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## Polyphenols in *Acacia mangium* and *Acacia auriculiformis* heartwood with reference to heart rot susceptibility

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**Abstract** The heartwood of *Acacia mangium* is vulnerable to heart rot and this is the first study to investigate the role of heartwood extractives in its susceptibility. *Acacia auriculiformis* was compared with *A. mangium* because it is rarely associated with heart rot. The heartwood extracts of both species were dominated by three flavonoids (2,3-*trans*-3,4',7,8-tetrahydroxyflavanone, teracacidin, and 4',7,8,-trihydroxyflavanone), which were purified and identified by nuclear magnetic resonance spectroscopy. The latter compound has not been previously reported in *A. mangium* and evidence for melacacidin is also newly reported. The mass spectrometric (MS) behavior of these compounds is given, for example teracacidin does not form molecular ions by either electrospray ionization or atmospheric-pressure chemical ionization. The nature of *Acacia* tannins was compared to quebracho tannin (composed of profisetinidins) using oxidative cleavage to enable MS detection but a negative reaction was obtained for both, which suggests the *Acacia* tannins may also be of the 5-deoxy proanthocyanidin type. The concentration of flavanones was less when *A. mangium* heartwood was decayed but the amount of proanthocyanidins was only slightly reduced and therefore these compounds may be more resistant to degradation by heart rot fungi. We found that the total phenol

content of *A. auriculiformis* was about fivefold that of *A. mangium*, and, while preliminary, this provides evidence for a role played by phenolic extractives in heart rot resistance of these *Acacia* species.

**Key words** Flavanones · Proanthocyanidins · LCMS · *Acacia* spp. · Heart rot

### Introduction

*Acacia mangium* is an important hardwood plantation tree grown in many areas of Asia, representing over 1 million ha of land use in the region.<sup>1</sup> One of the major impediments to using *A. mangium* for solid wood products is the prevalence of heart rot, which is fungal decay of heartwood in living trees.<sup>2–4</sup> Field studies have revealed that while *A. mangium* trees are susceptible to heart rot, *Acacia auriculiformis* and hybrids between the two species appear to be resistant.<sup>5,6</sup> Smaller branch sizes of *A. auriculiformis* have been suggested as a basis for the lower incidence of heart rot<sup>5</sup> because infection is established mostly through dead stubs and branches.<sup>5,7</sup> However, little research has been conducted on other factors that relate to decay resistance such as wood properties and extractives. The durability of heartwood in living trees is determined by a number of factors such as density, lignin content and type, water content, and especially extractives.<sup>8,9</sup>

Wood extractives from a variety of species of the large *Acacia* genus have been characterized and this has been thoroughly reviewed by Seigler.<sup>10</sup> *Acacia* heartwoods are dominated by flavonoids and proanthocyanidins (condensed tannins) with similar hydroxylation patterns.<sup>11</sup> A diversity of proanthocyanidin (PA) types have been found in acacia wood including procyanidins, profisetinidins (Fig. 1), prorobinetinidins, prodelphinidins, and proteracacinidins.<sup>10–12</sup> There have been few studies on the chemistry of *A. mangium* and *A. auriculiformis* heartwood extractives. Only two major flavonoids have been reported in *A. mangium* healthy heartwood,<sup>13,14</sup> which are 2,3-

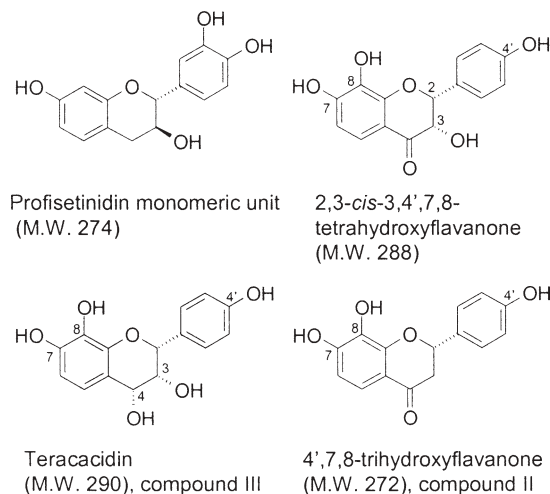
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**Fig. 1.** Structure of flavonoids found in *Acacia mangium* and *Acacia auriculiformis* and the profisetinidin subunit

*cis*-3,4',7,8-tetrahydroxyflavanone and teracacidin (Fig. 1). In *A. auriculiformis* heartwood, eight flavonoids have been identified, all having the 4',7,8-hydroxylation pattern, and include isoteracacidin (the 2,3-*cis*-3,4-*trans* isomer) and 4',7,8-trihydroxyflavanone (Fig. 1), in addition to the two reported for *A. mangium*.<sup>15</sup>

The aim of our studies was to characterize major compounds and conduct preliminary quantification of the wood extractives of *A. mangium* and *A. auriculiformis*, with emphasis on the wood from living trees rather than wood products. The nature of the condensed tannins has never been reported for either species. Quantitative comparison of the extracts between the two species also remains unreported to date. These studies are important to explore the role of extractives in heart rot resistance. Analysis of heart-rotted wood is also valuable to establish which compounds are degraded by the fungi and which are not. In addition to the methods of high performance liquid chromatography (HPLC) and nuclear magnetic resonance (NMR) spectroscopy used by Tachi et al.,<sup>13</sup> we used mass spectrometry with both electrospray ionization (ESI) and atmospheric pressure chemical ionization (APCI) to assist compound identification.

## Experimental

### Plant material

Australia. Wood samples were prepared from two 7-year-old trees each of freshly harvested *A. mangium* and *A. auriculiformis* grown in northern Queensland (Meunga, near Cardwell), where each species was grown in a hybridizing trial.<sup>16</sup> *A. mangium* tree 1 was seedlot 19256 from a Fiji seed production area. The Fiji orchard was dominated by seed collections from the Western Province in Papua New Guinea (PNG). This tree had a small amount of heart rot

but this was not near the heartwood sampled. Tree 2 was from CSIRO seedlot 17701 from the Claudie River in North Queensland. Both *A. auriculiformis* trees were from seedlot 19514 from a Melville Island seed orchard, which was based on seed from south-west PNG.

Indonesia. Wood samples were prepared from six freshly harvested *A. mangium* trees in plantations from two different regions of Sumatra (four trees in Riau and two in South Sumatra provinces). The trees were all 8 years old. The seed source of trees grown in these regions and descriptions of the soil and climate, have been described elsewhere.<sup>4</sup> Freshly harvested logs of trees with and without heart rot were sampled.

### Extractions

Large-scale extracts were prepared from heartwood of the four trees from Australia. Billets of wood were cut from 2 m above ground level directly after harvest. The wood was dried at 30–35°C in the dark, cut into disks, split, and then ground through a 1-mm mesh in a Wiley mill (Thomas Wiley Mill Model 4, Arthur H. Thomas Company, USA). The dried, ground heartwood (ca. 600 g) was extracted in 3.51 × 2 of 100% methanol (MeOH) at room temperature on a shaker in the dark. Each extraction was for 2 days and extracts were then filtered through a Whatman No. 5 filter paper. Extracts were taken to dryness on a rotary evaporator at 40°C.

Small-scale extracts were prepared from Indonesian plant material. Billets of wood were transported from the field in ice boxes and taken immediately to a laboratory (within 1 h). Samples of healthy sapwood, healthy inner and outer heartwood, and decayed and discolored heartwood were prepared using a chisel to make thin (<1 mm) shavings. These were weighed (ca. 100 mg fresh weight) and extracted in 1.5 ml × 2 of 70% aqueous acetone. Each extraction was for 24 h at 4°C in the dark. Samples were then stored at –20°C until being transported to Hobart for analysis.

### Fractionation and isolation

The large-scale extracts of tree 2 of both species were fractionated and purified using a schedule adapted from Tachi et al.<sup>13</sup> Thirteen grams of the bulk MeOH extract was dissolved in 10 ml of MeOH and extracted with 1 l of diethyl ether, ethyl acetate, and *n*-butanol, successively. Each successive extraction was performed at room temperature over 2 h. The three fractions and residue obtained are referred to as fractions A, B, C, and D respectively.

For the *A. mangium* extract, fraction A (2 g) was dissolved in *n*-hexane/acetone (1/1, v/v) and applied to a silica gel 60 column (Merck, Australia, 5 cm i.d. × 26 cm) and then eluted with *n*-hexane/acetone (7/3 and 5/5, v/v) and MeOH. The *n*-hexane/acetone (5/5, v/v) eluate was separated into three fractions and therefore five fractions were obtained in total (A-30, A-50-1, A-50-2, A-50-3 and M-100). The fraction A-50-3 was then further purified by preparative thin

layer chromatography (TLC) to isolate I and II ( $R_f$  0.16 and 0.36). Preparative TLC was completed using silica gel 60 plates (Merck, Germany) with a MeOH/dichloromethane (8/92) solvent. Bands were visualized with ultraviolet (UV) light (254 nm).

For *A. auriculiformis*, fraction A (3 g) was dissolved in 300 ml of ethyl acetate and extracted with 200 ml  $\times$  3 of water using a separation funnel. The water layer was collected and freeze-dried. To isolate III, the water-soluble fraction was further purified by preparative HPLC with a reversed phase Develosil ODS-10 column (20 mm i.d.  $\times$  250 mm, Nomura chemical, Japan) monitored at 280 nm. The solvent system used was as follows: a linear gradient elution for 40 min from 15% to 60% solvent B (MeOH) in solvent A [0.01% trifluoroacetic acid (TFA) in H<sub>2</sub>O] at a flow rate of 10 ml min<sup>-1</sup>.

#### Nuclear magnetic resonance (NMR) spectroscopy

<sup>1</sup>H-NMR and <sup>13</sup>C-NMR were recorded on a Jeol ALPHA-500 spectrometer (Jeol Tokyo, Japan). Chemical shifts are given in  $\delta$  values (parts per million), based on the <sup>1</sup>H and <sup>13</sup>C signals of the solvent (acetone-*d*<sub>6</sub>:  $\delta_H$  2.04,  $\delta_C$  29.8).

Compound I. MS  $m/z$ : 287 [M - H]<sup>-</sup>, 289 [M + H]<sup>+</sup>. <sup>1</sup>H-NMR (500 MHz, acetone-*d*<sub>6</sub>):  $\delta$  4.59 (1H, d,  $J$  = 12 Hz, H-2), 5.06 (1H, d,  $J$  = 12 Hz, H-3), 6.67 (1H, d,  $J$  = 8.5 Hz, H-6), 6.91 (2H, d,  $J$  = 8.5 Hz, H-3', 5'), 7.33 (1H, d,  $J$  = 8.5 Hz, H-5), 7.44 (2H, d,  $J$  = 8.5 Hz, H-2', 6'). <sup>13</sup>C-NMR (125 MHz, acetone-*d*<sub>6</sub>):  $\delta$  74.12 (C-2), 85.21 (C-3), 111.20 (C-6), 113.62 (C-4a), 115.79 (C-3', 5'), 119.06 (C-5), 129.25 (C-1'), 130.48 (C-2', 6'), 133.46 (C-8), 151.54 (C-8a), 153.18 (C-7), 158.74 (C-4'), 193.64 (C-4).

Compound II. MS  $m/z$ : 271 [M - H]<sup>-</sup>, 273 [M + H]<sup>+</sup>. <sup>1</sup>H-NMR (500 MHz, acetone-*d*<sub>6</sub>):  $\delta$  2.67 (1H, dd,  $J$  = 17, 2.5 Hz, H-3a), 3.08 (1H, dd,  $J$  = 17, 12.5 Hz, H-3b), 5.46 (1H, dd,  $J$  = 12.5, 2.5 Hz, H-2), 6.61 (1H, d,  $J$  = 8.5 Hz, H-6), 6.91 (2H, d,  $J$  = 8.5 Hz, H-3', 5'), 7.32 (1H, d,  $J$  = 8.5 Hz, H-5), 7.42 (2H, d,  $J$  = 8.5 Hz, H-2', 6'). <sup>13</sup>C-NMR (125 MHz, acetone-*d*<sub>6</sub>):  $\delta$  44.92 (C-3), 81.06 (C-2), 110.51 (C-6), 115.70 (C-4a), 116.07 (C-3', 5'), 118.73 (C-5), 129.12 (C-2', 6'), 131.17 (C-1'), 133.54 (C-8), 151.65 (C-7), 152.53 (C-8a), 158.60 (C-4'), 190.87 (C-4).

Compound III. MS  $m/z$ : 273 [M - H<sub>2</sub>O + H]<sup>+</sup>. <sup>1</sup>H-NMR (500 MHz, acetone-*d*<sub>6</sub>):  $\delta$  3.99 (1H, d,  $J$  = 4.0 Hz, H-3), 4.95 (1H, d,  $J$  = 4.0 Hz, H-4), 5.11 (1H, s, H-2), 6.47 (1H, d,  $J$  = 8.5 Hz, H-6), 6.82 (2H, d,  $J$  = 8.5 Hz, H-3'5, 5'5), 6.86 (1H, d,  $J$  = 8.5, H-5), 7.42 (2H, d,  $J$  = 8.5, H-2', 6'). <sup>13</sup>C-NMR (125 MHz, acetone-*d*<sub>6</sub>):  $\delta$  68.1 (C-4), 70.3 (C-3), 79.8 (C-2), 109.0 (C-6), 115.4 (C-3', 5'), 117.2 (C-4a), 118.7 (C-5), 129.5 (C-2', 6'), 130.8 (C-1'), 132.7 (C-8), 143.7 (C-8a), 145.7 (C-7), 157.8 (C-4').

#### Colorimetric assays

Total phenols were estimated by the Folin-Ciocalteu method as described previously.<sup>17</sup> Concentrations of total phenols were calculated with reference to a gallic acid standard curve.

A vanillin-hydrochloric acid colorimetric assay was used to determine flavanol content and was conducted with our previous method.<sup>18</sup>

To determine tannins, a protein precipitation method using bovine serum albumin (BSA, Wako, Japan) was used. This method was adapted from Kawamoto et al.,<sup>19</sup> and analyzed the BSA content of the supernatant liquid rather than the BSA content of precipitation. A 200- $\mu$ l volume of extract solution (2 mg ml<sup>-1</sup>, dissolved in 50% aqueous ethanol) was added to 200  $\mu$ l of BSA solution (2 mg ml<sup>-1</sup>, dissolved in 0.1 M acetate buffer, pH 5). After reacting at room temperature for 1 h, the solution was centrifuged (13000  $\times$  g, 2 min). The remaining BSA in the supernatant was determined by HPLC (CLASS M10A, Shimadzu, Japan) with a reversed phase Develosil 300 C4-HG-5 column (4.6 mm i.d.  $\times$  150 mm, Nomura chemical, Japan) monitored at 280 nm. The solvent system used was as follows: a linear gradient elution for 20 min from 20% to 80% solvent B (90% aqueous CH<sub>3</sub>CN containing 0.01% TFA) in solvent A (0.01% TFA in H<sub>2</sub>O) at flow rate of 1 ml min<sup>-1</sup>. The column temperature was 35°C.

#### HPLC-MS analysis

HPLC separations were carried out on a Waters Alliance 2690 using a Waters Nova Pak C18 column (3.9 mm i.d.  $\times$  150 mm). The mobile phase used three prepared solvents: A (water/acetic acid 98:2), B (MeOH/acetic acid 98:2), and C (100% *n*-hexane). The program used a linear gradient from initial conditions of 90% A and 10% B to 100% B over 6 min, then to 85% B and 15% C for 25 min. The flow rate was 0.8 ml min<sup>-1</sup>. Compounds were detected with a Waters 996 Photo-Diode Array detector, monitoring the range of 240–400 nm.

Mass spectrometry was carried out on a Finnigan LCQ (San Jose, CA, USA) with either electrospray ionization (ESI) or atmospheric-pressure chemical ionization (APCI) in either negative or positive ion mode using LCQ Navigator Version 1.2 software. The range from  $m/z$  125 to 1500 was scanned, with an AGC target value of  $2 \times 10^7$  and maximum ion injection time of 100 ms. The ESI operating conditions have been previously described.<sup>20</sup> APCI operating conditions were: sheath gas 50 psi, aux gas 5 psi, source current 6  $\mu$ A, capillary temperature 180°C, svaporizer temperature 460°C, capillary voltage -10V. Data-dependent MS-MS spectra were recorded as described above.

Semiquantification of individual compounds and proanthocyanidin-like polymers was performed using a rutin internal standard (Sigma, Sydney) with UV detection at 280 nm. To identify minor compounds (not purified and identified by NMR), HPLC retention times, UV spectra,

**Table 1.** Yield, total phenols, flavanol content, and tannin content for the large-scale methanol extracts and derived fractions of *Acacia mangium* and *Acacia auriculiformis*

Sample	Yield(%) <sup>a</sup>	Phenol content (%) <sup>b,c</sup>	Flavanol content (%) <sup>b,d</sup>	Tannin content ( $\mu\text{g BSA}/400\mu\text{g extract}$ ) <sup>e</sup>
<i>Acacia mangium</i>				
Bulk extract (1)	2.93	28.50	NA	NA
Bulk extract (2)	3.95	31.78	0.77	150.71
Fraction A (2)	0.94	38.39	2.10	25.71
Fraction B (2)	0.43	56.57	0.64	248.00
Fraction C (2)	0.37	21.29	0	320.96
Fraction D (2)	0.35	8.58	0	257.93
<i>Acacia auriculiformis</i>				
Bulk extract (1)	9.30	43.30	NA	NA
Bulk extract (2)	7.99	76.59	2.56	83.67
Fraction A (2)	4.54	75.96	0	9.24
Fraction B (2)	1.04	64.65	0	14.56
Fraction C (2)	0.82	20.85	2.73	78.69
Fraction D (2)	0.03	6.55	0.27	3.04

Figures in parentheses indicate tree number

NA, data not available; BSA, bovine serumalbumin

<sup>a</sup> Per dry weight of wood extracted

<sup>b</sup> Per dry weight of methanol extract

<sup>c</sup> Folin-Ciocalteu assay

<sup>d</sup> Vanillin-HCL test

<sup>e</sup> BSA assay

and MS data were used. Melacacidin from an *Acacia melanoxylon* extract was used as a standard.

### Depolymerization

Depolymerization of the acacia tannins was attempted by oxidative cleavage of the interflavan bond and nucleophilic trapping by phloroglucinol.<sup>21,22</sup> Bulk extract, fractions, or purified teracacidin (ca. 20mg) was dissolved in 100 $\mu\text{l}$  of 50% aqueous ethanol and placed in a 5-ml glass reaction vial (Alltech). To this 300 $\mu\text{l}$  of phloroglucinol reagent (80mg phloroglucinol in 0.5ml MeOH and 1.5ml 0.5M HCl) was added and the mixture was reacted for 5 min in a water bath at 100°C. The sample was then analyzed by negative ion LC-APCI-MS as above. The same method was tested on samples of quebracho tannin (kindly supplied by Prof. Ann Hagerman, Miami University, USA), which is dominated by profisetinidins.<sup>12</sup>

## Results and discussion

Extract yields and colorimetric assays of total phenols, flavanols, and tannins

Yields for the large-scale 100% MeOH extracts of *Acacia mangium* were an average of 2.5 times less than for *Acacia auriculiformis* (Table 1). For both species, of the fractions obtained from these samples, Fr-A (diethyl ether) contained the most extractives. Colorimetric tests revealed that for one sample of both species, flavanols were threefold and tannins were halved in *A. auriculiformis* when compared with *A. mangium*. Total phenols were an average of twofold higher in *A. auriculiformis* than *A. mangium* for the two

samples. In terms of extract yields per unit weight of wood, it was found that the amounts of phenols and tannins in the *A. auriculiformis* extract were about five times those in the *A. mangium* extract. While many more samples need to be analyzed to determine variability between different trees grown in different sites, this preliminary information supports the suggestion that phenolic extractives play a role in heart rot susceptibility.

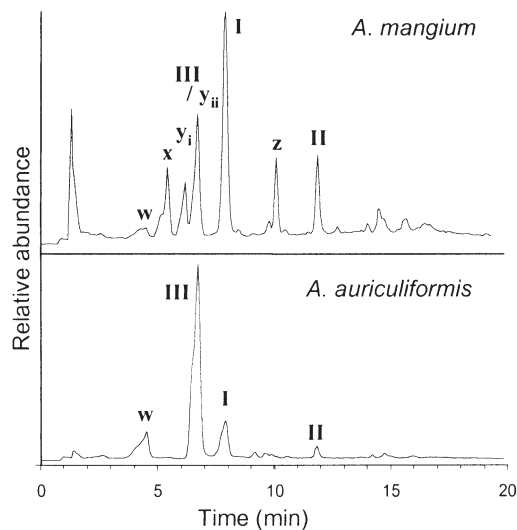
The presence of tannins in both *Acacia* species was confirmed by the BSA test which showed that fractions B, C, and D of *A. mangium* had the highest ability to precipitate protein (Table 1). The vanillin-HCl assay detected flavanols in the 100% MeOH extractives of both species but in low amounts (Table 1). This test poorly detects acacia and quebracho-type proanthocyanidins.<sup>18,22,23</sup> Proanthocyanidins are common in *Acacia* spp. wood and bark material<sup>10</sup> but Lange and Hashim<sup>24</sup> did not detect their presence in the polar extractive fractions of *A. mangium* wood using ethanol-hydrochloric acid. Therefore, it is assumed that the tannins in both *Acacia* species consist mainly of proanthocyanidins not sufficiently estimated by the vanillin-HCl assay.

HPLC-MS and NMR identification of flavonoids and proanthocyanidins

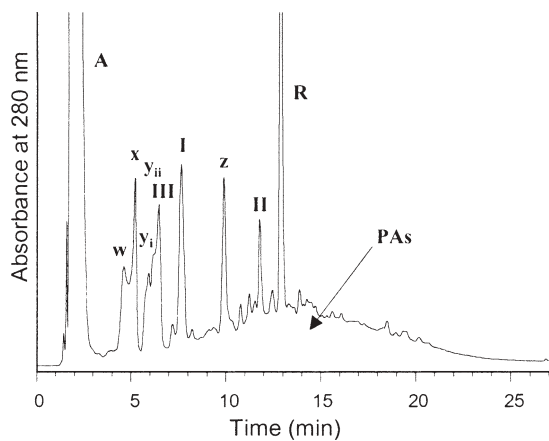
HPLC with MS or UV detection revealed that there were three main compounds detectable in both species (compounds I, II, III, see Figs. 2, 3). In *A. mangium* extracts from Australian or Indonesian trees, compound I was always dominant when detected by MS or UV but in *A. auriculiformis* compound III was the most abundant.

Compounds I, II, and III were purified and identified by NMR as 2,3-*trans*-3,4',7,8-tetrahydroxyflavanone; 4',7,8-trihydroxyflavanone; and teracacidin, respectively. Com-





**Fig. 2.** Positive-ion atmospheric-pressure chemical ionization-MS chromatogram of 100% MeOH extracts of *A. mangium* and *A. auriculiformis* heartwoods. I, 2,3-*trans*-3,4',7,8-tetrahydroxyflavanone; II, 4',7,8-trihydroxyflavanone; III, teracacidin; compounds w, x,  $y_i$ ,  $y_{ii}$ , z, see text



**Fig. 3.** HPLC-UV chromatogram (280 nm) of a 70% acetone extract of *A. mangium*. A, acetone; R, rutin internal standard; PAs, broad proanthocyanidin region

compound I is different to that reported by Tachi et al.,<sup>13</sup> because it is the 2,3-*trans*-isomer rather than the 2,3-*cis*-isomer previously reported. Analysis of the extracts by HPLC-MS revealed that while compounds I and II are detected as molecular ions using either APCI or ESI, teracacidin does not form a molecular ion by APCI and only a small amount by ESI. During negative ESI, the molecular ion (290) of teracacidin forms a dimer detected as  $[2M - H]^-$  ions at  $m/z$  579 which constitutes most of the compound. Other ions associated with the compound are  $m/z$  153 and 285 and a small amount of the molecular ion  $[M - H]^-$  at  $m/z$  289. Using positive-ion APCI, the molecular ion of teracacidin is dehydrated because of the instability of the 3,4-diol and is detected as a  $[M - H_2O + H]^+$  ion at  $m/z$  273, rather than  $[M + H]^+$  at  $m/z$  291.

Of the minor compounds, the material labeled as w in Figs. 2 and 3 had identical UV spectra and MS data to teracacidin but had an earlier retention time (see Figs. 2, 3). Isoteracacidin has been previously reported in *A. auriculiformis*<sup>15</sup> but was identified by paper chromatography and NMR spectroscopy. Because these methods were not used in our studies for compound w and no pure reference compound is available, identification of compound w as isoteracacidin cannot be confirmed. The compound labeled as x was identified as melacacidin, based on comparison with *Acacia melanoxylon* extracts. Melacacidin (MW 306) is a 3,4-diol (as is teracacidin) and its HPLC-MS behavior (K. Barry and N. Davies, 2001, unpublished data) was previously studied in *A. melanoxylon* heartwood in which it is the major extractive.<sup>20</sup> Melacacidin behaves in the same way as teracacidin by forming a dimer of  $[2M - H]^-$  at  $m/z$  611 during negative ESI and a dehydrated ion  $[M - H_2O + H]^+$  at  $m/z$  289 during positive APCI. Melacacidin was detected in small amounts in most of the Indonesian *A. mangium* samples and one Australian sample but not in the two Australian *A. auriculiformis* extracts. Compound w was detected in both *A. auriculiformis* samples and all *A. mangium* samples and showed identical ions by APCI and ESI-MS as found for teracacidin. The identification of melacacidin is previously unreported for *A. mangium*.

Peaks  $y_i$  and  $y_{ii}$  were routinely found in the extracts of Indonesian *A. mangium* samples but in only one of the Australian samples (Fig. 2). These peaks had  $[M + H]^+$  ions at  $m/z$  305 when detected by positive-ion APCI and  $[M - H]^-$  ions at  $m/z$  303 with negative-ion ESI indicating a MW of 304. The two chromatographic peaks ( $y_i$  and  $y_{ii}$ ) possibly represent two isomeric forms of one compound. The second peak ( $y_{ii}$ ) coeluted with teracacidin (III; Fig. 2). Lange and Hashim<sup>24</sup> identified taxifolin (MW 304) in *A. mangium* apparently by GC-MS, but  $y_i$  and  $y_{ii}$  have different UV spectra to taxifolin and different HPLC retention times. Compound z (Figs. 2, 3) was detected only in one Australian sample of *A. mangium* and also remains unidentified.

Proanthocyanin-like compounds were detected as an amalgamation of broad peaks on the HPLC-UV chromatogram (Fig. 3) but were not visible by LCMS (Fig. 2). While the BSA test detected tannins in the acacia extracts, the MW of peaks of PA-like compounds was beyond the detectable mass range of 2000 and therefore they must be large polymers. The depolymerization method used has been successful for a range of proanthocyanidins such as procyanidins<sup>21</sup> but our test with quebracho revealed that it was not effective for depolymerization of profisetinidin types as the extract remained unchanged. This is probably due to the lack of a 5-hydroxy group.<sup>25</sup> Negative-ion HPLC-APCI-MS analysis of the depolymerized samples from both species also revealed that the method was not effective in producing tannin products. While pure teracacidin did react during the test, resulting in an  $[M - H]^-$  ion at  $m/z$  397 that is representative of a subunit of MW 274 with a phloroglucinol attached (minus two hydrogens for the bond formed), fractions with tannin but no teracacidin (e.g., *A. mangium* fraction D) resulted in no reaction. While this test could not confirm the identity of the tannins, it is possible that they

**Table 2.** Concentration of proanthocyanidin-like compounds (PAs), and compounds I and II, 70% acetone extracts of *A. mangium*

	Sapwood	Heartwood	Discoloration	Decay
PAs	5.88 (0.92)	15.90 (1.26)	34.70 (3.47)	10.31 (3.81)
I	0	4.89 (1.19)	3.11 (0.90)	0.88 (0.88)
II	0	1.37 (0.25)	1.07 (0.09)	0.24 (0.24)
<i>n</i>	6	6	3	3

Values in parentheses are  $\pm$  SE. Concentrations given in mg rutin equivalent g FW wood<sup>-1</sup>

are quebracho-type tannins in both species. Further method development will be required to verify the nature of the tannins in the *Acacia* spp. samples.

#### Quantification by HPLC-UV

Two of the major flavonoids were quantified in the Indonesian *A. mangium* extracts in terms of rutin equivalents with UV detection at 280nm (Table 2). Compound I was detected in the highest amount at this wavelength, followed by II. Teracacidin (III) is poorly detected at this wavelength in comparison with the other compounds and, with the added complication of coelution with compound y<sub>ii</sub>, was not quantified. In all cases none or negligible amounts of I and II were found in the sapwood. Heartwood and discolored samples had the highest concentrations in all cases and showed no statistically significant differences when compared. The decayed wood had significantly lower concentrations of each compound suggesting that the compounds are degraded during the decay process.

Semiquantification of PA-like polymers in *A. mangium* was possible by integration of the characteristic amalgamation of compounds detected by HPLC-UV (Fig. 3) that were beyond the MS detection limits. The concentration of the PA-like compounds was significantly higher in the discolored wood. The heartwood had concentrations that were two-fold higher than the sapwood (Table 2). In contrast to the flavonoids (Table 2), the relative concentration of proanthocyanidins in the decayed wood was not decreased as much in proportion to the heartwood. That is, the concentration of compound I was ca. sixfold less in the decayed wood compared with heartwood, while the proanthocyanidins were only 1.5-fold less. This indicates that the proanthocyanidins are degraded to a lesser extent than the flavonoids in the decay process and supports a bioactive role for these compounds as discussed above.

#### Conclusions

The conclusions of this study are:

1. The heartwood of both species contains similar types of flavonoids.
2. The quantity of total phenols is approximately fivefold higher in *Acacia auriculiformis* than in *Acacia mangium*.

3. A similar amount of tannins was detected in *A. auriculiformis* wood compared with *A. mangium*; therefore, monomeric phenolics such as flavonoids may contribute to the relative heart rot resistance of *A. auriculiformis* compared with *A. mangium*.
4. The flavonoids I and II are degraded in *A. mangium* during the decay process, while the proanthocyanins are degraded to a lesser extent. The contribution of the components of the phenolic extracts to the heart rot resistance has been further explored with bioassays of heartrot fungi.<sup>28</sup>

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