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VERLAG PAUL PAREY · SPITALERSTRASSE 12 · HAMBURG 1

[1] *Faculty of Forestry University of Toronto, Toronto, Ontario, Canada*
[2] *Maritimes Forests Research Centre, Canadian Forestry Service, Fredericton, New Brunswick, Canada*

Identification of some compounds associated with resistance of Pinus densiflora to Fomes annosus

By M. T. Dumas[1,2], M. Hubbes[1], and G. M. Strunz[2]

Abstract

From the heartwood-free stemwood of *Pinus densiflora*, 7 compounds inhibitory to the linear growth of *Fomes annosus* were isolated. These were identified as pinosylvin, pinosylvin monomethyl ether, β-sitosterol, a cembrol-like diterpene alcohol, and three isomeric diterpene acids belonging to the abietane group, one of which is probably palustric acid. Other antifungal fractions contained complex mixtures and were not characterized.

1 Introduction

Fomes annosus (Fr.) Cke. [*Heterobasidion annosum* (Fr.) Bref.] is one of the most important pathogens affecting pines in the north temperate zone (Bega 1963; Gibbs 1972). The identification of species showing resistance to *F. annosus* has important implications for tree breeding programs concerned with minimizing damage to forest plantations by this pathogen.

A number of pine species and other conifers have displayed a measure of resistance to *F. annosus*, attributed, at least in part, to the presence of inhibitory compounds (see for example, references in Kuc and Shain 1977; Dumas and Hubbes 1980).

McGauley and Hubbes (1976) and Dumas and Hubbes (1979) found that the sapwood of *Pinus densiflora* (Sieb.) Zucc. was resistant to *F. annosus*. This resistance appeared to be due, at least in part, to the presence of inhibitory compounds extractable from the sapwood (Hubbes and McGauley 1976; Dumas and Hubbes 1980).

This paper reports the chemical identification of some of the fungitoxic compounds extracted from the sapwood of *P. densiflora*.

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2 Materials and methods

2.1 Collection of P. densiflora stems

The stems of two dominant *P. densiflora* trees, both 19 years old, were collected from the Ontario Forest Research Centre, Tree Breeding Research Plantation, Turkey Point, on May 10, 1978. The stems were cut into 1 metre sections and stored at −25°C until used.

2.2 Extraction and determination of extract concentration in the sapwood of P. densiflora

The frozen stem sections, which contained only sound sapwood, were debarked and reduced to shavings by means of a jointer. A sample whose fresh weight was equivalent to 225 g dry weight, was extracted three times at 4°C with 2 L portions of ethanol containing 10% acetic acid. The extracts were combined, and concentrated to 50 ml at 25°C on a Büchi Rotavapor. An equal volume of 10% aqueous acetic acid was added, and the pH of the extract was then adjusted to 8.5 by addition of sodium carbonate solution. The basic extract was extracted 3 times with an equal volume of chloroform, and the combined organic phases were concentrated in *vacuo*.[1] This extraction procedure was repeated with two additional 225 g batches of stemwood shavings. The concentration of extracted material in the sapwood was calculated as a mean of the three extractions.

2.3 Bioassay of the chloroform extract

The chloroform extract was tested for inhibition of the linear growth of *F. annosus* at the original concentration (0.993%), 0.5%, 0.1% and 0.05%. The test medium was 2% Bacto Difco agar, prepared in 15 ml portions and sterilized at 15 p.s.i. for 15 minutes. Aliquots of the extracts at their designated concentrations were taken to dryness on a Büchi Rotavapor at 25°C, and redissolved in 0.15 ml chloroform. Fifteen millilitres of the hot water agar was added to each concentration and 5 ml samples of the agar extract mixture were poured into 35 × 10 mm petri dishes. Control plates were treated with chloroform only. Each plate was inoculated with a 4 mm plug of *F. annosus*, taken from the edge of a 10 day old culture, and incubated at 22°C in the dark. The daily mycelial growth was measured. The inhibition tests were repeated 10 times.

2.4 Separation of the chloroform extract into fractions 1 to 4 by column chromatography

Eight hundred milligrams of the chloroform extract was loaded on to a Pharmacia column (2.5 × 50 cm) packed with 43 g silica gel GF 254 (Merck). Using acetone-chloroform-diethylamine (70:20:10 V/V/V) as eluent, fractions of 10.4 ml were collected. Fractions observed by thin-layer chromatography (t.l.c.) to be similar, were combined and evaporated to dryness on a Büchi Rotavapor at 25°C. The concentrations of the four resulting fractions were calculated as percentages of the sapwood dry weight.

2.5 Bioassay of the four fractions isolated by column chromatography

The 4 fractions were tested for inhibition of the linear growth of *F. annosus*. In addition to the assays at the original concentrations, fraction 1 was tested at 0.2%, fraction 2 at 0.02%,

[1] Extraction procedure adopted on the basis of earlier (misleading) evidence suggesting the presence of alkaloids.

fraction 3 at 0.005 % and fraction 4 at 0.05 %, 0.02 %, and 0.01 %. The procedure was the same as described above except that the samples were dissolved in 0.1 ml acetone, 5 ml of water agar was used as substrate, and the plates were inoculated with 3–4 mm plugs of *F. annosus*. Each inhibition test had 3 replicates, and the entire procedure, from separation to bioassay, was repeated 5 times.

2.6 Separation and bioassay of the constituents A to E of fraction 1

Fraction 1 was separated on the Pharmacia column (1.0 × 50 cm) packed to a height of 35 cm with LiChroprep Si60 (Merck) of mesh 25–40 µm. The column was eluted with acetone-hexane 10 : 90 V/V at a pressure of 1.0 kg cm^{-2}, using a Fluid Metering pump with pulse damper. The flow rate was 1.5 ml min^{-1} and 0.75 ml fractions were collected. Each fraction was spotted on 5 × 20 cm precoated t. l. c. plates (silica gel GF 254), which were developed in acetone-hexane 20 : 80 V/V to a height of 4 cm. Spots were visualized under ultraviolet light at 254 and 280 nm, then charred with sulfuric acid. Similar fractions were combined, concentrated *in vacuo*, then made up to a final volume of 5 ml in acetone. Five fractions, designated A, B, C, D, and E were separated. Their concentrations were calculated as percentages of the sapwood dry weight. Inhibition tests of A, B, C, D, and E were conducted at their original concentrations in the manner described in section 2.5.

2.7 Purification of constituents B, D, and E

Fractions A and C were found to be mixtures, while B, D, and E appeared to be relatively homogeneous. The latter components were rechromatographed separately on a column (1.1 × 24 cm) prepacked with Li Chroprep Si 60. The eluent was acetone-hexane 20 : 80 V/V; flow rate was 1.0 ml min^{-1} at 0.2 kg cm^{-2}. Fractions of 0.5 ml were collected, and each was monitored by t. l. c. using Merck 10 × 10 cm high performance t. l. c. plates, developed in acetone-hexane 20 : 80 V/V. Homogeneous identical fractions were combined and concentrated. The concentrated fraction in each case was tested for purity by two-dimensional development on a high performance t. l. c. plate (Solvent systems: acetone-hexane 20 : 80 V/V and ethyl acetate-hexane 20 : 80 V/V).

2.8 Separation, purification, and bioassay of compounds F and G isolated from fraction 2

Fraction 2 was separated into 2 components, designated F and G, in the same manner as described for B, D, and E (section 2.6, 2.7), except that both columns were eluted with acetone-hexane 20 : 80 V/V, and the second solvent for the two-dimensional t. l. c. was ethyl acetate-hexane 25 : 75 V/V. Bioassays of F and G were conducted as in Section 2.5 with the same number of replicates and repetitions. Compound F was tested at its original concentration only, while G was assayed at its original concentration, 0.025, 0.01, 0.005, and 0.001 %.

2.9 Separation, purification, and bioassay of compound H isolated from fraction 3

Fraction 3 was found to contain only one compound, designated H. The same general procedure was followed for its purification as described above for the other compounds, except that the first column was eluted with acetone-hexane 30 : 70 V/V, and the second column with the same solvents in the ratio 40 : 60 V/V. The solvent system for the t. l. c. was acetone-hexane 40 : 60 V/V, and for the two-dimensional t. l. c., the same followed by ethyl acetate-hexane 50 : 50 V/V.

The bioassay of H was conducted as above, at its original concentration, 0.005, 0.0025, and 0.001 %.

2.10 Separation, purification, and bioassays of components I, J, and K isolated from fraction 4

Fraction 4 was chromatographed on a Pharmacia column (2.5 × 50 cm) packed with 43 g silica gel GF 254. Using ethyl acetate-hexane 20:80 V/V as eluent, 5 ml fractions were collected. Pure fractions found to be identical by t.l.c. (ethyl acetate-hexane 20:80 V/V) were pooled, affording components I, J, and K.

Purification of I, J, and K was effected essentially as in Section 2.7 above, except that the column was eluted with ethyl acetate-hexane 20:80 V/V. The solvent systems for the two-dimensional t.l.c. were ethyl acetate-hexane 20:80 V/V and acetone-hexane 20:80 V/V.

Components I, J, and K were bioassayed against *F. annosus* at their original concentrations, as described in Section 2.5.

3 Results

3.1 Inhibition of F. annosus by the chloroform extract

The crude chloroform extract inhibited linear growth of *F. annosus* at all concentrations tested (Table 1).

Table 1

Inhibition of the linear growth of F. annosus by the chloroform extract from P. densiflora

Concentration of chloroform extract (% of dry wt sapwood)	% Inhibition relative to Controls
Original (0.993 ± 0.1[1])	37.5 ± 1.0[1]
0.5	28.9 ± 2.4
0.1	16.7 ± 1.1
0.05	8.8 ± 2.8

[1] Standard deviation

3.2 Bioassays of Fractions 1–4

The minor fractions, 2 and 3, were the most inhibitory to linear growth of *F. annosus* (Table 2). Inocula taken from plates showing complete inhibition were replaced on 4% malt agar and incubated at 22 °C in the dark. These inocula began to grow after they were transferred, showing that the action of fractions 2 and 3 is fungistatic.

Table 2

Percent inhibition of the linear growth of F. annosus by fractions 1–4

Fraction	Concentration (% of dry wt of sapwood)	% Inhibition relative to Controls
1	Original (0.60 ± 0.020)	85.7 ± 1.8
	0.20	41.1 ± 1.2
2	Original (0.070 ± 0.006)	100 ± 0
	0.02	100 ± 0
3	Original (0.012 ± 0.002)	100 ± 0
	0.005	100 ± 0
4	Original (0.120 ± 0.016)	84.4 ± 0.92
	0.05	28.8 ± 2.0
	0.02	20.3 ± 2.0
	0.01	15.2 ± 0.80

3.3 Identification (or characterization) and bioassay of constituents B and D–K

Since fractions A and C were complex mixtures and exhibited relatively poor inhibition of *F. annosus*, they were not investigated further.

Compound B, $C_{20}H_{34}O$, could be identified as a cembrol-type diterpene alcohol by comparison of its spectroscopic characteristics with published data (LISINA, REZVUKHIN, and PENTEGOVA 1965; KIMLAND and NORIN 1968). Although its mass spectrum showed striking similarities with that published for thunbergol, an isomer of cembrol (KIMLAND and NORIN 1968), it could be distinguished from the former by its ultraviolet absorption at 240 nm (methanol), which is indicative of a conjugated diene. Furthermore, although their nuclear magnetic resonance (n.m.r.) spectra were similar, they were not identical. In particular, the low field olefinic hydrogen doublet (J 15 Hz) of the disubstituted double bond occurs at δ 5.56 in contrast with that of thunbergol at δ 5.70. [The presence in this fraction of a small amount of an isomeric substance, possibly thunbergol, was suggested by a low intensity doublet (J 15 Hz) at δ 5.72 and a partly concealed signal (dd ?) at about δ 5.22.] The other hydrogen on the disubstituted double bond gives rise to a doublet of doublets at δ 5.28 (J 15 and 9 Hz), and the olefinic protons of the two trisubstituted double bonds appear as multiplets a δ 5.21 and 5.02. The protons of the two allylic methyl groups are manifested at δ 1.58 and δ 1.52. Of two signals at δ 1.28 and 1.26, the latter is believed to represent the methyl group attached to the carbinol carbon, while the former is attributed to a contaminant (from parafilm ?). A pair of doublets at 0.80 and 0.84 (J 7 Hz) account for the methyl hydrogens of the isopropyl group.

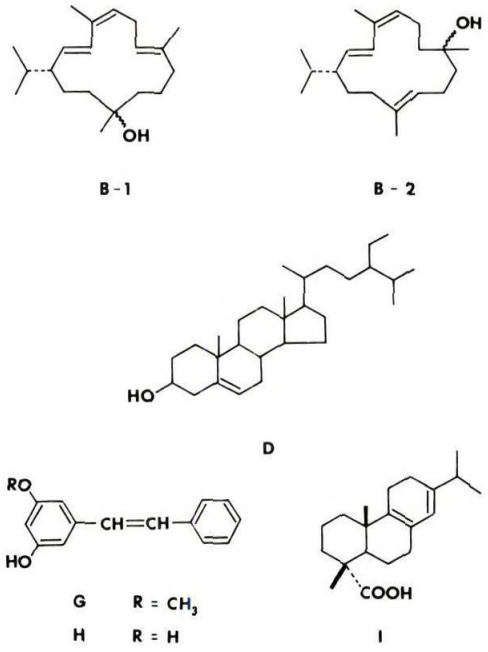

Fig. 1

While the spectral data for B are perhaps not irreconcilable with those reported for cembrol, B-1, Fig. 1 (LISINA, REZVUKHIN, and PENTEGOVA 1965), the small discrepancies observed could, on the other hand, readily be rationalized in terms of an isomeric formulation, for example, an epimeric structure, a geometrical isomer or the isomer B-2, considered by Professor PENTEGOVA's group to be a possible, though less likely, structure for cembrol. Indeed, the presence of a group of signals in the 360 MHz n.m.r. spectrum between ca. δ 1.8 and 2.3, integrating for more than 7 protons, may be cited as evidence favoring B-2, Fig. 1, (which possesses 7 allylic methylene or methine hydrogens in contrast with the 5 of structure B-1). Because of the presence of the contaminant however, the integration evidence cannot be considered conclusive, and rigorous characterization of B beyond "a cembrol-like diterpene alcohol" is not possible on the basis of data presently available.

Compound D, $C_{29}H_{50}O$, was identified as β-sitosterol on the basis of its spectra, and by comparison with an authentic sample (Fig. 1).

While t.l.c. analysis suggested that component E was homogeneous, high resolution mass spectrometry indicated that it consisted of two compounds, $C_{21}H_{30}O_2$ and $C_{21}H_{32}O_2$. These were not identified.

Fraction 2 was resolved into 2 apparently pure compounds, F and G. The former showed a substantial ion at m/e 330.2187 in the mass spectrum, corresponding to $C_{21}H_{30}O_3$, however some low intensity peaks at higher mass raise the possibility that this is not the molecular ion, and the identity of F was not pursued further.

Compound G was readily characterized as pinosylvin monomethyl ether by spectroscopic analysis, and by direct comparison with an authentic sample (Fig. 1).

Fraction 3 contained only one major compound, designated H, which was identified as pinosylvin (Fig. 1) by its spectra and by comparison with an authentic sample.

Fraction 4 was resolved into 3 isomeric diterpene acids, I, J, and K, $C_{20}H_{30}O_2$, whose spectral characteristics are indicative of the abietane group (ZINKEL, ZANK, and WESOLOWSKI 1971). Although two-dimensional t.l.c. suggested that these compounds were pure, mass and n.m.r. spectrometry showed that all, especially K, were contaminated to varying degrees by a dehydroproduct, $C_{20}H_{28}O_2$, possibly dehydroabietic acid. While J and K could not be unambiguously identified, spectroscopic analysis of I allowed it to be tentatively identified as palustric acid (Fig. 1). In particular, ultraviolet absorption at 265 nm and a signal at δ 5.40 in the n.m.r. spectrum (olefinic hydrogen), appear to distinguish it from related diterpene acids (ZINKEL, ZANK, and WESOLOWSKI 1971).

Each compound was assayed for its inhibition of the linear growth of *F. annosus*. As might be expected, the 2 stilbenes exhibited the strongest inhibition (Table 3). A fungistatic response occurred for pinosylvin monomethyl ether and pinosylvin at concentrations of 0.005 and 0.0025%, respectively.

Table 3

Percent inhibition of the linear growth of F. annosus by compounds B and D–K

Compound	Concentration (% of dry wt of sapwood)	% Inhibition relative to Controls
B (Cembrol-like)	Original (0.017 ± 0.001)	34.5 ± 2.94
D (β-Sitosterol)	Original (0.17 ± 0.007)	40.2 ± 0.460
E	Original (0.040 ± 0.006)	40.4 ± 2.38
F	Original (0.025 ± 0.006)	62.0 ± 1.4
G (Pinosylvin monomethyl ether)	Original (0.04 ± 0.008)	100 ± 0
	0.025	100 ± 0
	0.01	100 ± 0
	0.005	100 ± 0
	0.001	66.2 ± 2.1
H (Pinosylvin)	Original (0.012 ± 0.002)	100.0 ± 0
	0.005	100.0 ± 0
	0.0025	100.0 ± 0
	0.001	87.3 ± 1.45
I (Palustric acid, tentatively)	Original (0.022 ± 0.001)	24.15 ± 3.14
J (Diterpene acid)	Original (0.043 ± 0.003)	26.95 ± 1.43
K (Diterpene acid)	Original (0.032 ± 0.001)	61.40 ± 1.54

4 Discussion

The sapwood of *P. densiflora* contains compounds which inhibit the linear growth of *F. annosus*. Inhibitory material could be extracted with ethanol containing 10% acetic acid, and reextracted with chloroform after the initial extract was adjusted to pH 8.5. From the chloroform extract, 4 fractions inhibitory to *F. annosus* were obtained by a preliminary chromatographic separation. Fractions 2 and 3 exhibited the strongest inhibition (Table 2). Fraction 2 was composed of pinosylvin monomethyl ether and an unidentified compound designated F. Fraction 3 contained only one major compound identified as pinosylvin. This is the first report of the occurrence of these stilbenes in the sapwood of *P. densiflora*. They

have been isolated previously from the heartwood of this pine species by HATA and SOGO (1954) and ERDTMAN (1955).

The literature contains conflicting reports concerning the presence or absence of these stilbenes in normal sapwood. SHAIN (1967) could detect pinosylvin and its monomethyl ether in the reaction zone of *Pinus taeda* L. but not in the sapwood. JORGENSEN (1961) could not detect pinosylvin or pinosylvin monomethyl ether in normal sapwood from stem, branches, or roots of *P. resinosa* Ait. FALUSI (1980), on the other hand, did observe the presence of these stilbenes in the sapwood of some red pine trees. LINDSTEDT and MISIORNY (1951) found pinosylvin in the sapwood of *P. sylvestris* L., *P. mugo*, and *P. banksiana* Lamb. HART (1981) states that stilbenes occur in very small amounts as glycosides or methyl esters in the healthy tissues of leaves and sapwood. He does not however identify the stilbenes referred to, nor the tree species in which they were found.

The production of pinosylvin and its monomethyl ether as a response to injury or fungal invasion has been demonstrated. JORGENSEN (1961) and AYYAMPERUMAL (1971) found that these compounds accumulated in the sapwood of *P. resinosa* following mechanical or fungal injury, and SHAIN (1967) and PRIOR (1976) observed an increase in their concentration in *P. taeda* and *P. nigra* var. *maritima* (Ait.) Melville, respectively, following fungal invasion. For these reasons scrupulous care was exercised in collection and extraction of *P. densiflora* to ensure that no defects were present in the sapwood. Thus it can be concluded from the present study that pinosylvin and pinosylvin monomethyl ether are products of normal metabolic processes in the sapwood of *P. densiflora*.

The concentrations of these stilbenes found in the sapwood of *P. densiflora* were high enough to completely inhibit the linear growth of *F. annosus*. At their original concentrations, 0.012% pinosylvin and 0.04% pinosylvin monomethyl ether, both stilbenes were fungistatistic to *F. annosus* (Table 3). Pinosylvin was more inhibitory than its monomethyl ether: at 0.001%, the former inhibited *F. annosus* by 87.3%, whereas the same concentration of the monomethyl ether gave rise to 66.2% inhibition. RENNERFELT (1945) reported that the concentration of these stilbenes required for fungicidal activity against *F. annosus* was 0.02%, when tested in liquid media. RENNERFELT and NACHT (1955) found that these compounds were fungistatic at 0.005%. AYYAMPERUMAL (1971) concluded that the spores of *F. annosus* could not germinate in 50 ppm pinosylvin and 500 ppm pinosylvin monomethyl ether. GIBBS (1972) observed that pinosylvin monomethyl ether was more toxic to *F. annosus* than pinosylvin, when tested in malt extract agar.

It is apparent that the discrepancies reported in the amounts of these stilbenes required to inhibit *F. annosus* may be due to the type of medium used for the bioassays. This aspect of the interaction was reviewed thoroughly by HART and SHRIMPTON (1979). Differences may also be attributed to the strain of *F. annosus* used in the assays. SHAIN (1967) observed that when pinosylvins at 25 ppm was tested against 2 isolates of *F. annosus*, one was inhibited more than the other. In the present study, water agar was employed as the test substrate and the fast-growing strain 3201 of *F. annosus* [MCGAULEY and HUBBES (1976)] was used as the test organism. The response to pinosylvin and its monomethyl ether on water agar was found to be fungistatic. The same response was observed by DUMAS and HUBBES (1979) on fresh stem discs.

In addition to the stilbenes, other compounds inhibitory to the linear growth of *F. annosus*, including β-sitosterol and a cembrol-like diterpene alcohol were isolated from the stemwood of *P. densiflora* (Table 3). Palustric acid also was tentatively identified, and two isomeric diterpene acids, believed to belong to the same abietane group were partially characterized. (Control experiments showed that the resin acids were totally extracted into chloroform at pH 8.5 so that the amounts isolated reflect the actual concentrations present in the sapwood of *P. densiflora*).

β-sitosterol inhibited the growth of *F. annosus* by 40.2% at a concentration of 0.17%. The antifungal properties of this sterol against *Candida lipolytica* have been demonstrated by ARDITTI, FLICK, EHMANN, and FISCH (1975). Some other examples of plant sterols

possessing antifungal activity are found in papers by STOESSL, FISCH, and ARDITTI (1980) and STERNER (1974). The former authors observed that the sterol fraction from the roots of *Cymbidium* hybrids showed antifungal activity. STERNER (1974) indicated that one of the fractions involved in the resistance of balsam fir root centerwood to *Coniophora puteana* (Schum ex Fr.) Karst. was a sterol.

The cembrol-like diterpene alcohol at 0.017% concentration gave rise to 34.5% inhibition of *F. annosus*. Cembrol itself was first isolated from *P. sibirica* R. Mayr by LISINA, REZVUKHIN, and PENTAGOVA (1965), and a related isomeric substance, thunbergol, was discovered among the constituents of "pocket resin" from *Pseudotsuga menziesii* (Mirb.) Franco, by ERDTMAN, KIMLAND, NORIN, and DANIELS (1968).

The (presumed) palustric acid showed 24.2% inhibition at 0.022% concentration, and resin acids J and K at 0.043 and 0.032% gave rise to inhibition of 27.0 and 61.4%, respectively. This behavior is consistent with results on inhibition of *F. annosus* by resin acids reported by other researchers. Thus, HENDRICKS, EKMAN, and VON WEISSENBERG (1979) observed an inhibition of approximately 20% in the growth of *F. annosus* by palustric acid when tested at a concentration of 0.02%. Dehydroabietic acid and isopimaric acid were also reported to inhibit the growth of *F. annosus* (HARTMANN, RENZ, and JUNG 1981). SHAIN (1971) observed that abietic acid inhibited the linear growth of *F. annosus* by 60% when tested in malt agar at a concentration of 0.25%.

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Summary

The uninjured stemwood of *P. densiflora*, devoid of heartwood, was extracted with ethanol containing 10% acetic acid. The extract was concentrated, and an equal volume of 10% aqueous acetic acid was added. The pH was adjusted to 8.5 with sodium carbonate solution, and the mixture was extracted with chloroform.

The chloroform extract was separated into 4 fractions by chromatography. Fractions 1 and 4 at their original concentrations inhibited the linear growth of *F. annosus* by 85.7 and 84.4%, respectively. Fractions 2 and 3 were fungistatic to *F. annosus*.

Further chromatography separated some of the components of the fractions. From fraction 1, β-sitosterol and a cembrol-like diterpene alcohol were identified. Fraction 2 contained pinosylvin monomethyl ether and unidentified material. Fraction 3 was composed of a single compound, identified as pinosylvin. Fraction 4 contained three isomeric resin acids believed to belong to the abietane group, one of which was tentatively identified as palustric acid.

This is the first report of the occurrence of the stilbenes pinosylvin and pinosylvin monomethyl ether in the sound sapwood of *P. densiflora*.

Résumé

Identification de certains composés associés à la résistance du Pinus densiflora au Fomes annosus

On a extrait à l'éthanol contenant 10% d'acide acétique, du bois non-endommagé de troncs de *P. densiflora*. Cet extrait a été concentré et mélangé à un volume égal d'un soluté d'acide acétique à 10% puis, on a ajouté une solution de carbonate de sodium pour obtenir un pH de 8.5. On a ensuite extrait cette mixture au chloroforme et l'extrait e été séparé en 4 fractions par chromatographie. Les fractions 1 et 4, lorsqu'appliquées à leur concentration initiale, ont inhibé la croissance linéaire du *F. annosus* à 85.7 et 84.4% respectivement. Les fractions 2 et 3 se sont rélévées fongistatiques envers le *F. annosus*. On a aussi pu séparer, par chromatographie, certains des composants de ces fractions. On a identifié, dans la fraction 1, le β-sitostérol et un alcool diterpénique ressemblant à un cembrol. Dans la fraction 2, on a trouvé l'éther monométhylique de la pinosylvine et du matériel non-identifié. La pinosylvine est le seul composé présent dans la fraction 3, alors que la fraction 4 contient 3 isomères d'un acide résinique appartenant probablement au groupe des abiétanes. L'un de ces acides semble être l'acide palustrique. Cet article est le premier à rapporter la présence de stilbènes, la pinosylvine et son éther monométhylique, dans le bois d'aubier sain du *P. densiflora*.

Zusammenfassung

Identifizierung einiger Komponenten, die mit der Resistenz von Pinus densiflora gegenüber Fomes annosus in Verbindung stehen

Unverletztes Stammholz von *Pinus densiflora*, frei von Kernholz, wurde mit Äthanol, das 10% Essigsäure enthielt, extrahiert. Danach wurde der Extrakt konzentriert und ein gleiches Volumen von 10%-iger wässeriger Essigsäure hinzugefügt. Der pH-Wert wurde anschließend mit Natriumcarbonatlösung auf 8.5 gebracht und die Mischung mit Chloroform extrahiert.

Der Chloroformextrakt wurde chromatographisch in 4 Fraktionen zerlegt. In ihrer ursprünglichen Konzentration hemmten die Fraktionen 1 und 4 das lineare Wachstum von *Fomes annosus* um 85.7 bzw. 84.4%. Die Fraktionen 2 und 3 wirkten fungistatisch auf *F. annosus*.

Weitere Komponenten der Fraktionen wurden ebenfalls chromatographisch erhalten. Von Fraktion 1 wurde β-Sitosterol und ein Cembrol ähnlicher diterpenoider Alkohol erhalten. Fraktion 2 enthielt Pinosylvinmonomethyläther und daneben noch nicht identifiziertes Material. Fraktion 3 bestand aus einer einzigen Komponente: Pinosylvin. Fraktion 4 enthielt drei isomerische Harzsäuren, von denen angenommen wird, daß sie zur Gruppe der Abietane gehören. Eine der Säuren wurde vorläufig als Palustrinsäure identifiziert.

In der vorliegenden Arbeit wird erstmals über das Vorkommen der Stilbene Pinosylvin und Pinosylvinmonomethyläther im gesunden Splintholz von *Pinus densiflora* berichtet.

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Author's addresses: Dr. M. T. Dumas, Prof. Dr. M. Hubbes, Faculty of Forestry, University of Toronto, Toronto, Ontario. Dr. G. M. Strunz, Maritimes Forest Research Centre, Canadian Forestry Service, Fredericton, New Brunswick

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