodium acetate,[4] showed free hydroxyl groups at positions 5 and 7. The broad IR band around 3120 cm^{-1} and the carbonyl absorption at 1620 cm^{-1} confirmed the presence of a 5-hydroxyl group. These data and those from an examination of the hydrolysed components indicated the compound to be dihydrokaempferol 3-rhamnoside (engelitin) and the identity was confirmed by comparison with an authentic specimen. This compound has been previously isolated from the kino of *E. moluccana* Roxb. (syn. *E. hemiphloia* F. Muell. ex Benth.)[5] a eucalypt from the eastern States of Australia which has no close taxonomic relationship with the 3 species examined in this present study.

The kinos contain small amounts of 3 pairs of flavans with R_f values within the region 0·40–0·70 BAW and 0·35–0·75 6% acetic acid. Only trace amounts of ellagitannins were present and in some samples of *E. astringens*, small amounts of tetra- and tri-*O*-methyl ellagic acids were evident on the chromatograms. Engelitin was the only compound present in sufficient quantities for isolation.

The 2-D chromatograms of these three kinos are similar in appearance indicating that the composition of the kino can have some taxonomic value as these species are closely related.[6,7] Also, in contrast to many kinos such as those from *E. regnans* and related species, those three kinos examined turn brown on exposure to light instead of bright red.

It is noteworthy that the composition of the kino differs from the polyphenols in both the phloem and wood of *E. astringens*,[3] *E. lehmannii* and *E. platypus* (unpublished). The latter 2 species contain, in the heartwood, stilbenes, polymerized flavans, catechin, gallic and ellagic acids, ellagitannins and other components; the phloem contains ellagic and gallic acids, ellagitannins, methylellagic acids and glycosides (particularly with the latter species) and polymerized flavans.

Acid-degradation products of kino

In confirmation of earlier work[1] the acid-hydrolysed products of the kinos studied contained large amounts of red polymeric material, anthocyanidins, appreciable amounts of gallic acid and in some cases small amounts of ellagic acid. As these materials can also be found in the acid-hydrolysed products of wood and phloem they are not suitable for the purpose required.

Fresh kino samples yielded phloroglucinol (see also Ref. 1) which could be used as a means of chemical estimation of kino in young woody tissues. However, some samples

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[4] MABRY, T. J., MARKHAM, K. R. and THOMAS, M. B. (1970) in *The Systematic Identification of Flavonoids*, pp. 169–171, Springer, New York.

[5] HILLIS, W. E. and CARLE, A. (1963) *Australian J. Chem.* **16**, 147.

[6] BLAKELY, W. F. (1965) in *A Key to the Eucalypts*, 3rd Edn. Commonwealth of Australia Forestry and Timber Bureau, Canberra.

[7] PRYOR, L. D. and JOHNSON, L. A. S. (1971) in *A Classification of the Eucalypts*, The Australian National University, Canberra.

that had been stored for several years gave very small amounts of phloroglucinol and further study is needed to show the usefulness of phloroglucinol as an index of the amount of kino in mature wood of differing ages.

TABLE 1. SOME ACID-DEGRADATION PRODUCTS OF EUCALYPT KINOS

Compounds*	R_f (100) in		UV (254)	Appearance†	pNA‡
	BAW	6% HOAc		UV/NH_4OH	
Gallic acid	68	43	bu	s bu	br
Ellagic acid	38	2	m	y	f
Phloroglucinol	75	63	op	bu	or br
Unknown cpd **1**	79	32	w bu	bu	br
Unknown cpd **2**	92	10	y	s y	f br
Unknown cpd **3**	87	31	bu	bu	f br
Sublimation product of 1	81	73	op	op	f br

* Polymer and anthrocyanidins were also produced.
† bu—blue; br—brown; f—faint; m—mauve; op—opaque; or—orange; s—strong; y—yellow; w—white.
‡ Sprayed with diazotized *p*-nitroaniline.

All kinos so far examined yielded one or two distinctive compounds and occasionally traces of a third. *E. regnans* kino was chosen for detailed study because of its availability, its similarity with the kinos of most major species, and the absence of monomeric compounds which would complicate conclusions.

The monomeric degradation products were produced in small amounts and unknown compound **1** (Table 1) was a major one but only small amounts were obtained pure, m.p. 274–275. The IR spectrum showed a strong carboxyl band (1665 cm^{-1}), and the UV spectrum after the addition of $AlCl_3$ indicated the presence of vicinal hydroxyl groups (Table 2). None of the data matched those of dihydroxybenzoic acids.[8,9] Using calculations[9] for the effect of substituents on the principal ET band of substituted benzene derivatives, and as methoxyl bands (2830–2950 cm^{-1}) were absent in the IR spectrum, it is concluded that the compound is a trihydroxybenzoic acid, with the absence of hydroxyl *para* to the carboxyl group. (Calculations for salicylic and gallic acids closely matched observed values.) Compound **1** thus appears to be either 2,3,5- or 2,3,6-trihydroxybenzoic acid (m.p. 234·5–235·5 and 188·5–190·0 respectively). On sublimation, decarboxylation occurs, the calculated principal ET band in the UV has the wavelength corresponding to the decarboxylated

TABLE 2. UV SPECTRA OF UNKNOWN COMPOUND **1** AND SUBLIMATION PRODUCT A

Compound	MeOH	MeOH + NaOH	MeOH + $AlCl_3$	MeOH + $AlCl_3$ + HCl
1	251* (1·87)† 301 (0·23 sh) 334 (0·19)	261* (1·16) 282 (0·61 sh) 337 (0·45)	259* (1·44) 280 (0·62 sh) 320 (0·35)	251* (1·63) 301 (0·30 sh) 334 (0·21)
1a	293	‡	293	293

* λ_{max} (nm).
† Relative absorption in the one spectrum.
‡ Solution became quickly strong yellow with an indistinct spectrum.

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[8] COOPER-DRIVER, G., CORNER-ZAMODITS, J. J. and SWAIN, T. (1972) *Z. Naturforsch.* **276,** 943.

[9] SCOTT, A. I. (1964) in *Interpretation of the Ultraviolet Spectra of Natural Products*, pp. 94, 102, 109, 116, Pergamon, Oxford.

compound and a solution of the compound turns brown as expected for a substituted hydroquinone. Furthermore, whereas Compound **1** has an IR band (895 cm^{-1}) for isolated hydrogens in a phenyl ring,[10] the sublimed decomposition product had an additional band (820 cm^{-1}) for two adjacent hydrogens in a phenyl ring. Although these values support the view that Compound **1** is 2,3,5-trihydroxybenzoic acid, its m.p. is much higher.

The difficulties in isolating the unknown compounds make them unsuitable for the quantitative estimation of kino. None of these compounds is produced when leucoanthocyanin polymers from woody tissues are treated with acid and this indicates a significant difference in constitution and biosynthesis between them and kino polymers.

EXPERIMENTAL

Methods. 2-D PC on Whatman No. 1 paper with BAW (*n*-BuOH–27% HOAC, 1:1) and 6% HOAc. Location of phenols was with UV (254 nm and 365 nm) with NH_3, $FeCl_3$ (1%)–$K_3Fe(CN)_6$ (1%) (1:1, v/v), diazotized *p*-nitroaniline (0·05%) in aq. NaOAc (20%), vanillin in EtOH (10%) and conc. HCl (1:1). The presence of anthocyanidins was detected using Forestal solvent (HCl–HOAc–H_2O, 3:30:10). Sugars were chromatographed in BAW and EPW (EtOAc–pyridine–H_2O, 12:5:4) and detected with $AgNO_3$ in alkali and aniline phthalate. TLC was carried out on silica-gel (Merck GF 254), using ECF (EtOAc–$CHCl_3$–HCOOH, 2:10:1 v/v). Leucoanthocyanins were detected by heating the sample (10 mg) in *n*-BuOH–5 N HCl (1:1: 5 ml) at 100° for 30 min and chromatographing the reaction product in Forestal solvent. M.ps are uncorrected. The UV spectra were of MeOH solutions before and after the addition of NaOMe, NaOAc, $AlCl_3$, NaOAc and H_3BO_3, $AlCl_3$ and HCl.

Isolation of engelitin. Finely ground *E. astringens* kino (27 g) was ground to a powder and extracted at 100° 4 × with EtOAc (150 ml) for 10 min. The extract was evaporated in vacuum, dissolved in MeOH (20 ml), shaken with heptane (3 × 40 ml) filtered from dark brown insolubles, dispersed in H_2O (10 ml) refluxed for 15 min, filtered hot, cooled for 3 days and crystals (570 mg) removed and a further 282 mg (total 3·1%) recovered from the concentrated filtrate. Engelitin had R_f 0·79 in BAW, 0·50 in HOAc, 0·0 in ECF. The long needles had m.p. 172–176° (decomp.) (lit.[5] 172–175°), m.m.p., m.p. 172–176° (decomp.). Acid hydrolysis gave dihydrokaempferol (BAW, HOAc, and ECF and chromogenic sprays) and rhamnose. λ_{max} (nm) 294; λ_{min} 253 ν_{max} (KBr disk; cm^{-1}) 3350s, 2960w, 2930m, 2890w, 2700w sh, 1640s, 1590m, 1510m, 1460m, 1450m sh, 1375m, 1350m, 1290s, 1260s, 1225m, 1215m, 1200w, 1185m, 1160s, 1145s, 1115m, 1080s, 1060s, 1030s, 1015s, 995m, 970m, 910w, 885w, 870w, 845w, 820w, 805m, 760w, 735w, 690w, 670w, 650w, 640w, 630w, 595m, 555m, 520w.

Examination of acid-hydrolysed products of kino. Fresh *E. regnans* kino was extracted repeatedly with hot H_2O, and the soluble material (70% yield) freeze dried. It was refluxed with 2 N HCl for 3 hr and gave the same products as did the original kino heated for 7 hr. The insoluble material was separated, and the filtrate extracted with Et_2O to remove phloroglucinol, gallic acid, unknown compound **1** and other compounds, isoamyl alcohol removed the anthocyanidin and unknown compound **2** from the extracted aqueous layer which still contained polymeric material. The Et_2O extract was fractionated with No. 3 Whatman paper using BAW and the extracts of the appropriate bands then chromatographed with 6% HOAc until chromatographically pure material was obtained.

Unknown compound **1**. White crystals m.p. 274–275° with slow sublimation from 250°. ν_{max} (KBr disk, cm^{-1}): 3430m, 3250m, 1665s, 1610s, 1570m, 1510w, 1490m, 1400m, 1335s, 1260s, 1210w, 1180w, 1070s, 1040w, 1015w, 895w, 860w, 840w, 785m, 760w, 645w, 540w, 510w. It was extracted from Et_2O soln with 5% Na_2CO_3. Compound **1** was sublimed in vacuum at 100° to yield a product m.p. 166–168°. ν_{max} (KBr disk, cm^{-1}) 3500s, 3450s, 3260s, 3030m, 2920m, 2850w, 2700w, 2570w, 2450w, 1850w, 1725w, 1600w, 1510s, 1465s, 1350m, 1255m, 1240m, 1205s, 1185s, 1160w, 1095m, 1030w, 395w, 820w, 820s, 800w, 755s, 715m, 515w. The product turned dark brown on paper chromatograms.

Unknown compound **2**. This compound could not be extracted with Na_2CO_3 (5% soln) from ether and became orange on chromatograms.

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[10] NAKANISHI, K. (1962) in *Infrared Absorption Spectroscopy*, p. 27. Holden-Day, San Francisco.