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POLYPHENOLS OF *INTSIA* HEARTWOODS

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Key Word Index—*Intsia bijuga*; *I. palembanica*; Leguminosae; heartwood extractives; robinetin, 3,5,4′-tri- and 3,5,3′,4′-tetra-hydroxystilbenes, dihydromyricetin, myricetin, naringenin, leucocyanidin.

Abstract—Robinetin is the main polyphenol of the heartwood of *Intsia bijuga* and is accompanied by smaller amounts of 3,5,4′-tri- and 3,5,3′,4′-tetra-hydroxystilbenes, dihydromyricetin, myricetin and naringenin. The wood contains large amounts of water soluble polymers including leucocyanidin. The stilbenes are absent from the sapwood. Samples of *I. bijuga* and *I. palembanica* from several countries revealed differences in composition.

INTRODUCTION

Intsia bijuga (Colebr.) O.Ktze; (Leguminosae; subfamily Caesalpinioideae, common names include kwila, vesi) is a frequent species in the rain forests of several tropical countries. The brown heartwood of medium density (0·61 g/cm^3) has a high strength, a high durability to fungal attack, is resistant to termites and marine borers and is dimensionally stable. It could be used as a joinery and building timber in place of timbers which are becoming less readily available but has a disadvantage in some applications in that part of its extractives are water-soluble and can be readily leached out to stain adjacent material. In addition, during kraft pulping, pitch is produced and this doubtless is formed by the extractives. The wood when used for formwork also delays the setting of, or weakens, concrete.

This examination of the extractives was undertaken to ascertain the nature of the materials responsible for the above properties and to learn whether the disadvantages could be overcome.

RESULTS AND DISCUSSION

A heartwood sample of *I. bijuga* from Papua New Guinea contained 29·0% methanol extractives, which after dispersal into water were successively extracted with ether and ethyl acetate to yield 8·2 and 5·5% soluble solids. The remaining 15·3% (on heartwood basis) was water soluble. The monomeric components in the extracts are listed in Table 1. The amounts of components I-1 to I-7 were too small to enable separation and their chromatographic properties did not resemble known compounds.

Heartwood sawdust discolored cold (20°) water in 5 min and warm (50°) water removed 10% soluble material. Despite the low solubilities of pure robinetin and resveratrol these were present in the extract as well as the slightly water-soluble dihydromyricetin and naringenin. The polymeric material was possibly responsible for their solubilization in a manner similar to that encountered with ellagic acid in eucalypt extractives.

Little information could be learned about the readily water-soluble polymeric material which reacted strongly with ferric chloride. The production of cyanidin, and possibly a trace of pelargonidin, on heating with butanol–hydrochloric acid showed that polymeric leucoanthocyanins were present. Acid treatment produced water insoluble polymers but no other monomeric components other than robinetin were produced in more than trace amounts or were recognizable on chromatographic examination.

TABLE 1. SOME PROPERTIES OF POLYPHENOLS IN *Intsia bijuga* HEARTWOOD

Compound	R_f (× 100)* BAW	6HA	Colour‡ UV	Relative amount	λ_{max} (nm) MeOH	MeOH NaOAc	MeOH NaOAc H_3BO_3	MeOH $AlCl_3$
Robinetin	55	0	y.	+++	252	252	257	273
						270†	307†	
					318	318	316	316†
						331		
					368	376	388	452
Dihydromyricetin	68	28	op.	++	291	291†	291	314
					338†	325	331†	381
Resveratrol	82	0	bu.	++	306	306	306	—
					320	320	320	—
3,5,3',4'-Tetra-hydroxystilbene	62	0	bu.	+	304	—	306	—
					324	—	339	—
Naringenin	89	22	f.op.	+	286	283†	287	310
					334†	323	325†	374
Myricetin	73	0	f.y.	tr.	252	254	259	273
					308†	268†	306†	324†
						319		
					367	378	386	450
I-1	59	9	bu.	tr.				
I-2	72	15	bu.	tr.				
I-3	64	31	f.bu.	tr.				
I-4	66	38	op.	tr.				
I-5	69	50	or.	tr.				
I-6	87	43	f.bu.	tr.				
I-7	71	55	s-y	tr.				

* Taken from 2-D PCs. BAW = n-BuOH–HOAc–H_2O (6:1:2); 6HA = 6% HOAc.
† Shoulder.
‡ UV (254 nm) bu. = blue; f. = faint; op. = opaque; s-y = yellow after 1 day; y = yellow.

The yellow flecks readily visible in the vessels of most samples of *I. bijuga* were removed mechanically from some samples and found to be pure robinetin.

The change from the colourless sapwood to the brown heartwood in the Queensland and Papua New Guinea samples takes place over less than 5 rows of fibres. The yield (3·2%) of extractives from sapwood was much lower than that from heartwood (29·0%) and the composition was very different. Robinetin was either absent or present in faint trace amounts, the stilbenes were absent and myricetin and dihydromyricetin present in trace amounts. The most distinctive features of the sapwood extractives were 2 yellow fluorescent components (R_f above 0·95 in BAW; 0·0 in 6% HOAc) one of which gave an intense orange fluorescence in NH_3 vapour that was similar to that of chalcones. The data supports the view that heartwood extractives are formed *in situ* at the periphery.

A chromatographic comparison was made of extracts of heartwood samples of *I. bijuga* from Papua New Guinea (2 samples), West Irian, Fiji (5 samples), New Caledonia, Queensland, Australia and Madagascar (2 samples). With the exception of the latter, all 2-D chromatograms were very similar although the ratio of components to each other varied to a small degree. No geographical relationship with these small variations could be observed and all the chromatograms had a characteristic appearance. The chromatograms of the two samples from Madagascar were significantly different. Both lacked the two stilbenes which are distinctive in the other samples; one contained robinetin and myricetin but most other compounds were absent; in the other sample dihydromyricetin and some minor components were present. The chromatograms were different from those of *Afzelia africana*, which is closely related botanically, so that a mis-identification with *Afzelia* species of that region is unlikely.

Extracts of samples of *I. palembanica* from Indonesia, West Irian and Malaysia had very similar chromatograms which were readily distinguishable from those of *I. bijuga* from Fiji

and Papua New Guinea. Large amounts of robinetin and very small amounts of myricetin were present but apart from small amounts of Compound I–6 (Table 1) and trace amounts of 3,5,3′,4′-tetra-hydroxystilbenes little else was observed. Although the heartwoods of these 2 species are indistinguishable in visual appearance, and the anatomy is very similar or identical, the composition of their extractives is distinctly different.

There is little information on the wood extractives of the Caesalpinioideae. Although the 3,5,3′,4′-tetra- and 3,5,3′,4′,5′-penta-hydroxystilbenes have been isolated from *Vouacapoua*.[1] The 5-deoxyflavonoids common to other subfamilies of the Leguminosae are represented by robinetin. The tetra-hydroxystilbene and the *O*-dimethyl derivative of resveratrol have been found in the Lotoideae. Because of the variation of the occurrence of dihydromyricetin, the traces of myricetin and naringenin, and the stilbenes, the only compound recognized which may be of taxonomic significance in the Caesalpinioideae is robinetin.

The heartwoods of *Intsia* spp. have a high durability to fungal decay. When stilbenes are present in wood they have been considered responsible for this property in the past, but recently there has been evidence that has not supported this view.[2,3] It may be significant that *I. palembanica* with the same durability rating as *I. bijuga* lacks stilbenes. Robinetin does not have a high fungal toxicity[4] but the large amounts present and disposition of it in the wood[3] may play a significant role in this regard.

The easy water solubility of the *Intsia* polyphenols are responsible for some of the disadvantageous properties. Attention has been drawn to the retardation by similar extractives on the setting of concrete[5] but there are no data on the relative effect of the different components found in *I. bijuga*.

The constitution of the phenolic components of *Intsia* heartwood indicated that the formation of insoluble formaldehyde–phenolic components would reduce the migration of the extractives. Of the several methods tried, satisfactory stabilization of small samples was achieved by painting the surface with formaldehyde solution (40%) and after drying with ammonia (27%). Subsequent heating (above 100°) for a short period (1–2 min) rendered the components in the surface layers insoluble in water and the solvents used in common varnishes, although the colour of the timber was darkened.

Robinetin would possibly behave under pulping conditions in a manner similar to that shown by ellagitannins containing vicinal trihydroxy groupings.[6] Its destruction by alkaline oxidation should be readily achieved.

EXPERIMENTAL

Isolation. Ground sapwood and heartwood samples (1 g) from the same cross-section of *I. bijuga* from Papua New Guinea were extracted exhaustively with MeOH in a Soxhlet and the extract evaporated to dryness to yield respectively 3·2 and 29·0% solids. The heartwood extract was dissolved in MeOH and dispersed in H_2O and extracted with Et_2O and then EtOAc to yield 8·2 and 5·5% soluble material with 15·3% remaining in aqueous solution. Ground *I. bijuga* heartwood (626 g) obtained from the same sample was extracted in a Soxhlet with MeOH for about 1 week, with frequent renewals of solvent, and the extract evaporated in vacuum. The MeOH extract was redissolved in MeOH, extracted with heptane (0·6%

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solubles), allowed to stand for several days, the crystals removed, recrystallized from hot H_2O and 50% MeOH and identified as robinetin (I). The MeOH filtrate from I was evaporated in vacuum at 45°, dissolved in hot H_2O (200 ml), cooled, the precipitate of robinetin removed and the filtrate extracted with heptane (5 × 200 ml) then Et_2O (5 × 200 ml). The Et_2O extract was extracted with satd. $NaHCO_3$ solution (2 × 50 ml) and then with 5% Na_2CO_3 (2 × 50 ml) to remove further robinetin and the residue from the evaporated Et_2O was recrystallized from hot aq. MeOH and identified as resveratrol (3,5,4′-trihydroxystilbene) II. The mother liquor contained 3,4,3′,4′-tetrahydroxystilbene III and naringenin IV. The $NaHCO_3$ extract was acidified with HCl, extracted with Et_2O, the extract recrystallized from hot H_2O and further quantities of colourless crystals were collected from the mother liquor. It was identified as dihydromyricetin V. The aqueous material remaining after Et_2O extraction was extracted with EtOAc, but as chromatographic examination failed to show any new monomeric components it was not fractionated. The extracted aqueous residue was freeze-dried and subjected to acid treatment.

Methods of examination of components. The methods used for chromatographic and spectral examination of the extracts and components have been described.[7]

Robinetin I. The purified yellow crystals (yield 3·6%) had m.p. and m.m.p. above 350°, had R_fs and UV fluorescence identical with authentic material. The UV absorption spectra were identical with, and consistent for, robinetin (see Table 1). ν_{max} (cm^{-1} in KBr disks) 3450s, 3380s, 1600s, 1545m, 1520m, 1505w, 1465m, 1450w, 1415m, 1360m, 1305m, 1275s, 1235m, 1215m, 1190s, 1160m, 1120m, 1035m, 975w, 940w, 865w, 850w, 835w, 815w, 780w, 765w, 725w, 700w, 640w, 625w. The acetate had m.p. 221–223° (lit.[8] 225–226°).

Resveratrol II. Colourless crystals were obtained from aq. MeOH (yield 0·3%), with the same R_fs in different PC and TLC solvents,[9] colour reactions and UV spectra (Table 1) as authentic resveratrol and m.p. and m.m.p. of 265–267°. The acetate, recrystallized repeatedly from aq. MeOH, had dried m.p. 119–120° (lit.[10] 118–119°).

3,5,3′,4′-*Tetrahydroxystilbene* III. Attempts to isolate this compound from the mother liquor from the recrystallization of resveratrol by extraction of a BuOH solution with 0·1 M $NaHCO_3$ (pH 8·3) or with boric acid–NaOH buffers at pH 7·0, 8·0, 8·5, 9·0 were unsuccessful. Model experiments with resveratrol and 3,5,3′,4′-tetrahydroxystilbene behaved in a similar fashion. It was observed that the latter stilbene was more strongly fluorescent under UV than the former. The stilbene was identified co-chromatographically with authentic 3,5,3′,4′-tetrahydroxystilbene using three TLC solvents,[9] and paper chromatographic solvents of 30% HOAc, BAW, and BuOH–EtOH–H_2O (4:1:5 upper phase). The crude material was fractionated on No. 3 Whatman paper with BAW and the extract of the appropriate band gave UV absorption spectra identical with authentic material (Table 1).

Naringenin IV. A dull yellow band with a high R_f observed during the separation of the stilbenes II and III (above) was extracted. The compound present had the R_fs in TLC and PC solvents of naringenin compared simultaneously. It gave the specific magenta colour of flavanones with sodium borohydride in isopropanol followed by exposure to HCl[11]. The UV max. (Table 1) were identical with naringenin.

Dihydromyricetin (*ampeloptin*) V. A sample (3 mg) was heated with molten KOH for 30 sec, and after quenching in water, acidified, the aqueous material extracted with ether and the degradation products identified chromatographically by R_fs and with different spray reagents as phloroglucinol, gallic acid and a trace of protocatechuic acid. The R_fs of compound V in different solvents and colour reactions were identical with authentic dihydromyricetin. Its UV spectra (Table 1) are consistent with this constitution. The colourless crystals were repeatedly recrystallized from water (yield 0·2%) with m.p. brown 240° and black 250° (lit.[12] 247°,[13] 239–241°). ν_{max} (cm^{-1} in KBr disks) 3340s, 1635s, 1580s, 1535m, 1500w, 1465m, 1380 sh.m., 1355s, 1245s, 1210w, 1185s, 1150s, 1075m, 1020s, 995m, 950w, 830m, 810w, 750w, 720m, 680w, 660w, 645w, 625w, 580w, 540w, 525w. All attempts to prepare the acetate of this material or of authentic dihydromyricetin were unsuccessful.

Myricetin VI. Crude methanol extract was run on No. 3 Whatman paper using 6% HOAc, and the band R_f 0·0–0·10 was eluted and run again using BAW. The relevant band was eluted and purified and compared chromatographically and spectrally (Table 1) with authentic myricetin.

Ethyl acetate soluble extractives. Attempts to separate the small amount of the components in different solvents and on Sephadex G25 columns were unsuccessful. The only components recognized were those present in the Et_2O-soluble fraction and the remainder remained at the origin or was spread diffusely in the low R_f parts of the BAW and 6 HOAc chromatograms.

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Aqueous soluble residue. This material reacts strongly with $K_3Fe(CN)_6$–$FeCl_3$ reagent, and occupied the same region as the polymers in the ethyl acetate soluble fraction. It was heated in *n*-BuOH–HCl (5:1) at 100° for 30 min and chromatographed in different solvents. Cyanidin was identified chromatographically and pelargonidin appeared to be present.

Heptane soluble material. The pale coloured oil (0·6% yield) was not examined further.

Wood samples. Samples of *I. bijuga* and *I. palembanica* were obtained from the standard wood collection of the Forest Products Laboratory and the Fijian Forest Service.

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