Influence of Alkylresorcinols from Rye and Related Compounds on the Growth of Food-borne Molds

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ABSTRACT

The influence of 5-*n*-alkylresorcinols (AR) from rye cultivar Halo (15–23 C atoms in the side chain), 5-pentadecylresorcinol, and 4-*n*-hexylresorcinol on the growth of the bread molds *Aspergillus parasiticus, A. versicolor, Penicillium chrysogenum,* and *P. roqueforti* was studied. After five days of growth, the spreading of *A. parasiticus* and of *P. roqueforti* was reduced by 10 mg of AR per milliliter of agar medium, whereas the other molds were not influenced by this concentration. 5-Pentadecylresorcinol was totally inhibitory at 100 mg/ml and was nearly as active at 10 and 1 mg/ml as the corresponding levels of AR. The reduction in bread mold growth on rye bread as compared to wheat bread does not appear to result from the relative high levels of AR in rye. 4-*n*-Hexylresorcinol acted as a strong inhibitor at 1 mg/ml medium thus demonstrating its powerful antimicrobial properties.

5-*n*-Hexylresorcinols (AR) naturally occur in several families of higher plants (e.g., in Gramineae, Anacardiaceae, Proteaceae, Myrosinaceae, Ginkgoaceae) and in *Azotobacter vinelandii* (Reusch and Sadoff 1979). Rye contains a valuable protein, yet it is not used extensively as a feed for young animals because it is less palatable than other cereals, resulting in a reduced feed intake and slower weight gains (Evans et al 1973). Wieringa (1967) isolated mixtures of AR in rye oil with distinct growth-reducing properties. When rye oil was added to the feed of swine or rats, reduced food consumption and a lower weight gain were observed. Using 5-pentadecylresorcinol as a model substance for AR as an addition to animal feed, Sedlet et al (1984) found that approximately 70% of the growth depression of rats was due to decreased food intake, and 30% was attributed to a toxic effect of the pentadecylresorcinol.

A possible damage to human health has been discussed for European countries with high bread consumption (Verdeal and Lorenz 1977), yet there are no toxicologic studies of AR in humans.

Identification studies showed that the AR from cereal grains comprise a group of resorcinol derivates with a straight, predominantly saturated hydrocarbon chain of 15–25 carbon atoms at the fifth position (Wieringa 1967). In a comparison study, rye contained the highest and wheat the lowest concentrations of AR, whereas triticale had intermediate levels (Verdeal and Lorenz 1977). According to Wieringa (1967), AR are localized in the pericarp layer of the rye caryopsis. Bread, as the final product of rye processing, contains only 78% of the AR content of the whole grain (Verdeal and Lorenz 1977).

Little is known about the biological importance of AR for the plants that produce them. It is assumed that these compounds—similar to other phenolic derivates (Picman et al 1984)—exert an antimicrobial effect, thus protecting the grain against an invasion by harmful microorganisms (Musehold 1980). Several compounds chemically related to the AR of grains possess strong antimicrobial properties: e.g., 2-*n*-hexyl-5-*n*-propylresorcinol as an antibiotic of *Pseudomonas* sp. (Kanda et al 1975, Kitahara and Kanda 1975), which is active against bacteria and dermatophytes; and 4-*n*-hexylresorcinol (4-hexyl-1,3-dihydroxybenzene), which selectively inhibits fungi (Eastwood 1944, Urakubo 1955), such as *Trichophyton, Sporotrichum, Microsporum,* and *Epidermophyton* (in a concentration of 1:100,000) (Leao and Eichbaum 1948). 4-*n*-Hexylresorcinol is fungistatic against *Aspergillus niger, Penicillium simplicissimum, Trichothecium roseum*, and various dermatophytes (Zsolnai 1960, Lipnicki 1963).

Unpublished studies in this laboratory showed that bread molds form smaller colonies on rye bread than on wheat bread. Because the growth depression on rye breads could be caused by the relatively high AR content in rye grains, a study was underaken to evaluate the antifungal effects of AR towards common bread molds. The known antifungal compound 4-*n*-hexylresorcinol, the AR model substance 5-pentadecylresorcinol, and AR were tested to determine their effects on growth of common bread molds.

MATERIALS AND METHODS

Organisms

The following molds were used in this study: *Aspergillus parasiticus* (NRRL 2999), producer of aflatoxins; *Aspergillus versicolor* (strain 519 Karlsruhe), producer of sterigmatocystin; *Penicillium chrysogenum* (strain Sp 465 Kulmbach), producer of citrinin; *Penicillium roqueforti* (strain Sp 1446 Kulmbach), producer of PR toxin. Stock cultures were maintained on malt extract agar (Difco) at 20 ± 1°C.

Chemicals

The AR mixture was prepared by E. Kerber (Stuttgart) from the rye cultivar Halo and had the following side chain composition: 17 C atoms (32.6%), 19 C atoms (29.5%), 15 C atoms (14.3%), 21 C atoms (14.0%), and 23 C atoms (9.7%). Side chains with less than 15 C atoms and more than 23 C atoms were not detected.

The chemicals for comparison were 4-*n*-hexylresorcinol (krist. rein; Serva, Heidelberg, Germany) and 5-pentadecylresorcinol (tech., 85%; Aldrich, Milwaukee, WI).

Malt extract agar containing different concentrations of the three compounds was prepared in the following manner: 1 g of AR was dissolved in 10 ml of acetone resulting in a solution with 100 mg AR/1 ml. From this solution 1 ml was added to 9 ml of molten malt extract agar resulting in agar containing 10 mg of AR per milliliter. One milliliter of the 100 mg/ml AR solution was diluted with 9 ml of acetone yielding an AR solution with 10 mg/ml. From this solution malt extract agar with 1 mg AR/ml was made. The preparation of malt extract agars with the different levels of 4-*n*-hexylresorcinol and 5-pentadecylresorcinol was performed in a corresponding manner. Controls were malt extract agar without additions (control) and malt extract agar with 1 ml of acetone in 9 ml of agar (acetone control), this being the volume of solvent added to the medium in the resorcinol experiments. For all the media, enough petri dishes were prepared to ensure at least two dishes for each mold.

Determination of Fungal Growth

The petri dishes were inoculated by aseptically transferring spore material from stock cultures of the single molds onto the

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TABLE I
Effects of Alkylresorcinols, 5-Pentadecylresorcinol, and 4-*n*-Hexylresorcinol on Growth of Molds

Test Compound and Concentration	Colony Radius[a] (mm) After 5 and 10 Days of							
	A. parasiticus		*A. versicolor*		*P. chrysogenum*		*P. roqueforti*	
	5	10	5	10	5	10	5	10
Control (agar)	16.0	23.3	5.0	10.0	8.3	13.8	10.0	26.0
Acetone control	7.5	26.0	2.5	7.5	4.5	10.5	8.0	25.5
Alkylresorcinols								
10 mg/ml	1.0	20.0	2.0	7.5	5.0	9.0	5.0	24.7
1 mg/ml	9.5	20.5	3.0	7.5	6.0	10.4	7.8	24.2
5-Pentadecylrescorcinol								
100 mg/ml	0	0	0	0	0	0	0	0
10 mg/ml	7.0	22.0	3.5	8.0	8.0	14.0	9.6	18.0
1 mg/ml	13.0	19.5	3.5	7.0	6.0	12.8	8.5	17.0
4-*n*-Hexylresorcinol								
1 mg/ml	0	0	0	0	0	0	0	0
0.1 mg/ml	2.2	14.8	0.8	2.0	1.5	4.5	0	0
0.01 mg/ml	3.6	18.0	3.4	7.5	4.5	9.5	5.5	21.8

[a]Average values of duplicate experiments are given.

surface of the agar media. The incubation was performed in the dark at 20 ± 1°C. Within an incubation period of 10 days the growth was measured by determining the length of the hyphae of the developing colonies. Duplicate experiments were conducted. With each medium and with the four molds at least two petri dishes were inoculated and analyzed.

RESULTS

The single measurements of hyphal development in the individual duplicate experiments gave results differing only ±1 mm from each other. Therefore, duplicate tests were sufficient. The growth rates of the four bread molds under the influence of the different resorcinols are summarized in Table I.

Controls

All fungi grew better on pure malt extract agar than on agar supplemented with acetone. This solvent was not replaced by one with lesser toxicity because of the extreme water insolubility of the AR. The controls with acetone must therefore be regarded as the basis for comparison.

Alkylresorcinols

Compared with the acetone control medium, the growth of *A. parasiticus* was distinctly reduced by additions of 10 mg/ml alkylresorcinols to nutrient agar and to a lower degree by addition of 1 mg/ml, whereas the other three molds obviously were not influenced by these levels. Media with higher concentrations of AR could not be prepared because of the insolubility of the AR in the aqueous medium.

5-Pentadecylresorcinol

5-Pentadecylresorcinol is often used as a standard in AR studies. The highest concentration (100 mg/ml) completely inhibited the growth of all four molds, whereas additions of 10 and 1 mg/ml gave different results. *A. parasiticus* was slightly inhibited by 10 mg/ml and slightly enhanced in growth by 1 mg/ml. *A. versicolor* was not influenced by either concentration, whereas *P. chrysogenum* and *P. roqueforti* developed larger colonies under the influence of 10 and 1 mg/ml.

4-*n*-Hexylresorcinol

The four molds were completely inhibited in growth on media with 100, 10, and 1 mg 4-*n*-hexylresorcinol per milliliter of medium. *P. roqueforti* was especially sensitive to this compound; even 0.1 mg/ml did not allow any growth. This concentration strongly reduced the growth of the other three fungi, and 0.01 mg/ml slightly reduced the spreading of the colonies of *A. parasiticus* and *P. roqueforti.*

DISCUSSION

Only growth of *A. parasiticus* NRRL 2999 was inhibited by 10 mg of AR from rye cultivar Halo per 1 ml of malt extract agar medium, whereas *A. versicolor, P. chrysogenum*, and *P. roqueforti* were not affected by additions of 10 and 1 mg AR/ml. It must be concluded from these results that the antifungal properties of AR in the concentrations tested are neglegible. According to Verdeal and Lorenz (1977) and Weipert and El Baya (1977), rye grains have an average AR content of 0.10–0.15%. Considering an average loss of 20% during the preparation of bread (Verdeal and Lorenz 1977), rye bread has an AR content of less than 0.1%. In the present study the malt extract agars had additions of 1% (10 mg/ml) or 0.1% (1 mg/ml). The low growth-inhibiting activity of these agars suggests that the slower growth of bread molds on rye bread compared with wheat bread is not due to the AR content in rye bread.

The question remains open whether AR have antifungal properties protecting the cereal grain against the attack of fungi. The molds tested in this study belong to the "storage flora" of grains. In the field, however, the grains are invaded by "field fungi," such as species of *Alternaria, Cladosporium, Fusarium* and others. These fungi may be more sensitive towards AR than the storage molds.

Under the influence of 10 and 1 mg/ml 5-pentadecylresorcinol, *A. parasiticus* and *P. chrysogenum* developed larger colonies than at corresponding levels of AR. In *A. versicolor* and *P. roqueforti* the effects of both compounds were similar. Hexylresorcinol proved to be the most effective substance against the four molds tested. Even additions as low as 0.1 mg/ml distinctly reduced the fungal growth. In *A. parasiticus* and *P. roqueforti* an inhibiting effect was observed with 0.01 mg/ml. The inhibiting levels correspond to the data given by Leao and Eichbaum (1948) for *A. niger, A. nidulans*, and *Penicillium* sp.

It is known that fungi are able to metabolize 5-methylresorcinol (Sahasrabudhe et al 1986) and can degrade *n*-alkylbenzenes when the side chains of these substances have more than a minimum length (Fedorak and Westlake 1986). For *Penicillim* spp. the minimum side chain length is 9 C atoms. This leads to the hypothesis that 5-pentadecylresorcinol and AR are metabolized by the bread molds, leading to formation of less toxic compounds. On the other side, hexylresorcinol, with a relatively short side chain, is less intensively attacked, thus retaining its antifungal activity.

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