Holz als Roh- und Werkstoff 59 (2001) 61–66 © Springer-Verlag 2001

The composition of the extractives from unaffected and heartrot affected heartwood of *Acacia mangium* Willd

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Yield and composition of the extractives from sound and heartrot affected wood of *A. mangium* has been studied by a successive extraction of the wood with a series of solvents with increasing polarity, followed by GC, GC/MS and DC studies of the fractions obtained. The study of the extractives of sound and affected wood with respect to yield and composition cannot explain the influence of the heartrot fungi on wood components in detail. Differences mainly have been found in the more polar fractions (acetone/water and ethanol/water, respectively). The content of lipophilic extractive constituents is with about 0.6–0.7% rather low and does not differ between sound and affected heartwood. The composition of the lipophilic extractives is not very different either.

Die Zusammensetzung der Extrakte aus gesundem und aus von Kernfäule befallenem Kernholz von Acacia mangium Willd

Die Ausbeute und die Zusammensetzung der Extraktstoffe von gesundem und Kernfäule-befallenem Holz von *A. mangium* wurde untersucht, wobei sukzessiv mit einer Reihe von Lösungsmitteln ansteigender Polarität extrahiert und die einzelnen Fraktionen mit Hilfe von GC, GC/MS und DC untersucht wurden. Die Untersuchung der Extrakte aus gesundem und befallenem Holz zeigte, daß Extraktausbeuten und Zusammensetzung den Einfluß des Pilzbefalls auf die Holzkomponenten nicht detailliert beschreiben Können. Unterschied in Ausbeute und Zusammensetzung der Extrakte wurden hauptsächlich in den polaren Extraktfraktionen (Aceton/Wasser- und Ethanol/Wasser-Extrakte) gefunden. Der Gehalt an lipophilen Extraktbestandteilen ist mit etwa 0.6–0.7% eher niedrig, gesundes und befallenes Holz unterscheiden sich praktisch nicht. Auch die Zusammensetzung der lipophilen Extrakte ist kaum verschieden zwischen gesundem und befallenem Holz.

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1 Introduction

A. mangium is one of the major compensatory plantation species grown in Malaysia that was initially planted in Sabah in 1966 (Logan and Balodis 1982). These trees were planted with the aim of providing future utility timbers and fibre for pulp and paper industry (Othman and Seng 1993; Peh et al. 1982; Kader and Sui 1989). It is a fast growing tree and could be planted in places of low fertility soil where other species could not survive (Pinyopusarerk et al. 1993).

The occurrence of heartrot was first reported in 1981 in Sabah (Gibson 1981). The extent and severity of the occurrence of heartrot studied by Sudin et al. (1993) showed that for an eight year old *A. mangium* tree, the attack could be as high as 48.7%. It was reported by Ito (1991) that about 50% of the infection occured through dead branch stubs, through poorly healed wounds of dead branch stubs, roots, and split bark. Studies were also accomplished investigating the occurrence, symptoms, and influence of silvicultural treatments on heartrot (Lee et al. 1988; Lee and Zakaria 1992; Lee 1993; Chong 1993). Possible fungi species responsible for heartrot are *Phialophora, Trichoderma, Rhinocladiella, Thelaviospis and Paecilomyces* that probably occur (Lee et al. 1988). The incidence is higher in bigger diameter class trees and had correlation with site condition and seed sources and age (Ito and Nanis 1994; Ito 1998). The resistance of *A. mangium* exposed to white rot and brown rot showed great variations (Ujang and Amburgey 1993). The intensity of the induced wound to the eight year old *A. mangium* tree showed that the intensity of the wound reaction is low compared with European hardwoods (Schmitt and Liese 1994). Studies on the wood properties between sound and heartrot affected samples of *A. mangium* showed that heartrot does not affect the fibre length since it commenced at the pith where most cells were already fully developed. (Sulaiman et al. 1998).

Extractives on the other hand, have been known to play an important role towards wood properties (Immamura 1989). Extractives studies on *A. mangium* trees as possible sources for cement-bonded particle boards the wood of *A. mangium* unfortunately showed a tendency to inhibit cement hardening (Tachi et al. 1988). Two flavonoids have been isolated from the wood (Tachi et al. 1989). The same authors found a major extractive component, teracidin, with a 7,8-dihydroxyl group in a proanthocyanidin stucture to have a strong inhibitory effect in cement hardening. Although extractives of many Acacia species have

been examined by Harbone (1989) and also by Hillis (1977), few data are available on the study of extractives on the differences between unaffected and heartrot affected *A. mangium,* if any. Therefore the objective of this study was to look for such differences in extractives composition.

2
Materials and methods

Heartrot affected and unaffected sapwood and heartwood samples from *Acacia mangium* were obtained from Byram Forest Reserve, Pulau Pinang, Malaysia. The air dried samples were chipped and milled to pass a sieve with 0.5 mm grating. 100 g of air dry wood meal of each sample were extracted for 10 h using a Twisselmann extractor. The method of successive extraction of wood meal and related materials with petroleum ether, diethyl ether, acetone/water (9:1), ethanol/ water (8:2) and hot water has been described elsewhere (Kubel et al. 1988; Weissmann et al. 1992; Lange 1992a, 1992b). For the determination of hot water solubles (hot water extract) the method TAPPI Standard T 207 om-93 (TAPPI 1996) has been used. After each extraction the extracted woodmeal was air dried before it was extracted with the next, more polar solvent.

Petroleum ether extractives were separated into a free acid fraction and a fraction of the neutral parts by extracting the petroleum ether solution with sodium carbonate solution – by that means the free acids are removed from the petroleum ether solution. The petroleum ether solution after this extraction was washed with water, dried over sodium sulfate and then the solvent was distilled off to yield the neutrals. The sodium carbonate solution was acidified with sulfuric acid to yield the free fatty acids, which were extracted from the acidified solution with diethyl ether, the ether solution then was washed thoroughly with water, dried over sodium sulfate and the solvent was distilled off to yield the fraction of free acids.

The fraction of the neutrals was saponified with boiling ethanolic KOH solution for one hour. After that the ethanol mainly was distilled off, and water was added to the reaction mixture in order to dissolve the mixture in the water. After cooling the solution was extracted with diethyl ether to yield the fraction of the unsaponifiables. The aqueous solution then was acidified with sulfuric acid to yield the acids from the saponification of the ester parts of the neutrals (glycerides and/or sterol esters etc.). The separation of the unsaponifiables into hydrocarbons and hydroxyl-containing compounds was accomplished with solid phase extraction using disposal cartridges (Aminopropyl-Bond Elut, Analytichem International). The hydrocarbons were eluted from the cartridges with hexane, and successively the hydoxyls were eluted with chloroform (Kaluzny et al. 1985). All fractions have been studied using GC or GC/MS. For GC studies of the fatty acid fractions the fractions have been esterified with diazo methane to yield the methyl esters. The composition of other fractions from petroleum ether extractives have been studied without any derivatization.

GLC were performed using a Chompack CP 9001 chromatograph equipped with FID and fused silica capillary columns DB 5, 30 m.

From the extraction of woodmeal with diethyl ether only the yield has been determined. The composition of these fractions have not been studied. Commonly, these fractions of a successive extraction of wood meals contain partly oxidised fats and fat accompanying compounds as well as, in some cases, low molecular phenolics or phenolic acids.

The acetone- and ethanol-water extractives were separated into three fractions. In the first step 100 mg of the extract was shaken with 20 ml of warm water (45 °C) for 1.5 h. Insoluble parts have been filtered off using porous glass filter funnel. The filtrates were further separated into carbohydrates and soluble phenolics, using disposal cartridges C18 modified silica gel (Bond Elut C18, Analytichem International). Carbohydrates were eluted with water and the phenolics stripped off with methanol.

3
Results and discussion

3.1
Successive Extractions

The yields of the extractions are listed in Table 1. As can be seen from the data, there is a difference in extractives contents of heartwood and sapwood of *Acacia mangium,* mainly in the contents of polar constituents (acetone/water plus ethanol/water fractions). This is quite regular in the case of wood and can be found with most species. From the heartwood, the content of petroleum ether extractives is in the normal range of variation and there is no difference between unaffected and heartrot affected samples. Concerning the diethyl ether extractives, the difference between inner and outer heartwood is within the normal range of variation. A distinct difference is to be seen between unaffected and heartrot affected heartwood samples. The diethyl ether extractives content decreases by about 45%. On the other hand, there is an increase of diethyl ether extractives in unaffected and heartrot affected sapwood. There is no conclusive explanation for this difference between sapwood and heartwood. The differ-

Table 1. Yields of extractives (based on o.d.wood) from *Acacia mangium* wood samples, obtained by a successive extraction with solvents of increasing polarities
Tabelle 1. Extraktausbeuten der sukzessiven Extraktion von Holzproben aus *Acacia mangium* mit Lösungsmitteln steigender Polarität

Sample no.	1 (%)	2 (%)	3 (%)	4 (%)	5 (%)	6 (%)
Extract						
Petroleum ether	0.34	0.52	0.70	0.59	0.65	0.76
Diethyl ether	0.62	1.16	2.63	1.47	2.40	1.26
Acetone/water 9:1	1.41	1.62	9.63	12.48	7.08	10.22
Ethanol/water 8:2	1.44	1.15	0.41	0.26	1.09	1.57
hot water	1.9	0.9	2.1	1.0	2.8	4.4

sample 1: unaffected sapwood
sample 2: heartrot affected sapwood
sample 3: unaffected outer heartwood
sample 4: heartrot affected outer heartwood
sample 5: unaffected inner heartwood
sample 6: heartrot affected inner heartwood

ences between the yields of diethyl ether extractives from affected and unaffected heartwood samples may be due to enzymatically catalyzed condensation reactions.

About 87–97% of the hydrophilic components are being extracted with acetone/ water, only 3–13% of the polar constituents have been removed successively by an ethanol/water extraction. Concerning the polar constituents, there are more extractives present in the outer heartwood than in the inner (unaffected wood samples). An increase can be recognized between unaffected and heartrot affected heartwood samples. This increase amounts to 30% in acetone-water extractives of outer heartwood and 44% of inner heartwood, respectively.

Table 2. Composition of petroleum ether extractives from *Acacia mangium* wood samples
Tabelle 2. Die Zusammensetzung der Petroletherextrakte der Holzproben aus *Acacia mangium*

	Sample number					
	1 (%)	2 (%)	3 (%)	4 (%)	5 (%)	6 (%)
petroleum ether extractives	0.34	0.52	0.70	0.59	0.65	0.76
free acids	34	18	18	16	28	43
neutrals	65	68	61	71	71	35
acids from neutrals*	86	64	62	63	57	72
unsaponifiables	14	35	36	35	30	25
hydrocarbons	27	21	19	16	10	12
hydroxyl cpds.	58	65	60	50	72	72

* after saponification
sample 1: unaffected sapwood
sample 2: heartrot affected sapwood
sample 3: unaffected outer heartwood
sample 4: heartrot affected outer heartwood
sample 5: unaffected inner heartwood
sample 6: heartrot affected inner heartwood

3.2
Petroleum ether extractives

The petroleum ether extractives have been fractionated into fractions of free fatty acids, fatty acids from neutrals after saponification of the the neutrals and fractions of hydrocarbons as well as of hydroxyl containing compounds from the unsaponifiables. Separation methods have been described in chapter 2. Yields of fraction are shown in Table 2.

3.2.1
Free fatty acids and acids from neutrals after saponification

The mixtures of fatty acids (free acids as well as acids from neutrals) are rich in higher acids, cerotic (C-26) and montanic acid (C-28), which commonly are constituents of wax esters like bees wax or lanolin and which are not often found to be constituents of petroleum ether extractives from woods. The fatty acid composition from sapwood neutrals is quite different from that of heartwood neutrals, about 36–39% of the fatty acids from sapwood neutrals consist of unsaturated C-18 acid, while in the acids from heartwood neutrals only 6–9% unsaturated C-18 acids have been found. Data on fatty acid composition are given in Table 3. As can be seen from this table, the sapwood samples contain small amounts of the saturated C-30 (melissic acid), C-32, C-34 and C-36 acids. During separation of free fatty acids from samples 5 and 6, a remarkable amount of a precipitate has been observed while extracting the solution with sodium carbonate. This precipitate has been filtered off, thoroughly washed and dried.

Table 3. Composition of fatty acid fractions from petroleum ether extractives from the heartwood of *Acacia mangium*
Tabelle 3. Die Zusammensetzung der Fettsäurefraktionen aus den Petroletherextrakten des Kernholzes von *Acacia mangium*

	Rt_{rel}*	Free acids		Acids from neutral parts					
		3 (%)	4 (%)	1 (%)	2 (%)	3 (%)	4 (%)	5 (%)	6 (%)
Fatty acid									
C-12	0.57	?	0.7	?	?	0.8	1.0	0.7	0.5
C-14	0.78	?	tr.(?)	?	?	1.7	2.0	0.6	0.7
C-16	1.00	1.7	1.0	16.7	17.0	1.9	2.4	2.1	2.8
C-18, unsat.	1.19	2.5	0.8	39.1	36.2	6.1	7.4	5.7	8.8
C-18, sat.	1.22	0.7	0.7	4.4	2.9	0.5	0.6	0.5	0.7
C-20	1.43	1.2	0.7	1.0	0.9	0.4	0.5	0.5	0.5
C-22	1.64	2.8	3.2	1.9	2.2	1.3	1.5	2.2	1.8
C-24	1.92	9.9	14.6	3.7	6.3	5.9	6.6	9.8	8.1
C-26	2.32	26.3	31.1	7.2	11.4	21.5	20.1	27.1	22.2
C-28	2.90	29.0	31.6	6.7	10.4	28.6	28.3	24.7	28.4
C-30	3.36	–	–	1.4	0.8	0.2	0.2	0.1	0.1
C-32	4.06	–	–	1.4	0.9	tr.(?)	tr.(?)	?	?
C-34	5.02	–	–	1.5	0.7	–	–	–	–
C-36	6.31	–	–	1.1	0.5	–	–	–	–

*$Rt_{\text{acid C-16}}$ = 11.78 min., column: DB-5, T = 150–280 °C, 7.5 °C/min, 60 min. constant at 280 °C. Injector 250 °C, Detector: 280 °C
sample 1: unaffected sapwood
sample 2: heartrot affected sapwood
sample 3: unaffected outer heartwood
sample 4: heartrot affected outer heartwood
sample 5: unaffected inner heartwood
sample 6: heartrot affected inner heartwood

By IR-studies this solid has been found to contain carboxyl groups but has not been identified yet. Therefore no composition of the free fatty acid mixtures is being given in Table 3 for the samples 4 and 5.

3.2.2
Hydrocarbons from unsaponifiables

The hydrocarbon fractions, which have been separated from the unsaponifiables, are complex mixtures of hydrocarbons with more than 130 components (see Table 4.). Among these the n-paraffins of the methane series appear in form of a Gauss-like distribution with eikosane (C-20) being the maximum component. The series of compounds from tetradecane (C-14) to octakosane (C-28) have been identified. Furthermore, the components U-1 to U-12 have been registered but remained unidentified. Possibly these components are branched iso-paraffins. Besides, one third or more of each fraction consists of about 100 unidentified minor components (each cpd. <0.5%), probably also hydrocarbons. The sapwood fractions (1 and 2) have not been studied in detail.

Table 4. Composition of hydrocarbon fractions of petroleum ether extractives from the heartwood of *Acacia mangium*
Tabelle 4. Zusammensetzung der Kohlenwasserstoff-Fraktion des Petroletherextrakts aus dem Kernholz von *Acacia mangium*

Compound	Rt_{rel}*	Sample number			
		3 (%)	4 (%)	5 (%)	6 (%)
Hydrocarbons					
C-14	0.53	0.4	0.4	1.2	0.6
U-1	0.67	2.5	2.6	5.0	3.9
C-15	0.74	0.4	0.4	1.8	1.4
U-2	0.87	0.4	0.4	0.8	0.7
C-16	1.00	1.0	1.0	1.2	1.4
U-3	1.13	1.0	1.1	1.0	1.0
C-17	1.26	1.2	1.2	1.0	1.3
U-4	1.38	1.1	1.1	0.8	1.1
C-18	1.51	1.2	1.2	1.0	1.3
U-5	1.62	1.8	1.8	1.3	1.7
C-19	1.75	3.5	3.2	2.0	3.0
U-6.0	1.87	5.4	5.2	3.2	4.2
U-6.1	1.92	2.4	2.3	1.8	2.3
U.6.2	1.95	1.9	1.3	1.5	2.0
U-6.3	1.97	1.5	1.8	0.7	0.5
C-20	2.01	5.7	5.2	3.2	4.5
U-7.0	2.06	2.9	2.9	2.2	2.8
U-7.1	2.11	1.7	1.4	1.2	1.7
U-7.2	2.13	1.4	1.4	1.3	1.6
U-7.3	2.17	5.6	5.2	3.5	4.9
U-7.4	2.23	2.9	3.1	2.5	2.8
C-21	2.29	1.7	1.4	1.3	1.7
U-8.0	2.36	3.6	3.3	2.3	3.1
U-8.1	2.50	1.4	1.7	1.0	1.4
C-22	2.59	3.8	3.2	2.2	2.8
U-9.0	2.75	1.2	1.2	1.0	0.4
U-9.1	2.79	1.2	1.2	1.5	1.0
C-23	2.86	2.3	2.1	1.7	1.7
U-10	2.96	1.2	1.7	1.0	0.2
C-24	3.10	0.7	0.7	0.5	0.1
U-11	3.20	1.9	2.1	1.2	1.3
C-25	3.34	0.8	1.3	2.2	0.4
C-26	3.62	0.8	1.0	0.7	0.7
C-27	3.92	0.1	0.1	tr.	tr.
C-28	4.13	0.5	0.5	0.3	0.5
minor components		32.9	31.4	44.9	40.0

* Rt_{C-16} = 10.31 min. T = 150–270 °C, 3.0 °C/min., constant at 270 °C for 40 min
injector: 250 °C, detector 280 °C
sample 3: unaffected outer heartwood
sample 4: heartrot affected outer heartwood
sample 5: unaffected inner heartwood
sample 6: heartrot affected inner heartwood

3.2.3
Hydroxyl fraction from unsaponifiables

In the hydroxyl fractions 3–6, β-sitosterol in amounts of about 25% has been determined. Besides 1.2–2.6% of tetrakosanol as well as small amounts (0.1–0.3%) of the corresponding C-12, C-18 and C-22 fatty alcohols have been identified. Two further major components of these fractions (about 25% each) with Rt_{rel} of 0.79 and of 1.07 (relatively to β-sitosterol = 1.00; β-sitosterol = 74.57 min.) have not yet been identified. From the magnitude of their retention times these components might belong to the group of triterpene alcohols.

3.3
Diethyl ether extractives

From these extractives, the amount of extractives has been determined only, the composition of fractions have not been studied. In studies on Central European hardwood extractives (*Quercus robur, Fagus sylvatica*), carried out by one of us together with other coworkers, the amounts of diethyl ether extractives were in the range of the petroleum ether extractives (Kubel et al. 1988; Weissmann et al. 1989). In the case of *A. mangium* the yields of diethyl ether extractives are three times higher than those of the petroleum ether extraction. Mainly partly oxidised fats and fat accompanying compounds have been found to be the components of these fractions in the above mentioned studies of European heartwoods. Since low molecular phenolic compounds, such as pinosylvin monomethyl ether (component of extractives of pine wood) are soluble in and extractable with diethyl ether, phenolic compounds with similar solvent parameters may also be present in diethyl ether extractives of heartwood.

3.4
Polar extractives

The acetone/water and ethanol/water extractives have been separated into two fractions, insolubles and water solubles fraction by shaking the extractives with warm water. The water soluble parts were further separated into carbohydrates and soluble phenolics, using disposal cartridges C18 modified silica gel (Bond Elut C18, Analytichem International). By this procedure three fractions each have been obtained from the acetone/water and ethanol/water extractives. Yields are given in Table 5. The composition of the hot water extractives has not been studied. These extractives may consist, at least in parts, of artifacts (hydrolized hemicelluloses).

As can be seen from the data of Table 5., a heartrot attack to the heartwood of *A. mangium* led to an increase

Table 5. Composition of polar extractives fractions from *Acacia mangium* wood samples
Tabelle 5. Die Zusammensetzung der polaren Extraktfraktionen der Kernholzproben von *Acacia mangium*

Fractions	Sample number			
	3 (%)	4 (%)	5 (%)	6 (%)
Acetone/water 9:1	9.63	12.48	7.08	10.22
Water insolubles	13	10	45	28
carbohydrates	13	9	13	11
phenolics	74	81	42	61
Ethanol/water 8:2	0.41	0.26	1.09	1.57
Water insolubles	15	9	3	2
carbohydrates	41	43	42	41
phenolics	44	48	55	57
Acetone/water + Ethanol water	10.01	12.74	8.17	11.79
Water insolubles	13	10	40	25
carbohydrates	14	10	17	15
phenolics	73	80	44	60

sample 1: unaffected sapwood
sample 2: heartrot affected sapwood
sample 3: unaffected outer heartwood
sample 4: heartrot affected outer heartwood
sample 5: unaffected inner heartwood
sample 6: heartrot affected inner heartwood

in acetone/water and ethanol/water extractives. On the other hand, there is a strong decrease in primary water insoluble and carbohydrate parts among the polar extractives and a slight decrease in primary soluble phenolics.

This becomes evident, when the three components of samples 3 and 4 as well as of samples 5 and 6 are related on equal amounts of total extractives:

Acetone/water + Ethanol water	10.01	10.01	8.17	8.17
	(3)	(4)	(5)	(6)
1. Water insolubles	13	8	40	17
2. carbohydrates	14	8	17	10
3. soluble phenolics	73	63	44	42
Sum of 1, 2, 3	100	79	100	69

The increase of polar extractives is probably due to a hydrolysis of parts of the lignocellulosics, but also the water insoluble components of the polar extractives may have been enzymatically hydrolyzed by the heartrot fungus to yield soluble phenolics. Parts of the carbohydrates, soluble phenolics and even from the water insoluble matter should have been additionally metabolized.

Only preliminary investigations have been carried out in this study concerning the composition of the three fractions 'water insolubles', 'carbohydrates' and 'soluble phenolics'. No colour reaction has been observed by reacting the water insolubles with ethanolic hydrochloric acid. Therefore, no proanthocyanidins are present in these fractions. Commonly the water insolubles from heartwoods consist of 'Brauns lignins', often misleadingly designated as 'Brauns native lignins'. Brauns (1939) extracted wood meal with ethanol and precipitated the lignin with water. Freudenberg (Freudenberg and Neish 1968) used acetone/water mixtures for the extraction. In studies of the *Quercus robur* wood extractives (Weissmann et al. 1989), the IR-spectra of the water insolubles corresponded to those of hardwood organolv lignins. In the water insolubles from *Fagus sylvatica* wood catechins (proanthocyanidins) are present, probably in addition to Brauns lignins, a deep red colour has been observed by reacting the insolubles with ethanolic hydrochloric acid (Kubel et al. 1988).

In the carbohydrate fractions of the heartwood extractives, only small amounts of glucose and of *myo*-inosite have been detected.

In the fractions of soluble phenolics no catechin and/or *epi*-catechin have been detected by DC-studies, this result corresponds to the fact that no proanthocyanidins have been found among the water insoluble matter. The presence of taxifolin (dihydroquercetin) could be established but the presence of quercetin is uncertain.

4 Conclusions

Differences between the extractives of sound and of heartrot affected heartwood of *A. mangium* mainly can be observed by an increase of the polar extractives and a decrease of ether extractives in affected heartwood. Possibly ether soluble components do participate in enzymatically induced condensation reactions which lead to higher amounts of polar extractives. But there will also be hydrolysis reactions with lignocellulosics to a certain extend; reaction products of this type will also contribute to the increase of the amount of polar extractives. On the other hand, water insoluble polar extractives in parts are obviously also hydrolyzed by the heartrot fungus to yield soluble phenolic components. The study of the extractives of sound and affected wood with respect to yield and composition cannot explain the influence of the heartrot fungi on wood components in detail.

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