

ANTI-FUNGAL PROPERTIES OF THE PYROLIGNEOUS LIQUORS FROM THE PYROLYSIS OF SOFTWOOD BARK

Daniel Mourant

Lead Investigator
Département de génie chimique
Université Laval
Ste-Foy, Québec
Canada G1K 7P4

Dian-Qing Yang

Research Scientist
Forintek Canada Corp, East Division
319 rue Franquet
Ste-Foy, Québec
Canada, G1P 4R4

Xiao Lu

Research Associate

and

Christian Roy

Professor
Département de génie chimique
Université Laval
Ste-Foy, Québec
Canada G1K 7P4

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ABSTRACT

Thermal decomposition of balsam fir and white spruce mixed bark residues at 450°C and under vacuum (<20 kPa abs.) results in high yields of pyroligneous liquors rich in phenols. The pyrolytic aqueous condensate fractionation in four distinct parts was accomplished by a liquid-liquid extraction method. Each fraction was tested for its anti-fungal properties.

Petri dish bioassays were conducted using two brown-rot fungi (*Postia placenta* and *Gloeophyllum trabeum*) and two white-rot fungi (*Irpex lacteus* and *Trametes versicolor*). The fraction obtained by ethyl ether extraction and containing organic acids, phenols, and phenol-derivatives (3.0% by weight), benzenediols, (3.9% by weight) and a variety of other products (quinones, furans, etc.), produced the largest inhibition of the decay fungi, while the neutral fraction, leftovers of the precipitation of the aforementioned fraction, showed no inhibition effects. *T. versicolor* was most sensitive to these fractions, while *I. lacteus* was the least. The addition of CuSO₄ to the water-soluble organic compounds improved anti-fungal activity.

Keywords: Pyrolysis, aqueous phase, phenols, decay, bark, bioassays, wood preservation.

INTRODUCTION

Tighter environmental laws in many countries, like the withdrawal of chromium copper arsenate (CCA) by the industry for domestic use,

have encouraged researchers to look for products that are safer for both the environment and consumers, while effective against various deteriorating agents.

One avenue that seems promising is the use of

biomass as a source of natural wood preservatives. A number of studies have been performed with tannin extractives from bark or wood, but leachability of these compounds limits their use. The fixation of tannins can be achieved with various metals (Laks et al. 1988; Couto 1996; Suzuki et al. 1997; Scalbert et al. 1998). Tannins are also used as a phenol substitute in resin formulation, but this inclusion is less for their antifungal properties than for financial reasons (Sellers 1999). Far less explored is the use of pyrolytic condensates, which can be obtained from biomass, in particular bark residues. Bark wastes are a disposal issue for mills and identifying alternative uses would reduce environmental impact associated with these residues (Suzuki et al. 1997; Pakdel et al. 1997; Boucher et al. 2000; Freel and Graham 2000; Meier et al. 2001).

Pyrolysis is a process in which biomass is thermally decomposed in the absence of oxygen. The reactions are performed either in the presence of an inert gas or under vacuum. The end products are charcoal, gas, pyrolytic oils and, in this process, an aqueous phase also called pyrolygneous liquor. The pyrolytic water and oils constitute a condensate of organic compounds derived from the thermal decomposition of the biomass.

In the particular pyrolysis system configuration used in this study, softwood bark is fed into a reactor operating under vacuum and is coupled to two condensing columns, producing a pyrolysis oil phase and an aqueous phase in amounts that are defined by the feedstock moisture, the residence time of the feed inside the reactor, the temperature, and several other operating parameters (Pakdel et al. 1997). The nature of the bark also has an impact on the composition and the quality of the products.

Wood bark itself contains a large quantity of natural anti-fungal compounds. During pyrolysis, the lighter fragments evolve and are preserved if their contact time inside the hot chamber is short. Such conditions are present when the pyrolysis is conducted under vacuum. The higher temperatures create new products by breaking the more complex molecules that are

recovered after their condensation in the condensing units. The use of the pyrolytic aqueous phase is a good base for developing a waterborne preservative as this aqueous phase must be treated before discharge.

Copper toxicity against wood-destroying organisms is well established, and the addition of copper compounds like CuSO_4 to pyrolysis condensates may increase their anti-fungal efficiency. Copper is widely used for formulation of new wood preservatives that are replacing CCA.

MATERIALS AND METHODS

Pyrolysis

A bark mixture was obtained from a large pulp and paper plant in Quebec City (Papiers Stadacona Inc.) and was composed of balsam fir (*Abies balsamea*—70%), white spruce (*Picea glauca*—28%), and larch (*Larix laricina*—2%). The fresh bark was air-dried to a moisture content of approximately 16%, and the feedstock was shredded to a mesh size less than 25 mm. The H52 pyrolysis run was performed at 450°C and at a total pressure of 20 kPa in a process development unit at a throughput capacity of 34 kg/h. A total of 206 kg of feedstock was converted. The resulting aqueous phase weighed 56 kg with a moisture content of 80.9%, and an organic content of 19.1%. The pyrolysis oil yield was 31.4% on an anhydrous feedstock basis. The highly acidic aqueous phase was adjusted to a pH of 4 by addition of 0.1M NaOH to approximate the pH of wood. The pyrolysis process has been described elsewhere (Roy et al. 1997a,b, Roy et al. 1999)

Fungal culture

The brown-rot fungi used for the petri dish test were *Postia placenta* (Fries) M. Larsen et Lombard (ATCC 11538) and *Gloeophyllum trabeum* (Pers.: Fr.) Murr. (ATCC 11539), while the white-rot fungi were *Irpex lacteus* (Fr.:Fr.) Fr. (ATCC 11245) and *Trametes versicolor* (L.:Fr.) Pilát (ATCC 12679) according to AWPA standards for testing new wood preser-

vatives (AWPA 1991). These fungi were inoculated in malt extract broth (1.5%) and were incubated for two weeks at 25°C. Mycelia were harvested by centrifugation at 7000 rpm for 15 min, and mycelial pellets were homogenized in sterile water. The mycelial suspensions were then kept at 4°C until needed for the test.

an extraction was conducted with ethyl acetate to obtain the “phenolic” fraction, as it is supposed to be a purified version of the ethyl ether fraction. The remaining aqueous phase was then extracted with ethyl acetate to obtain another more polar fraction, which was identified as the ethyl acetate fraction.

Aqueous phase fractionation

As shown in Fig. 1, liquid-liquid extraction methods were used to separate four groups of organic compounds in the aqueous phase. The first step involved an extraction with ethyl ether. The organic phase thus obtained was divided into two parts; one part was retained and identified as the ethyl ether fraction, and the other part was treated with 1M NaOH to precipitate the phenols as phenolates. The remaining organic phase was evaporated to obtain the neutral fraction. After re-acidification with HCl to pH 4,

GC/MS analysis

The GC/MS analysis of the fractions was performed with a Hewlett-Packard model 5890 gas chromatograph with split injection at 290°C. The separation was achieved using a 30-m × 0.25-mm i.d. Hewlett Packard HP5-5MS fused silica capillary column with 0.25-mm film thickness. Helium was the carrier gas with a flow of about 1 ml min⁻¹. The initial oven temperature was 50°C for 2 min, then programmed to increase to 290°C at 10°C min⁻¹. The end of the column was directly introduced in the ion source

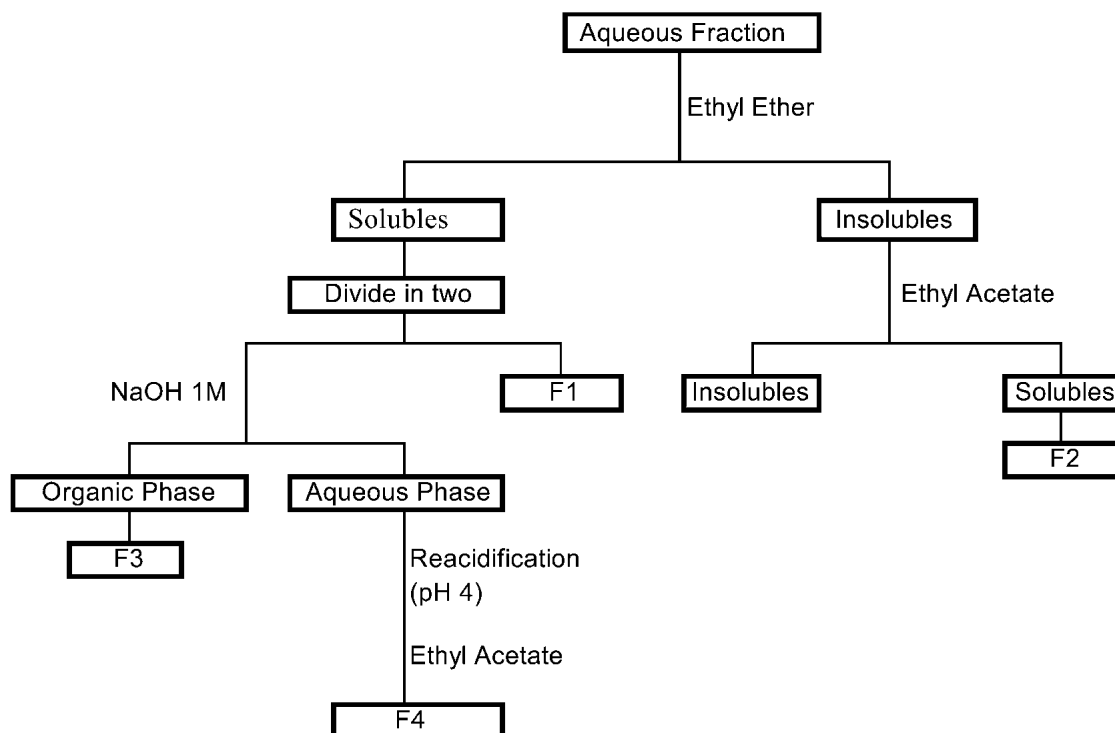


FIG. 1. Fractionation of the pyrolytic aqueous phase by liquid-liquid extraction, where F1 = ethyl ether fraction, F2 = ethyl acetate fraction, F3 = neutral compounds fraction and F4 = phenolic fraction.

of a Hewlett-Packard model 5970 series quadrupole mass selective detector. The mass spectrometer typical operating conditions were: transfer line 270°C, ion source 250°C, electron energy 70 eV. Data acquisition was carried out with a PC base G1034C Chemstation software and NBS mass spectra library database. The mass range of $m/z = 30\text{--}350$ Dalton was scanned every second.

Inhibition test

The test was conducted in petri dishes containing malt extract-agar (1.5%) in which three holes were punched in the periphery and a fourth in the middle. An aliquot of 80 μl of the tested fraction was added to each of the peripheral holes, while 0.1 ml of the ground fungus was added in the central hole. The petri dishes were then sealed with parafilm and incubated at 25°C. Distance between the peripheral holes and the edge of the growing mycelium was measured after 7 and 14 days. The inhibition was then calculated with the formula:

$$\text{Inh. \%} = (1 - [d_t - d_i]/d_t) \times 100 \quad (1)$$

where d_t is the total distance between the central hole and the peripheral hole and d_i is the distance between the mycelial rim of fungal growth and the peripheral hole. In the case where mycelial growth did not reach the peripheral holes in control plates, another formula was applied:

$$\text{Inh. \%} = (1 - [d_t - d_i - d_{\text{ref}}]/[d_t - d_{\text{ref}}]) \times 100 \quad (2)$$

where d_{ref} is the distance between the mycelial rim and the peripheral hole in the control plates. The same experiment was conducted with the fractions supplemented with CuSO_4 .

The four fractions were tested after being diluted in deionized water at 0.05, 0.10, 0.15, and 0.20 g/ml. Five petri dishes were prepared for each treatment. Deionized water was used as control when the fractions were used and a 5% CuSO_4 solution was used when the fractions were amended with the salt.

RESULTS AND DISCUSSION

Chemical analysis

The highest yield (4.49%), based on the whole aqueous phase, was obtained from the ethyl ether extraction, while the lowest yield was the neutral compounds (1.33%).

The spectra revealed several well-defined peaks in the ethyl ether, in the ethyl acetate, and in the “phenolic” fractions while the neutral fraction exhibited only a few peaks. While a good number of peaks were readily identified, the presence of background noise or small peaks with very low spectral similarity to any compounds in the database complicated the analysis of certain fractions, thus reflecting the inherent complexity of the pyroligneous liquor (Pakdel et al. 1994; Diebold 1999). Large similarities were present in the ethyl ether and phenolic fractions, which is normal as this last fraction originates from the ethyl ether fraction after removing the neutral compounds. Many compounds were found in both fractions, especially phenols and their derivatives. The difference between the quantities of compounds in those two fractions could be attributed to losses during manipulations. Fifteen of the most abundant compounds found in the fractions are presented in Table 1.

The ethyl ether and the “phenolic” fractions contained a high content of phenolic compounds present as diols (mainly benzenediol and its derivatives, which were the most important compounds found in these two fractions). The ethyl acetate fraction was rich in ketonic compounds and other polar compounds, which were not soluble in ethyl ether. The most abundant product found in the ethyl acetate fraction was 3,4-dihydro-2H-pyran, although a few phenolic compounds, mostly 2-methoxyphenol and 2-methoxy-4-propylphenol, were also present. The neutral fraction mainly contained 2-furanmethanol and 2,3-dimethylcyclopent-2-en-1-one, but almost no phenols.

Fungal inhibition

There were significant differences among these four fractions in their ability to affect fun-

TABLE 1. Major compounds found in the fractions identified by GC/MS and having a similarity of 50% or more with the database. Weight is calculated by comparison with the internal standard, anthracene.

Compounds	Weight of chemical compound (mg)			
	Ethyl ether	Ethyl acetate	Neutral	Phenolic
Butyl acetate		0.385	0.174	2.391
2-cyclopenten-1-one		0.812		
2-furancarboxaldehyde	2.009		0.03	0.047
3,4-dihydro-2H-pyran	0.539	1.727		
Phenol	1.257			1.767
3-methyl-1,2-cyclopentanedione	1.742	0.861	0.024	2.104
3-methylphenol	1.236			1.129
2-methoxyphenol	2.183	0.09		2.296
2-methoxy-4-methylphenol	1.151			1.432
1,2-benzendiol	5.917	0.139		4.935
5-[hydroxymethyl]-2-furancarboxaldehyde	0.626	1.352		
3-methyl-1,2-benzendiol	1.072			
4-ethyl-2-methoxyphenol	0.893			0.399
4-methyl-1,2-benzendiol	2.419			2.123
4-hydroxy-3-methoxybenzenacetic acid	1.09	0.118		0.939
Anthracene (internal standard)	0.258	0.263	0.263	0.258

gal growth (Table 2) when compared with student's t-test at $\alpha = 0.05$. This statistical test of comparison between means was used to verify any differences between all the results obtained. The ethyl ether fraction at 0.20 g/ml completely inhibited the growth of the two brown-rot fungi and *T. versicolor*, and produced 98.4% inhibition against *I. lacteus*. *P. placenta* and *T. versicolor* were still unable to grow in 0.15 g/ml of this fraction, but *G. trabeum* and *I. lacteus* grew to a lesser extent than the controls.

The neutral fraction produced no inhibition of the four fungi tested. The ethyl acetate fraction showed inhibition against *G. trabeum*, but not the other three fungi. The "phenolic" fraction exhibited some inhibition of *P. placenta* and *T. versicolor* at 0.20 g/ml, but was less effective against the other two fungi, especially *I. lacteus*, which appeared to be the most resistant fungus to pyrolytic oil fractions. This was expected as *I. lacteus* is known to be tolerant of organic-based wood preservatives such as creosote.

The presence of 4-ethyl-2-methoxyphenol in both ethyl ether and "phenolic" fractions and of 4-propyl-2-methoxyphenol in the ethyl ether fraction is interesting. Suzuki et al. (1997) suspected that those two compounds might have some preservation effects. Most phenolic com-

pounds have disinfectant abilities, which would explain why the ethyl ether fraction, which has a higher phenolic content, was more effective than the "phenolic" fraction. The 4-ethyl-2-methoxyphenol content in the ethyl ether fraction was more than twice that of the "phenolic" fraction. 4-propyl-2-methoxyphenol was also found in the ethyl acetate fraction, which could explain its somewhat medium performance against decay fungi. This result could also explain why certain fungi could be more affected by certain phenolic compounds than others, such as in the case of *G. trabeum*, which was affected by the ethyl acetate fraction at a concentration of 0.20 g/ml.

The addition of 0.05 g/ml CuSO_4 to the fractions increased the efficiency of the less active fractions or of the less concentrated fractions. No statistical difference was found with the already effective ethyl ether fraction at higher concentrations. Improvement was observed for all the other fractions, which was attributed to the anti-fungal properties of CuSO_4 . No significant differences were observed with brown-rot fungi due to the high standard deviations among the data of all fractions after student t-tests were performed. In the case of white-rot fungi, a statistical difference was observed between the control and all the other fractions against *T. ver-*

TABLE 2. Growth inhibition of brown-rot fungi, *G. trabeum* and *P. placenta*, and white-rot fungi, *I. lacteus* and *T. versicolor*, by the four fractions of pyrolytic aqueous phase at various concentrations, with and without copper sulphate, after 14 days of incubation at 25°C.

Fungi and fractions	Concentration of fractions in deionized water (g/ml)							
	0.20		0.15		0.10		0.05	
	No Cu	Cu	No Cu	Cu	No Cu	Cu	No Cu	Cu
	Growth inhibition %							
<i>G. trabeum</i>								
Ethyl ether	100 ^A (0)	100 ^A (0)	64 ^{B:H} (43)	100 ^A (0)	0 ^L (0)	100 ^A (0)	0 ^L (0)	100 ^A (0)
Ethyl acetate	64 ^{A:I} (47)	100 ^A (0)	0 ^L (0)	100 ^A (0)	0 ^L (0)	100 ^A (0)	0 ^L (0)	99 ^{A:B} (1)
Neutral	0 ^L (0)	100 ^A (1)	0 ^L (0)	99 ^{A:B} (2)	0 ^L (0)	99 ^{A:B} (3)	0 ^L (0)	97 ^{A:C} (4)
Phenolic	81 ^{A:G} (40)	100 ^A (0)	0 ^L (0)	100 ^A (0)	0 ^L (0)	99 ^{A:B} (3)	0 ^L (0)	97 ^{A:B} (4)
Control	0 ^L (0)	98 ^{A:B} (3)						
<i>P. placenta</i>								
Ethyl ether	100 ^A (0)	100 ^A (0)	100 ^A (0)	100 ^A (0)	37 ^{G:H} (16)	95 ^{A:D} (11)	0 ^L (0)	100 ^A (0)
Ethyl acetate	12 ^{H:K} (12)	98 ^{A:C} (5)	0 ^L (0)	97 ^{A:C} (5)	0 ^L (0)	85 ^{A:F} (22)	0 ^L (0)	89 ^{A:E} (17)
Neutral	0 ^L (0)	79 ^{A:H} (41)	0 ^L (0)	80 ^{A:H} (41)	0 ^L (0)	57 ^{A:J} (49)	0 ^L (0)	98 ^{A:B} (3)
Phenolic	100 ^A (0)	100 ^A (0)	94 ^{A:D} (11)	100 ^A (0)	37 ^{G:I} (23)	100 ^A (0)	0 ^L (0)	87 ^{A:G} (28)
Control	0 ^L (0)	79 ^{A:H} (40)						
<i>I. lacteus</i>								
Ethyl ether	98 ^{A:B} (4)	100 ^A (0)	37 ^{F:J} (31)	97 ^{A:B} (3)	0 ^L (0)	94 ^{B:C} (4)	0 ^L (0)	94 ^{B:C} (4)
Ethyl acetate	0 ^L (0)	96 ^B (2)	0 ^L (0)	98 ^{A:B} (3)	0 ^L (0)	92 ^{B:D} (3)	0 ^L (0)	90 ^{B:D} (6)
Neutral	0 ^L (0)	87 ^{C:D} (4)	0 ^L (0)	89 ^{C:D} (6)	0 ^L (0)	90 ^{C:D} (4)	0 ^L (0)	88 ^{C:D} (4)
Phenolic	73 ^{A:H} (39)	95 ^{B:C} (3)	0 ^L (0)	93 ^{B:D} (4)	0 ^L (0)	93 ^{B:D} (4)	0 ^L (0)	90 ^{B:D} (6)
Control	0 ^L (0)	87 ^{C:D} (6)						
<i>T. versicolor</i>								
Ethyl ether	100 ^A (0)	100 ^A (0)	100 ^A (0)	100 ^A (0)	63 ^{D:G} (22)	100 ^A (0)	0 ^L (0)	100 ^A (0)
Ethyl acetate	6 ^{I:L} (12)	100 ^A (0)	1 ^{K:L} (4)	100 ^A (0)	0 ^L (0)	99 ^{A:B} (2)	0 ^L (0)	89 ^{B:D} (7)
Neutral	0 ^L (0)	100 ^A (0)	0 ^L (0)	88 ^{B:D} (7)	0 ^L (0)	90 ^{B:D} (7)	0 ^L (0)	79 ^{D:E} (8)
Phenolic	100 ^A (0)	100 ^A (0)	24 ^{G:L} (29)	99 ^{A:B} (4)	4 ^{J:L} (7)	100 ^A (0)	0 ^L (0)	98 ^{A:B} (3)
Control	0 ^L (0)	71 ^{E:F} (8)						

Note: All results are means of 15 replicates. Standard deviations are in brackets. Means followed by the same letter(s) are not significantly different at $\alpha = 0.05$, according to Student's t-test. Letters separated by a colon (:) include all others in between.

sicolor. No statistical differences were observed for *I. lacteus* between the control and the neutral fraction, the ethyl acetate fraction at the concentrations of 0.10 and 0.05 g/ml, and the "phenolic" fraction at 0.05 g/ml. These data imply that copper can be the sole agent responsible for the observed inhibition. The statistical difference between the control and the observed samples, especially against *T. versicolor*, might be explained by a synergetic effect between the copper sulphate and some compounds of the pyrolytic fractions, thus enhancing the anti-fungal capacity of the mixture. The high standard deviations associated mostly with *P. placenta* when tested against fractions amended with copper may be due to the copper tolerance of this

fungus. The high standard deviations were especially apparent in the ethyl acetate and neutral fractions where most of the anti-fungal property was related to the copper.

Further research should be undertaken with lower concentrations of CuSO_4 to determine the optimal combinations of CuSO_4 and the pyrolytic aqueous fraction. Statistical analysis could also determine what percentage of the inhibition can be attributed to copper sulphate and the pyrolytic fractions. Too often, wide standard deviations made it statistically impossible to separate distinct inhibition from a concentration to another. Therefore, more samples should be tested, reducing the probabilities of such events.

CONCLUSIONS

Chemical fractions obtained from softwood bark pyrolytic liquors exhibited variable activity against selected decay fungi and this activity was enhanced by the addition of copper. The most efficient fraction was the one obtained with ethyl ether, particularly rich in phenolics, at higher concentrations. 4-ethyl-2-methoxyphenol and 4-propyl-2-methoxyphenol might have a predominant role in the fungal inhibition. Further testing in wood blocks is recommended.

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