In: Llewellyn, G.C.; Dashek, W.V.; O'Rear, C.E., eds. Biodeterioration research 4: Mycotoxins, wood decay, plant stress, biocorrosion, and general biodeterioration: Proceedings of 4th meeting of the Pan American Biodeterioration Society; 1991 August 20–25; as an electronic symposium. New York: Plenum Press: 317-332; 1994.

CHEMICAL COMPOSITION AND FUNGITOXIC ACTIVITIES OF PINE CONE EXTRACTIVES

J.A. MICALES*, J.S. HAN, USDA Forest Service, Forest Products Laboratory, Madison, WI, USA

J.L. DAVIS, and R.A. YOUNG, Department of Forestry, University of Wisconsin, Madison, WI, USA

INTRODUCTION

Pine cones, a renewable resource, are not used to their potential. Large quantities of cones are produced annually throughout the world, especially in pine plantations grown for the pulp and paper industry. They are collected, dried to facilitate seed release, and generally discarded. Some nurseries grind the cones into mulch or they may be sold for crafts, but consumer demand for cones is small compared to by-products from other industries. New uses for pine cones could provide additional income for forest landowners.

A better understanding of the chemical composition of pine cones may lead to the discovery of desirable products. Hot water extracts of Pinus parviflora Sieb et Zucc. cones are a popular folk medicine in Japan, especially for the treatment of gastric cancer (Nagata et al., 1990). Medical researchers have found tannins and other lignin-related compounds that appear to have antibacterial, antiviral, and antitumor properties in extracts from pine cones (Fukuchi et al., 1989a, Harada et al., 1991; Kurakata et al., 1989; Nagata et al., 1990; O-Hara et al., 1990; Sakagami et al., 1991). Pine cones are also known to contain monoterpenes (Mattson and Strauss, 1986; Kossuth and Biggs, 1981) and diterpenoid resin acids (Kossuth and Biggs, 1981).

Pine cones accumulating on the forest floor for many years appear to resist decay. Whether this resistance is due to fungitoxic extractives in the

cones, or some other mechanism, such as lignin content or lack of contact with soil moisture, is unknown. The possibility of unidentified fungitoxic extractives should be explored. Currently, new wood preservatives are needed to replace traditional compounds that have been banned or restricted due to their general toxicity. Compounds derived from natural products may have less impact on the environment than synthetic agents and would be more acceptable to consumers. This project was designed to study the chemical composition of pine cones and to determine whether cones from four different Pinus species contain any extractives active against the fungi that cause mold, sapstain, and wood decay.

MATERIALS AND METHODS

Extraction and Analysis

Pine cones from lodgepole pine (Pinus contorta Dougl.), Jeffrey pine (Pinus jeffreyi Grev. & Balf.), and sugar pine (Pinus lambertiana Dougl.) were collected from Toiyabe National Forest near Carson City, Nevada. They were air dried and ground to pass through a 6-mm screen and then stored at -4C until needed. Fresh cones off the tree and 1-year-old oxidized cones from the ground were collected from Scots pine (Pinus sylvestris L.) in the University of Wisconsin Arboretum, Madison, Wisconsin, and were processed immediately upon collection.

The ground samples were analyzed according to Pettersen and Schwandt's method (Petterson and Schwandt, 1991) for sugars and the TAPPI standard method (TAPPI, 1989) for lignin. Resin acids were removed by Soxhlet extraction using diethyl ether for 24 hr. The samples were then extracted with acetone and methanol.

Resin Acid Analysis

The diethyl ether extracts from each species were further separated using DEAE-Sephadex column chromatography (Zinkel, 1983; Zinkel and Rowe, 1964). The extracts were eluted sequentially to obtain a neutral fraction (by eluting with a 89% diethyl ether, 10% methanol, 1% water), a CO_2 fraction (by eluting with a 89% diethyl ether, 10% methanol and 1% water saturated with carbon dioxide), and an acetic acid fraction (by eluting with 88% diethyl ether, 10% methanol, 1% water and 1% acetic acid). The fractions were methylated with diazomethane to form methyl esters prior to gas chromatography. Fractions were analyzed by DB-1 (Han and Zinkel, 1990) and BDS (Han and Zinkel, 1991) fused

silica capillary columns. Compounds were identified by comparing the retention times of their methyl esters with those of standard samples of softwood extractives.

Culture Maintenance

The following fungal species and isolates were used in this study: Ceratocystis coerulescens (Muench) Bakshi, C256; Aureobasidium pullulans (deBary) G. Arnaud, MDX18; Trichoderma harzianum Rifai, ATCC20476; Postia placenta (Fr.) M. Lars. & Lomb., MAD698; Neolentinus lepideus (Fr.:Fr.) Redhead and Ginns, MAD534; Gloeophyllum saepiarium (Wulfen:Fr.) P. Karst., FD18; Gloeophyllum trabeum (Pers. ex Fr.) Murr., MAD617; Trametes versicolor (L.:Fr.) Pilat, MAD697; and Schizophyllum commune Fr., MAD566S. The isolates were maintained on 2% malt-extract agar (MEA) slants at 4C for the duration of the study.

Fungal Growth Inhibition

The neutral and CO_2 fractions of the diethyl ether extracts were evaluated for their abilities to inhibit mold, sapstain, and wood-decay fungi. The fractions were dissolved in absolute ethanol to final concentrations of 50 or 100 µg/ml (depending on the amount of sample available). These stock solutions were further diluted to final concentrations of 5, 10, 50, and 100 µg/ml by adding 0.5 ml of stock solution to 250 ml of autoclaved, molten 2% MEA. Control cultures contained 0.5 ml absolute ethanol in 250 ml media. The media were inoculated with mycelial plugs from the margins of 14-day-old colonies of the fungi grown on 2% MEA. Two replicate plates were made for each concentration and each fungal species. The cultures were incubated at 27C. Colony diameters were measured at 4 and 7 days, depending on the growth rate of the fungus. The percentage growth inhibition was calculated based on the diameter growth of control colonies.

The ability of nine known resin acids to inhibit fungal growth was also determined. Pimaric, isopimaric, sandaracopimaric, levopimaric, dehydroabietic, abietic, neoabietic, palustric, and lambertianic acids were obtained from the resin acid collection of the Forest Products Laboratory. (Some of the resin acids were purchased, some were donated, and others were by-products from specific experiments. All the resin acids were purified using appropriate combinations of silver nitrate-alumina column and gas liquid chromatography. All the samples were analyzed by DB–1 and BDS capillary columns prior to the actual

experiments.) Stock solutions were prepared by dissolving the resin acids in absolute ethanol. They were then incorporated into 2% MEA for a final concentration of 200 µg/ml. All the resin acids, with the exceptions of pimaric and isopimaric, were further diluted to final concentrations of 5, 10, and 50 µg/ml in the medium. Control media contained 0.5 ml absolute ethanol per milliliter. The media were inoculated with plugs from the margins of 14-day-old colonies of fungi growing on MEA. Two replicate plates were made for each concentration and each fungal species. The cultures were incubated at 27C. Colony diameters were measured after 3, 6, 8, or 10 days, depending on the growth rate of the fungus. The percentage growth inhibition was calculated by comparing these growth rates with the radial growth rate of colonies on control media.

Table 1. Chemical Analysis of Scot Pine Cone.

Chemicals	Content* (%)
Glucose	46.2
Mannose	24.6
Galactose	4.0
Xylose	3.5
Arabino	0.0
Klason lignin	23.8
Acid soluble lignin	0.7
Ash	0.4
Ethanol/toluene extractives	6.4

*Sugars and lignin given as a percentage of extractive-free wood.

RESULTS

Chemical Composition of Pine Cones

Table 1 shows the chemical composition of Scots pine cones. The cones contained large amounts of glucose (46%) and mannose (25%), and minor quantities of galactose and xylose. The cones also contained significant levels of Klason lignin (24%) but only barely detectable quantities of acid-soluble lignin (0.7%). Ethanol/toluene extractives made up 6% of the sample.

Identification of Extractves

Analysis of the neutral fractions of diethyl ether extracts of four pine cones by gas chromatography (using a DB-1 column) revealed the presence of about six primary compounds that have not been identified. The acetic acid fractions of diethyl ether extracts of the same four samples contained approximately 15 unidentified compounds. The CO_2 fractions of diethyl ether extracts contained nearly 24 resin acids. The 16 that were identified are presented in Table 2. The different species of pine contained different distributions of resin acids, but there were common patterns. All four species contained > 18% of dehydroabietic acid.

Lodgepole, Jeffrey, and sugar pine also exhibited significant quantities (> 23%) of isopimaric acid. Pimaric acid made up 15% of the extractives of Scots pine but < 2% in the other species. Lodgepole pine contained much higher levels of abietic acid than the other species (14.3% compared with 3% to 6%). Similarly, sugar pine was the only species that possessed a large quantity (25%) of lambertianic acid. Most other resin acids detected were present at very low levels in all four species.

The resin acid compositions of fresh and dried cones from Scots pine are shown in Table 3. The concentrations of pimaric, isopimaric, and sandaracopimaric acids were relatively constant. Upon drying, quantities of levopimaric, palustric, abietic, and neoabietic acids decreased, while dehydroabietic acid substantially increased.

Fungal Growth Inhibition

The neutral fraction of diethyl ether extracts from Scots pine cones exhibited little fungitoxic activity (Table 4). Growth reductions were observed for C. coerulescens, a sapstain fungus, and Trametes versicolor, a white-rot fungus, but growth of the other sapstain, mold, and wood-decay fungi was not affected. Higher levels of fungitoxic activity were present in the CO_2 fraction of diethyl ether extracts derived from Scots pine cones (Table 5). Aureobasidium pullulans, a sapstain fungus, and P. placenta, a brown-rot fungus, displayed no sensitivity to a 50- μ g/ml preparation, but all other species tested were inhibited by 14% to 53%.

The fungitoxic properties of the neutral and CO_2 fractions of diethyl ether extracts from cones of sugar, lodgepole, and Jeffrey pines are shown in Table 6. The neutral fractions from these species displayed little fungitoxic activity. At

50 μ g/ml, the growth of two white-rot fungi was slightly inhibited (2% to 17%) by neutral fractions of cones from all three species. The neutral fractions from sugar and lodgepole cones inhibited the radial growth of the brown-rot fungus G. trabeum, but only by 5% to 9% at 50 μ g/ml. The neutral fraction from Jeffrey pine cones was inactive against G. trabeum but inhibited the growth of G. saepiarium by 17%.. Similar to Scots pine, the CO_2 fractions from sugar, lodgepole, and Jeffrey pine cones exhibited greater fungitoxic activity than did the neutral fraction. The CO_2 fraction from sugar pine inhibited the growth of G. Trabeum, G. saepiarium, Trametes versicolor, and S. commune by 28% to 44% at 100 μ g/ml. The CO_2 fractions from Jeffrey and lodgepole pines displayed similar inhibitory properties against G. trabeum, Trametes versicolor, and S. commune, but were not active against G. saepiarium.

The fungitoxicity of nine known resin acids is shown in Table 7, Pimaric and isopimaric acid had little or no effect on the growth of C. coerulescens, Trichoderma harzianum, G. trabeum, or Trametes versicolor at 200 μ g/ml. Sandracopimaric, levopimaric, dehydroabietic, abietic, neoabietic, palustric, and lambertianic acids all displayed some fungitoxic activity, The growth of Trichoderma harzianum was resistant to some of these compounds. The fungitoxicity of these active compounds was determined for other mold, sapstain, and decay fungi (Table 8). Abietic, dehydroabietic, and levopimaric acids exhibited the greatest fungitoxicity; in most cases, dehydroabietic acid was the most effective compound. Dehydroabietic acid was particularly inhibitory to S. Commune, reducing growth 75% at 50 μ g/ml. This was greater than the inhibition caused by any of the CO_2 fractions. Lambertianic, palustric, and sandracopimaric acids were the least effective compounds, although all the resin acids had some activity against G. trabeum. The presence of lambertianic, levopimaric, palustric, and sandaroacopimaric acids in the media selected for a nonpigmented colony type of C. coerulescens. This nonpigmented phenotype was more resistant to these compounds than was the commonly expressed, pigmented colony type.

The fungitoxicity of the CO_2 fractions is not due solely to the presence of known resin acids. The portion of fungitoxicity of the CO_2 fractions that could be derived from abietic, dehydroabietic, and sandaracopimaric acids is calculated in Table 9. The greatest amount of fungitoxicity is probably due to dehydroabietic acid, but in most cases additional compounds, or interactions

among compounds, must also be involved. The toxicity of isocupressaic and acetylisocupressaic acids, which were also identified in the CO_2 fraction of Scots pine (Table 2), could not be determined since the purified compounds were not available for comparison. The presence of these and other unidentified extractives may account for the remaining fungitoxic effect.

Table 2. Resin Acids in Pine Cones.

Resin acid	Content (%)			
	Lodgepole pine	Jeffrey pine	Sugar pine	Scots pine
Myrcecommunic acid	0.7	0.0	0.3	0.0
Secodehydroabietic acid	0.2	1.9	0.9	0.5
Pimaric acid	1.5	1.3	0.3	14.6
Sandaracopimaric acid	2.3	2.5	3.0	7.0
Isopimaric acid	34.8	31.0	22.5	5.9
Levopimaric acid	3.6	1.0	0.4	0.9
Palustric acid	2.7	0.4	0.5	5.0
Lambertianic acid	0.4	0.3	25.3	0.0
Dehydroabietic acid	19.5	41.1	18.3	34.0
Imbricataloaic acid	0.5	2.6	0.3	0.0
Abietic acid	14.3	3.9	3.7	6.2
Neoabietic acid	3.7	0.0	0.0	3.2
Imbricatoloaic acid	0.0	2.1	0.0	0.0
Isocupressaic acid	0.9	0.7	0.6	1.4
Acetylimbricatoloaic acid	0.0	1.6	3.0	3.4
Acetoxyisocupressaic acid	1.4	2.1	0.0	0.0

Table 3. Resin Acids in Fresh and Oxidized Scots Pine Cones.

	Content (%)	
Resin acid	Fresh	Oxidized
Pimaric acid	16.7	18.3
Sandaracopimaric acid	6.5	8.7
Levopimaric Acid	3.6	0.9
Palustric acid	17.0	5.2
Isopimaric acid	7.9	7.2
Abietic Acid	13.4	8.6
Dehydroabietic acid	11.8	47.0
Neoabietic acid	23.0	4.1

Table 4. Inhibition of Colony Diameter Growth of Wood-Rotting and Sapstain/Mold Fungi by Neutral Fraction of Diethyl Ether Extracts From Scots Pine Cones.

Fungus	Decay/mold	Inhibition[a] (%) 10 μg/ml	50 μg/ml	100 μg/ml
Ceratocystis coerulescens	Sapstain	0.0	18.7	28.4
Aureobasidium pullulans	Sapstain	0.0	0.0	0.0
Trichoderma harzianum	Mold	0.0	0.0	0.0
Postia placenta	Brown rot	0.0	0.0	0.0
Neolentinus lepidius	Brown rot	0.0	0.0	0.0
Gloeophyllum trabeum	Brown rot	0.0	0.0	0.0
Trametes versicolor	White rot	27.9	13.0	16.9
Schizophyllum commune	White rot	0.0	0.0	0.0

[a]Each reading represents the average of two replicate measurements each of two replicate plates.

Table 5. Inhibition of Colony Diameter Growth of Wood-Rotting and Sapstain/Mold Fungi by CO_2 Fraction of Diethyl Ether Extracts From Scots Pine Cones.

Fungus	Decay/mold	Inhibition[a,b] (%) 5 μg/ml	10 μg/ml	50 μg/ml
Ceratocystis coerulescens	Sapstain	0.0	11.3	36.7
Aureobasidium pullulans	Sapstain	0.0	0.0	0.0
Trichoderma harzianum	Mold	0.0	0.0	38.0
Postia placenta	Brown rot	0.0	0.0	0.0
Lentinus lepidius	Brown rot	6.2	8.2	19.2
Gloeophyllum trabeum	Brown rot	0.0	0.0	16.1
Gloeophyllum saepiarium	Brown rot	0.0	0.0	14.4
Trametes versicolor	White rot	0.0	0.0	21.7
Schizophyllum commune	White rot	0.0	0.0	53.1

[a]Sufficient compound was not available to test 100 μg/ml.
[b]Each reading represents the average of two replicate measurements each of two replicate plates.

Table 6. Inhibition of Colony Diameter Growth of Wood-Rotting Fungi by Neutraland CO_2 Fractions of Diethyl Ether Extracts of Pine Cones.[a]

Fungus	Concentration µg/ml	Inhibition (%) Neutral Sugar pine	Neutral Jeffrey pine	Neutral Lodge pole pine	CO_2 Sugar pine	CO_2 Jeffrey pine	CO_2 Lodge pole pine
Brown-rotters							
Gloeophyllum trabeum	100	ND[b]	ND	ND	36.3	46.4	42.9
	50	4.8	0.0	9.0	21.9	39.3	33
	10	0.0	0.0	0.0	2.4	17.9	8.9
	5	0.0	0.0	2.3	0.0	7.1	5.4
Gloeophyllum saepiarium	100	ND	ND	ND	44.2	1.8	0.0
	50	0.0	17.4	0.0	48.8	1.8	0.0
	10	0.0	17.4	0.0	0.0	0.0	0.0
	5	0.0	10.9	0.0	ND	0.0	0.0
White-rotters							
Trametes versicolor	100	ND	ND	ND	27.1	30.5	25.9
	50	8.5	16.7	6.4	20.8	23.7	20.7
	10	0.0	0.0	0.0	10.4	15.2	10.3
	5	0.0	0.0	6.4	8.3	8.5	6.9
Schizophyllum commune	100	ND	ND	ND	27.7	40.7	25.0
	50	4.2	2.1	6.5	20.8	23.7	20.7
	10	0.0	0.0	0.0	0.0	20.4	19.2
	5	0.0	0.0	0.0	6.4	22.2	7.6

[a] Each meqasurement based on the diamater growth of two replicate colonies. Colonies incubated at 27C for 4 to 7 days, depending on the growth rate of the organism.
[b] ND, not determined. Sufficient neutral fraction was not available to test 100 µg/ml.

Table 7. Inhibition of Radial Growth of White-Rot, Brown-Rot, Mold, and Sapstain Fungi (5 Day Growth) by Resin Acids (200 µg/ml).

Resin acid	Inhibition (%) Ceratocystis coerulescens (sapstain)	Trichoderma harzianum (mold)	Gloeophyllum trabeum (brown-rot)	Trametes versicolor (white-rot)
Pimaric acid	0	0	0	0
Isopimaric acid	0	10	0	0
Sandaracopimaric acid	26	0	15	11
Levopimaric acid	25	66	64	37
Dehydroabietic acid	17	49	17	37
Abietic acid	42	15	42	26
Neoabietic acid	41	35	41	33
Palustric acid	19	4	16	19
Lambertianic acid	26	2	12	14

DISCUSSION

The chemical composition of Scots pine cones generally resembled that of solid pine wood (Pettersen, 1984). The cones contained large quantities of glucose, probably derived from cellulose, and smaller quantities of mannose, galactose, and xylose, probably derived from hemicellulose. They also contained significant amounts of lignin and ethanol/toluene extractives. The yields of extractives increased as the polarity of the solvent increased. This suggests an abundance of polyphenols and tannins in the cones and a smaller amount of terpenes, fats, and waxes. The CO_2 fractions obtained by column chromatography contained resin acids; 16 were identified by gas chromatography.

Table 8. Fungitoxic Effect of CO_2 Fraction From Scots Pine Attributable to Known Resin Acids.*

Organism	Fungitoxic effect (%)		
	Abietic acid	Dehydroabietic acid	Sandaracopimaric acid
C. coerulescens	8.4	50.9	0.0
T. harzianum	3.9	44.7	2.0
N. lepidius	4.8	33.8	1.8
G. trabeum	12.7	88.8	10.0
G. saepiarium	7.7	42.4	1.0
T. versicolor	9.1	65.9	1.6
S. commune	4.4	48.0	0.0

*Percentage of fungitoxic effect calculated by (expected inhibition) ÷ (actual inhibition (Table 5)) X 100, where *expected inhibition* = (*inhibition* by pure resin acid (Table 8)) X (concentration of resin acid in CO_2 fraction (Table 2)). Pimaric and isopimaric acids are not included since they were not fungitoxic at 50 μg/ml.

The toxicity of diterpenes, of which resin acids are one group, is well-known from water pollution studies associated with paper mills. The fungitoxicity of individual resin acids has not been widely studied. The LD_{50}s of abietic and dehydroabietic acids to mice have been reported as 2.5 g/kg and 3 to 4 g/kg, respectively. The median lethal concentrations to rainbow trout, measured after 96 hr bioassays, were as follows: dehydroabietic acid -1.1 g/kg; pimaric acid -0.8 g/kg; abietic acid - 0.7 g/kg; palustric acid -0.5 g/kg; and isopimaric acid - 0.4 g/kg (Zinkel and Russell, 1989). Apparently these compounds are relatively nontoxic to vertebrates.

Certain resin acids detected in the pine cone extracts were fungitoxic to representative mold, sapstain, and wood-decay fungi. Levopimaric, dehydroabietic, abietic, and neoabietic acids inhibited the mycelial growth of this assortment of fungi from 15% to 64% at 200 μ g/ml. Pimaric, isopimaric, and sandaracopimaric acids displayed only limited activity. This difference in toxicity can be explained by the chemistry of these compounds. Pimaric, isopimaric, and sandaracopimaric acids belong to the pimarane group of resin acids. In these compounds, C-15, C-16, and C-17 are attached to C-13 as vinyl and methyl groups (Fig. 1). The other five resin acids with the greatest fungitoxicity belong to the abietane group of compounds. These acids have isopropyl groups from C-15, C-16, and C-17 attached at C-13 (Fig. 2). Apparently this isopropyl group is required for fungitoxicity.

A previous study (Walter et al., 1989) showed that some resin acids are more stable than others. Levopimaric acid tends to be somewhat unstable, while dehydroabietic acid is usually less reactive. This reactivity can be explained by the chemical structure of the compounds. Levopimaric acid can be oxidized into dehydroabietic acid, either directly or with abietic acid as an intermediary step. Neoabietic, abietic, and palustric acids, which are isomers, can also be oxidized into dehydroabietic acid (Fig. 2). These differences in stability are reflected in theresin acid composition of the pine cones and in the changes observed in resin acid composition of Scots pine cones upon drying. All four species contained significant quantities of dehydroabietic acid and only minor quantities of levopimaric acid. The substantial increase in dehydroabietic acid and simultaneous decrease in levopimaric, palustric, abietic, and neoabietic acids upon cone drying supports the explanation based on oxidation sequences. However, dried cones from lodgepole pine contained substantial levels of abietic acid; reduced forms of resin acids can also exist in nature.

In most cases, the amount of fungitoxicity exhibited by the CO_2 fractions was higher than could be explained based on the content of the known resin acids alone. The response of the fungi to the preparations from different species of Pinus was variable; some fungi were more sensitive than others to extracts from the different pines. These two observations support the idea that other compounds besides the known resin acids may be fungitoxic. The identity of these additional fungitoxic agents may vary among pines. For example, the CO_2 fraction from sugar pine was the only preparation with significant activity against

the brown-rot fungus G. saepiarium. Sugar pine was the only species that produced measurable quantities of lambertianic acid, but this compound was not Active against G. saepiarium; some other agent must also be involved. The combination of two or more resin acids with even low levels of fungitoxicity could greatly increase the efficacy of the preparation and the range of target organisms it is active against, perhaps due to synergistic effects. None of the CO_2 fractions from different Pinus species inhibited fungal growth entirely, but it is possible that some fungitoxic agents are present in very small quantities and would be more toxic at higher concentrations. Purifying these compounds further would be necessary to produce a more effective wood preservative.

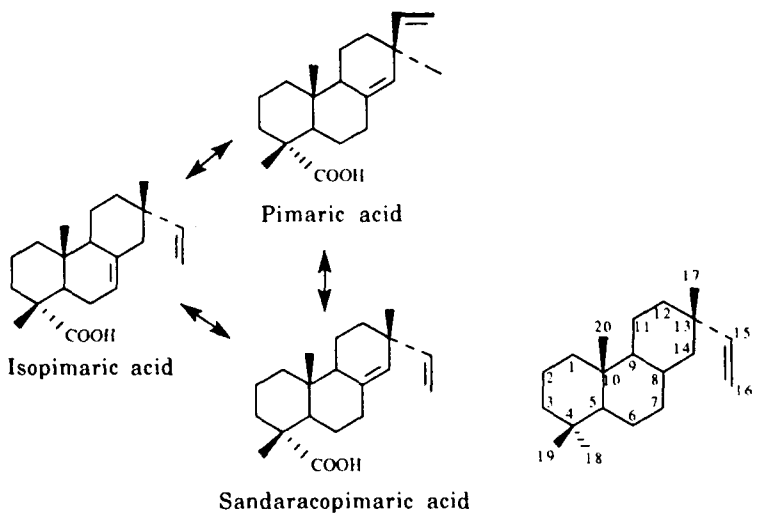

Figure 1. Isomerization of Pimarane Group Resin Acids.

Incorporating a compound into an agar medium may not be the best means of evaluating fungitoxicity (Hart and Hillis, 1974; Scheffer and Cowling, 1966), Agar growth media provide an extremely artificial testing environment. Compounds that successfully prevent the growth of fungi in agar may be ineffective in wood due to problems of penetration, inactivation, or differences in fungal physiology. More research is needed to properly evaluate the

preservative potential of these preparations. Formulation research would also be needed to maximize the fungitoxic effect of any compound. For example, resin acid soaps have been shown to have an LD_{50} of 2 mg/l to Daphnia pulex; free acid forms did not cause mortality at 6 mg/l (Zinkel and Russell, 1989). This demonstrates how different formulations of a compound or group of compounds can affect fungitoxicity.

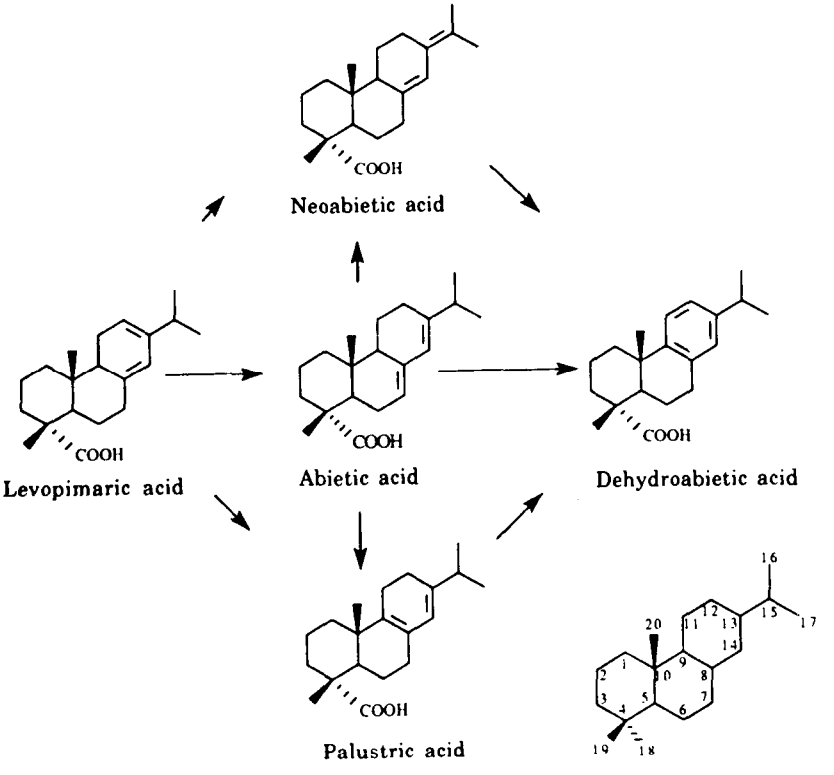

Figure 2. Isomerization and Oxidation of Abietane Group Resin Acids.

Further research is needed to identify the remaining unknown extractives present in pine cones and to evaluate the true potential of resin acids as wood preservatives. Associating the isopropyl group with fungitoxicity is an important first step towards understanding the mode of action of resin acids and for predicting the fungitoxic activity of individual compounds.

SUMMARY

The CO_2 fraction of diethyl ether extracts of four species of pine cones inhibited the growth of representative mold, sapstain, and wood-decay fungi. The fractions contained more than 24 resin acids; 16 were identified by gas chromatography as diterpenoid resin acids. A large portion of the fungitoxicity of the CO_2 fractions is probably due to the presence of abietane resin acids. The abietane resin acids have an isopropyl group attached to C-13; this moiety may be responsible for the fungitoxicity of these compounds. Pimaric, isopimaric, and sandaracopimaric acids, which have methyl and vinyl groups attached to C-13, displayed little biological activity. The fungitoxicity of the CO_2 fractions was higher than could be explained by the presence of known resin acids alone; additional resin acids and other extractives found in low concentrations may add to the fungitoxicity of the preparation.

Acknowledgments

A portion of this research was supported by the Grainger Foundation. The authors also thank Adrian Richter (Forest Products Laboratory, retired) and Barry Stern (Toiyabe National Forest) for collecting the pine cones.

Note: The use of trade or firm names in this publication is for reader information and does not imply endorsement by the U.S. Department of Agriculture of any product or service.

REFERENCES

Fukuchi, K., Sakagami, H., Ikeda, M., Kawazoe, Y., Oh-Hara, T., Konno, K., Ichikawa, S., Hata, N., Kondo, H. and Nonoymama, M. (1989a). Inhibition of Herpes simplex virus infection by pine cone antitumor substances. Anticancer Res. 9, 313-318.

Fukuchi, K., Sakagami, H., Okuda, T., Hatano, T., Tanuma, S.I., Kitajima, K., Inoue, Y., Inoue, S., Ichikawa, S (1989b). Inhibition of Herpes simplex virus infection by tannins and related compounds. Antiviral Res. 11, 285-298.

Han, J.S. and Zinkel, D.F. (1990). Gas chromatography of resin acids with a methyl silicon fused-silica capillary column. Naval Stores Rev. 100, 11-15.

Han, J.S. and Zinkel, D.F. (1991). Gas chromatography of resin acids with a BDS fused-silica capillary column. Naval Stores Rev. 101, 13-16.

Harada, H., Sakagami, H., Nagata, K., Oh-Hara, T., Ishihama, A., Hata, N.and Misawa, Y. (1991). Possible involvement of lignin structure in anti-influenza virus activity. Antiviral Res. 15, 41-50.

Hart, J.A. and Hillis, W.E. (1974). Inhibition of wood-rotting fungi by stilbenes and other polyphenols in Eucalyptus sideroxylon. Phytopathology, 64, 939-948.

Kossuth, S.V. and Biggs, R.H. (1981). Role of apophysis and outer scale tissue in pine cone opening. Forest Sci. 27, 828-836.

Kurakata, Y., Sakagami, H., Takeda, M., Konno, K., Kitajima, K., Ichikawa, S.,Hata, N., and Sato, T. (1989). Mitogenic activity of pine cone extracts against cultures splenocytes from normal and tumor-bearing animals. Anticancer Res. 9, 961-966.

Mattson, W.J. and Strauss, S.H. (1986). Are cone volatiles involved in cone finding by the red pine cone beetle, Conophthorus resinosae (Coleoptera: Scolytidae)? In: Proceedings of the 2nd Conference of the Cone and Seed Insects Working Party S2.07-01. pp. 185-204, September 3-5, 1986., Braincon, France.

Nagata, K., Sakagami, H., Harada, H., Nonoyama, M., Ishihama, A., Konno, K. (1990). Inhibition of influenza virus infection by pine cone antitumor substances. Antiviral Res. 13, 11-21.

Oh-Hara, T., Sakagami, H., Kawazoe, Y., Kaiya, T., Komatsu, N., Ohsawa, N., Fujimaki, M., Tanuma, S.I., and Konno, K. (1990) Antimicrobial spectrum of lignin-related pine cone extracts of Pinus parviflora Sieb. et Zucc. In Vivo 4, 7-12.

Petterson, R.C. (1984). The chemical composition of wood, In: The Chemistry of Solid Wood, R. Rowell (Ed.), pp. 57-126. American Chemical Society, Washington, D.C.

Petterson, R. C., and Schwandt, V. H. (1991). Wood sugar analysis by anion chromatography. J. Wood Chem. Technol. 11, 495-502.

Pullianen, E. (1972). Summer nutrition of crossbills (Loxia pytyopsittacus, L. curvirostra, and L. leucoptera) in northeastern Lapland in 197. Annales Zoologici Fennici 9, 28-31.

Sakagami, H., Kawazoe, Y., Komatsu, N., Simpson, A., Nonoyama, M., Konno,

K.,Yoshida, T., Kuroiwa, Y., and Tanuma, S.I. (1991). Antitumor, anitviral and immunopotentiating activities of pine cone extracts: potential medicinal efficacy of natural and synthetic lignin-related materials. Anticancer Res. 11, 881-888.

Sakagami., H., Oh-Hara, T., Kaiya, T., Kawazoe, Y., Nonoyama, M., Konno, K. (1989). Molecular species of the antitumor and antiviral fraction from pine cone extract. Anitcancer Res. 9, 1593-1598.

Schaffer, T.C. and Cowling, E.B. (1966). Natural resistance of wood to microbial deterioration. Ann. Rev. Phytopathol. 4, 147-170.

TAPPI (1989). Official Test Method T-222 OM-88. Acid-insoluble lignin wood and pulp. Technical Association of Pulp and Paper Industry, Atlanta, GA. TAPPI Test Methods, Vol. 1.

Walter, J., Han, J.S., and Zinkel, D.F. (1989). Fate of resin acids in Kraft pulping. Naval Stores Rev. 99, 17-19.

Young, R.D. and Davis, J.L. (1990). Utilization of pine cones. Report submitted to the Grainger Foundation. 23 p.

Zinkel, D.F. 1983. Quantitative separation of ether-soluble acidic and neutral materials. J. Wood Chem. Technol. 3, 131-143.

Zinkel, D.F. and Rowe, J.W. (1964). A rapid method for the quantitative separation without alteration of ether-soluble acidic and neutral materials. Analytical Chem. 36, 11601161.

Zinkel, D.F. and Russell, J. (eds.) (1989). Naval Stores Production, Chemistry, and Utilization. New York, Pulp Chemicals Association. 1,060 p.