IRG/WP 96-10l60

THE INTERNATIONAL RESEARCH GROUP ON WOOD PRESERVATION

Section 1 Biology

Ibuprofen Inhibits in vitro Growth of Brown-rot Fungi

By

Carol A. Clausen

USDA, Forest Products Laboratory, Madison, WI, U.S.A.

Prepared for the 27th Annual Meeting

Guadaloupe, French West Indies

19-24 May 1996

IRG Secretariat
BOX 5607
S-114 86 Stockholm
Sweden

Ibuprofen inhibits in vitro growth of brown-rot fungi

by
Carol A. Clausen

USDA Forest Service, Forest Products Laboratory
One Gifford Pinchot Drive,
Madison, Wisconsin, USA 53705

SUMMARY

Ibuprofen, a phenyl propionic acid derivative (2-(4-isobutylphenyl)propionic acid) inhibited growth & in vitro of 17 brown-rot fungi at a concentration of 100 ug/mL, while exhibiting no growth inhibition for 8 white-rot fungi at the same concentration. Propionic acid did not inhibit fungal growth in vitro. Morphological changes were noted in fungi that were able to grow in the presence of ibuprofen.

Key words: brown-rot fungi, white-rot fungi, ibuprofen

INTRODUCTION

Brown-rot fungi make up only 7% of all wood rotting basidiomycetes, but they are the most destructive wood rotting organisms (Gilbertson and Ryvarden, 1986). Researchers continue to look for new methods to inihibit biodeterioration of wood by these organisms. Chemicals with antifungal properties are typically screened against representatives of both brown-rot and white-rot fungi. When screening compounds for antimycotic activity, differences in the mechanism of decay by these two groups of decay fungi are often revealed.

Ibuprofen, an anti-inflammatory inhibitor of prostaglandin biosynthesis, has been widely accepted as an oral pain reliever (Reynolds, 1989). Sanyal et al., 1993, recently found that ibuprofen displayed antimycotic activity against pathogenic fungi of medical importance. Various dermatophytes were remarkably susceptible to ibuprofen in vitro. However, the mode of antimycotic action for dermatophytes remains unknown.

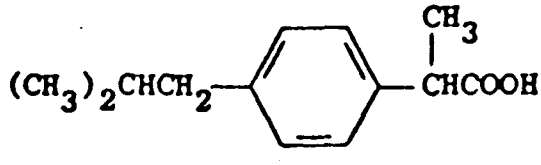

Figure 1. 2-(4-isobutylphenyl)-propionic acid

Ibuprofen is a phenyl propionic acid derivative (Figure 1). Propionic acid, an effective biocide used to protect stored grains from microbiological attack, is also a known inhibitor of fungal growth when sprayed on chip piles. at a concentration of 2% (Eslyn 1973, Springer et al. , 1977). Propionic acid was tested at the same concentrations as ibuprofen for antimycotic effects.

The objective of this study was to examine the ability of brown- and white-rot fungi to grow at various concentrations of ibuprofen. It was shown that ibuprofen exhibits antimycotic activity against a group of basidiomycetous fungi responsible for brown-rot decay of wood, while having no affect on white-rotters at the same concentrations. Propionic acid was not inhibitory for either group of decay organisms at the same concentration as ibuprofen.

MATERIALS AND METHODS

Culture conditions The seventeen brown-rot and eight white-rot fungi used in this study are listed and identified in Table 1. Isolates were maintained on 2% malt agar.

Minimum inhibitory concentration (MIC) The MIC was defined as the minimum inhibitory concentration which inhibited fungal growth on agar plates after 3 wk incubation.

Plate cultures A stock solution of ibuprofen (Sigma) prepared in methanol was added at 100 ug/ml or 200 ug/ml to 2% malt agar which had been cooled to 45°c. Propionic acid (Sigma) was added to pre-cooled malt agar at the same concentrations. Petri plates were inoculated with a 0.4 cm disk of test fungus cut from the advancing zone of an agar plate. The agar disk was trimmed with a sterile scalpel to remove excess agar. Plates were incubated at 27°C, 70% RH, and diameters of colonies were measured at 4 d intervals for 3 wk. Control plates were untreated.

Enzymatic inactivation Four, 125ml Erlenmeyer flasks containing 25 ml Bailey's Minimal Medium (Highley, 1973) with 0.5% cellobiose were inoculated with agar plugs of *Phanerochaete chrysosporium* ME-461 and incubated for 3 wk at 27°C, 70% RH. Culture filtrates were pooled, and filtered through Whatman No. 1 filter paper and a 0.2 um Millipore filter. Dilutions of the filter sterilized culture filtrate (1:1, 1:2, 1:5, and 1:10) were mixed (1:1) with inhibitory concentrations of ibuprofen in 2% malt agar precooled to 45°C. Plates were incubated 24 h at 25°C before inoculation with *Postia placenta* Mad-698. Controls included culture filtrate without ibuprofen and ibuprofen treatment without culture filtrate.

Mode of action After 3 wk incubation, agar plugs from the brown-rot isolates showing no growth were transferred to fresh malt agar to see if ibuprofen was fungicidal or fungistatic at concentrations which inhibited growth. Transferred isolates were incubated at conditions previously described.

RESULTS

The MIC of ibuprofen (100 ug/ml) totally inhibited growth of 15/17 brown-rot isolates (Table 1). Only N. lepideus and A. serialis showed slight growth after 15 d, but they also showed morphological changes (Figure 2). White-rot isolates showed a slight delay in growth (7 d) at 100 ug/ml ibuprofen, but 8/8 isolates tolerated this concentration of treatment (Table 1). Three of the eight white-rot isolates, P. brevispora, P. chrysosporium ME-461, and X. frustulatus were tolerant of 200 ug/ml ibuprofen. White-rot fungi also demonstrated morphological changes in gross cultural characteristics (Figure 2). Agar plugs from test plates showing no growth after 3 wk were transferred to fresh malt agar and incubated for an additional 3 wk. The minimum inhibitory concentration of ibuprofen was fungicidal to the brown-rot isolates in this study.

Propionic acid slightly slowed the mycelial growth of all organisms at 100 and 200 ug/ml compared to the controls. All organisms tested were able to grow on agar and were morphologically similar to control plates except for S. incrassata Mad-563. Propionic acid was fungicidal to S. incrassata at 100 ug/ml.

Culture filtrate from P. chrysosporium ME-461 did not inactivate the inhibitory effects of ibuprofen on growth of the brown-rot isolate P. placenta Mad-698.

DISCUSSION

Ibuprofen was fungicidal to 15/17 brown-rot isolates at a minimum inhibitory concentration of 100 ug/ml, but did not inhibit white-rot fungal growth. Ibuprofen's antifungal mode of action on brown-rot fungi is unknown, as it is for dermatophytes. White-rot organisms have a mechanism for overcoming the inhibitory effects of ibuprofen, though some morphological changes were noted. The two brown-rot isolates that were able to grow in the presence of ibuprofen also showed an unusual cultural morphology. Preliminary results in a soil block test (ASTM, 1991) did not show a clear inhibition of brown-rot fungi in wood at the same concentrations that were inhibitory in agar (results not shown). Further testing is necessary to confirm this result.

Propionic acid is known to be an effective fungal inhibitor on chip piles at 2% (Eslyn 1973, Springer et al. 1977). It was compared to ibuprofen at the inhibitory concentration because of it's reported antifungal properties, and because ibuprofen is a propionic acid derivative. However, the MIC of ibuprofen was 198-fold less than the effective concentration of propionic acid reported by Eslyn and Springer et al. At 100 ug/ml, propionic acid did not affect growth or morphology of any organism tested with the exception of S. incrassata Mad-563. Propionic acid was fungicidal to S. incrassata at 100 ug/ml.

It is well documented that white rot organisms possess the ability to degrade a wide range of compounds with their lignolytic system (Field et al. , 1993, Paszczynski et al. , 1992). The culture filtrate of P. chrysosporium ME-461 was tested for enzymatic inactivation of ibuprofen. Various amounts of filter-sterilized culture filtrate were mixed with 100 ug/ml ibuprofen in precooled agar and incubated 24 h at 25°C. Plates, which were then inoculated with P. placenta Mad-698, exhibited inhibition of the brown-rot organism suggesting that ibuprofen's Inhibitory effects on P. placenta were not enzymatically inactivated by P. chrysosporium culture filtrate. More parameters need to be examined before it can be stated with certainty that enzymatic activity is not responsible for the ability of white-rotters to grow unhindered at concentrations of ibuprofen that are inhibitory to brown-rotters.

In conclusion, ibuprofen was fungicidal to 15 isolates of brown-rot fungi at 100 ug/ml. Growth of 8 isolates of white-rot fungi was not affected. Two brown-rot fungi grew slowly at this concentration with notable morphological changes. White-rot fungi also exhibited morphological changes.

Propionic acid was fungicidal to S. incrassata at 100 ug/ml, but all other brown-rot and white-rot isolates grew normally in the presence of propionic acid. Further studies are needed to confirm the mechanism of action for brown-rot inhibition by ibuprofen that is either lacking or inactivated by white-rot fungi.

REFERENCES

ASTM (1991) Standard test method for assessing natural decay resitance. ASTM Desig. D 2017-81. Annual Book of Standards. American Society for Testing and Materials, Philadelphia, PA.

Eslyn, W.E. (1973) Propionic acid-A potential control for deterioration of wood chips stored in outside piles. TAPPI **56**, 152-153.

Field, J.A., de Jong, E., Feijoo-Costa, G., & de Bont, J.A.M. (1993) Screening for lignolytic fungi applicable to the biodegradation of xenobiotics. TIBTECH **11**, 44-49.

Gilbertson, R.L. and Ryvarden, L. (1986) North American Polypores, Abortiporus-Lindtneria, Vol. 1. Gronlands Grafiske A/S, Oslo, Norway.

Highley, T.L. (1973) Influence of carbon source on cellulase activity of white-rot and brown-rot fungi. Wood and Fiber Sci. **5**, 50-58.

Paszczynski, A., Pasti-Grigsby, M.B., Goszczynski, S., Crawford, R.L., & Crawford, D.L. (1992) Mineralization of sulfonated azo dyes and sulfanilic acid by Phanerochaete chrysosporium and Streptomyces chromofuscus. Appl. Environ. Microbiol. **58**(11), 3598-3604.

Reynolds, J.E.F. (1989) Analgesic and anti-inflammatory agents. In: Martindale: The Extra Pharmacopoeia. 29th ed., pp. 1-46. The Pharmaceutical Press: London.

Sanyal, A.K., Roy, D., Chowdhury, B., & Banerjee, A.B. (1993) Ibuprofen, a unique anti-inflammatory compound with antifungal activity against dermatophytes. Lett. Appl. Micro. **17**, 109-111.

Springer, E.L., Feist, W.C., Zoch, L.L., & Hajney, G.J. (1977) Evaluation of chemical treatments to prevent deterioration of wood chips during storage. TAPPI **60**, 93-97.

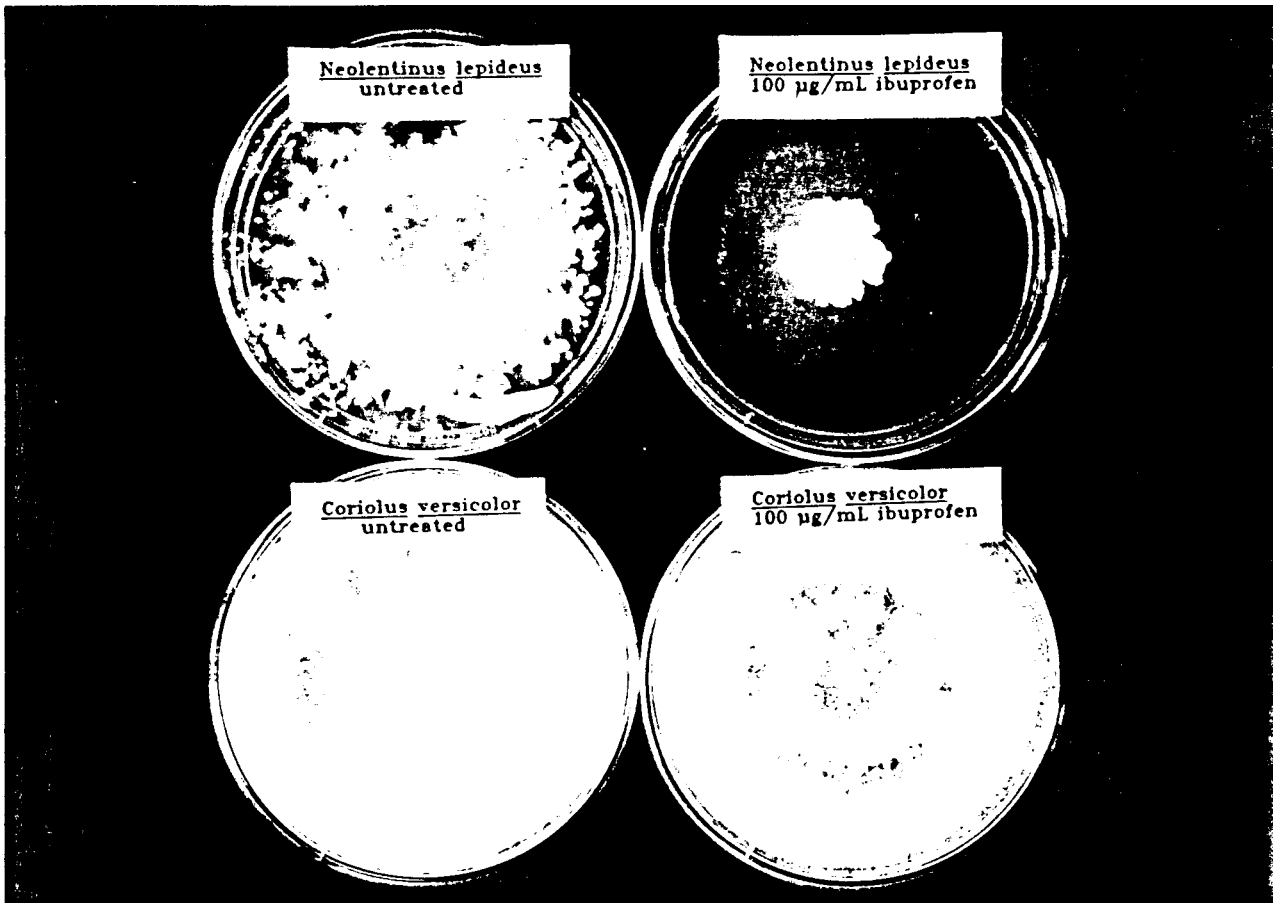

Figure 2. The brown-rot fungus, Neolentinus lepideus Mad-534 (top) and white-rot fungus, Trametes versicolor Mad-697 (bottom) show morphological changes in the presence of 100 ug/ml ibuprofen (right) compared to untreated controls (left).

Table 1. Growth inhibition of brown-rot and white-rot fungi on agar supplemented with ibuprofen after 21 days incubation.

	Control Growth[a] (cm)	Ibuprofen[b]
Brown-rot Isolates:		
Coniophora puteana (Schum.: Fr.) Karst. (Mad-515)	8.0	0
Postia placenta (Fr.) Lars. & Lomb. (Mad-698)	8.0	0
Neolentinus lepideus (Fr.: Fr.) Redhead et Ginns (Mad-534)	8.0	3.8
Gloeophyllum trabeum (Pers.: Fr.) Murr. (Mad-617)	8.0	0
Serpula incrassata (Berk. & Curt.) Murr. (Mad-563)	8.0	0
Anrodia xantha (Fr.: Fr.) Ryv. (Mad 5096-35)	8.0	0
Laetiporus sulphureus (Fr.) Murr. (W) Sh-27-R	8.0	0
Laetiporus sulphureus (Fr.) Murr. (E) Boat-206	8.0	0
Postia placenta (Fr.) Lars. & Lomb. (ME-20)	8.0	0
Antrodia carbonica (Overh.) Gilbn. & Ryv. (HHB-5104)	8.0	0
Antrodia serialis (Fr.: Fr.) Donk (FP-104443)	8.0	2.7
Antrodia vaillantii (DC:Fr.) Ryv. (FP-90877)	8.0	0
Leucogyrophana arizonica J. Ginns (RLG-9902)	5.0	0
Sparassis crispa Wulf.: Fr. (KJM-279)	3.4	0
Phaeolus schweinitzii (Fr.) Pat. (105389)	8.0	0
Fomitopsis pinicola (Fr.) Karst. (105877)	8.0	0
Fomitopsis meliae (Underw.) Gilbn. (FP-10002)	8.0	0
White-rot Isolates:		
Trametes versicolor (L.: Fr.) Pil. (Mad-697)	8.0	2.8
Phlebia brevispora Nakas. (HHB-7030)	8.0	8.0
Irpex lacteus (Fr.: Fr.) Fr. (HHB-7328)	8.0	8.0
Bjerkandera adusta (Fr.) Karst. (L-15359)	8.0	8.0
Phanerochaete chrysosporium Burds. (ME-461)	8.0	8.0
Phanerochaete chrysosporium Burds. (ME-446)	8.0	8.0
Ganoderma applanatum (Pers.) Pat. (HHB-7823-S)	8.0	8.0
Xylobolus frustulatus (Pers.: Fr.) Boid. (FP-24059)	8.0	6.8

[a] Maximum growth (8.0 cm) = Petri plate diameter

[b] 100 ug/ml