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Antifungal Activity of Octyl Gallate: Structural Criteria and Mode of Action

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Abstract—Octyl gallate (3,4,5-trihydroxybenzoate) was found to possess antifungal activity against *Saccharomyces cerevisiae* and *Zygosaccharomyces bailii*, in addition to its potent antioxidant activity. Catechol moiety is essential to elicit this activity. The primary fungicidal activity of octyl gallate comes from its ability to act as a nonionic surface-active agent (surfactant). The length of the alkyl chain is not a major contributor but plays an important role in eliciting the activity. © 2001 Elsevier Science Ltd. All rights reserved.

In our continuing search for antimicrobial agents, propyl (C_3) (**1**), octyl (C_8) (**2**), and dodecyl (C_{12}) (**3**) gallates (see Fig. 1 for structures) were selected because these three are currently permitted for use as antioxidant additives in food and cosmetic products.[1] Their antimicrobial activity was first tested against the 16 selected microorganisms and the results are listed in Table 1. In general, Gram-positive bacteria and fungi were susceptible while Gram-negative bacteria were mostly resistant to the gallates. Among the three gallates tested, octyl gallate was found to exhibit fungicidal activity against *Saccharomyces cerevisiae*, *Zygosaccharomyces bailii*, *Candida albicans*, and *Aspergillus niger*, and was noticed to be the only active compound against these fungi. Hence, the current study was centered on modes of fungicidal action of octyl gallate against *S. cerevisiae* as an example. The activity against *Z. bailii*, a food spoilage yeast species, was also studied but to a lesser extent. Octyl gallate was noted to be effective against *S. cerevisiae* with both minimum inhibitory concentration (MIC) and minimum fungicidal concentration (MFC) of 25 μg/mL (89 μM), suggesting no residual fungistatic activity. The same gallate also exhibited antifungal activity against *Z. bailii* with both MIC and MFC of 50 μg/mL (177 μM). In contrast, neither propyl nor dodecyl (lauryl) gallates showed any fungicidal activity against the fungi tested. Notably, the fungicidal activity of octyl gallate against *S. cerevisiae* and *Z. bailii* was not influenced by the pH values.

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The head and tail structure and nonspecific antimicrobial activity of octyl gallate are similar to those of long chain alkanols in many aspects.[2] However, additional functions may need to be considered for alkyl gallates. For example, the ester group did not exist in the alkanol structure and may be involved in eliciting the additional activity. Since alkanols themselves exhibit the antifungal activity against *S. cerevisiae*,[3] the possibility of *S. cerevisiae* exuding an esterase that hydrolyzes alkyl gallates to gallic acid and the corresponding alkanols, was first taken into account. This possibility can be readily ruled out since none of the alkyl benzoates, 4-hydroxybenzoates or 3,5-dihydroxybenzoates, exhibited any antifungal activity against *S. cerevisiae*.

Analogues of octyl gallate were synthesized[4] and tested for their antifungal activity against *S. cerevisiae* for comparison. Among them, octyl protocatechuate (3,4-dihydroxybenzoate) (**4**) is the only compound to exhibit the activity. As a result, both octyl 3,4,5-trihydroxybenzoate and octyl 3,4-dihydroxybenzoate showed the activity and the potency is almost comparable. On the other hand, octyl benzoate (**5**), octyl 2-hydroxybenzoate (**6**), octyl 3-hydroxybenzoate (**7**), octyl 4-hydroxybenzoate (**8**), and octyl 3,5-dihydroxybenzoate (**9**) exhibited no antifungal activity, indicating that catechol or pyrogallol moieties are largely associated with the activity. This can be supported by the fact that octyl 3-hydroxy-4-methoxybenzoate (**10**) and octyl 4-hydroxy-3-methoxybenzoate (**11**) no longer showed any antifungal activity. On the basis of the data obtained, it appears that a catechol moiety is prerequisite to elicit

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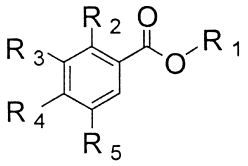
the antifungal activity. It should be noted however that octyl 2,3-dihydroxybenzoate (**12**) did not exhibit any activity. On the other hand, geranyl gallate (**13**) showed antifungal activity against *S. cerevisiae* with an MFC of 50 μg/mL. The rationale for this comes from the fact that the alkyl chain length of geraniol is approximately as the same as octanol.[2] The antifungal mechanism of these benzoates seems to be associated with their specific structural features but the precise mechanism remains unclear. In addition, the ester group is essentially not related to the activity since 3,4-dihydroxyphenyl decanoate (**14**) and heptyl 3,4-dihydroxycinnamate (caffate) (**15**) also exhibited similar antifungal activity.



1. $R_1=(CH_2)_2CH_3$, $R_2=H$, $R_3=R_4=R_5=OH$
2. $R_1=(CH_2)_7CH_3$, $R_2=H$, $R_3=R_4=R_5=OH$
3. $R_1=(CH_2)_{11}CH_3$, $R_2=H$, $R_3=R_4=R_5=OH$
4. $R_1=(CH_2)_7CH_3$, $R_2=R_5=H$, $R_3=R_4=OH$
5. $R_1=(CH_2)_7CH_3$, $R_2=R_3=R_4=R_5=H$
6. $R_1=(CH_2)_7CH_3$, $R_2=OH$, $R_3=R_4=R_5=H$
7. $R_1=(CH_2)_7CH_3$, $R_3=OH$, $R_2=R_4=R_5=H$
8. $R_1=(CH_2)_7CH_3$, $R_4=OH$, $R_2=R_3=R_5=H$
9. $R_1=(CH_2)_7CH_3$, $R_3=R_5=OH$, $R_2=R_4=H$
10. $R_1=(CH_2)_7CH_3$, $R_3=OH$, $R_4=OCH_3$, $R_2=R_5=H$
11. $R_1=(CH_2)_7CH_3$, $R_3=OCH_3$, $R_4=OH$, $R_2=R_5=H$
12. $R_1=(CH_2)_7CH_3$, $R_2=R_3=OH$, $R_4=R_5=H$

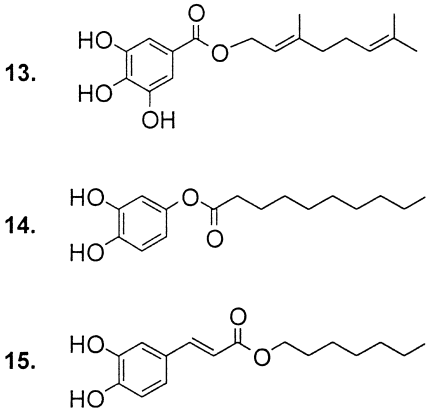


Figure 1. Chemical structures of gallates and related compounds.

The addition of glucose to an unbuffered suspension of *S. cerevisiae* cells results in the extrusion of acid. The change in external pH upon the addition of glucose is characteristic of yeast cells. This acid extruded could be due to the action of the plasma membrane H^+-ATPase.[5] The activation of the H^+-ATPase by glucose is not yet fully understood at a molecular basis, but the maintenance of internal pH homeostasis is essential for the cell to survive since intracellular pH is important for the activity of a number of enzymes with pH optima.[6,7] This glucose-induced medium acidification process was inhibited by octyl gallate. The inhibition was presumably caused by its inhibition of the H^+-ATPase. Notably, antifungal octyl gallate inhibited this glucose induced acidification but neither propyl nor dodecyl gallates showed this activity as shown in Figure 2.

The head and tail structure of octyl gallate is similar to those of alkanols of which primary antifungal action comes from their ability to disrupt the native membrane-associated function of the integral proteins as nonionic surfactants.[2] Therefore, the mode of antifungal action of octyl gallate can be expected to act as a nonionic surfactant and thus it would first approach the binding site with the electronegativity of the oxygen atoms. The oxygens are hydrogen bond acceptors that will disrupt existing hydrogen bonds but the binding site may not be specific. The intrinsic proteins of immediately surrounding membranes are suggested to be held in position by hydrogen bonding, as well as by hydrophobic and electrostatic forces, and that hydrogen bonding also mediates the penetration of membranes by proteins. As mentioned above, hydrogen bonds may be disrupted by octyl gallate, and redirected. Thereby the conformation of the protein may be changed, and consequently the H^+-ATPase in particular may lose its

Table 1. Antimicrobial activity[11] of propyl (C_3), octyl (C_8), and dodecyl (C_{12}) gallates

Microorganisms tested[12]	C_3	C_8	C_{12}
Bacillus subtilis	800 (*1600*)[a]	12.5 (*25*)	12.5 (*25*)
Brevibacterium ammoniagenes	1600 (*3200*)	25 (*50*)	12.5 (*25*)
Micrococcus luteus	1600 (*3200*)	12.5 (*25*)	12.5 (*25*)
Streptococcus mutans	400 (*800*)	50 (*50*)	100 (*100*)
Staphylococcus aureus	1600 (*3200*)	25 (*50*)	12.5 (*25*)
S. aureus (MRSA)	1600 (*3200*)	25 (*50*)	12.5 (*25*)
Propionibacterium acnes	800 (*800*)	25 (*25*)	6.25 (*6.25*)
Escherichia coli	1600 (*1600*)	>800 (*>800*)	>800 (*>800*)
Pseudomonas aeruginosa	3200 (*>3200*)	>800 (*>800*)	>800 (*>800*)
Enterobacter aerogenes	3200 (*>3200*)	>800 (*>800*)	>800 (*>800*)
Proteus vulgaris	400 (*400*)	25 (*50*)	>800 (*>800*)
Salmonella choleraesuis	1600 (*>3200*)	12.5 (*12.5*)	25 (*50*)
Saccharomyces cerevisiae	3200 (*>3200*)	25 (*25*)	>1600 (*>1600*)
Zygosaccharomyces bailii	>3200 (*>3200*)	50 (*50*)	>1600 (*>1600*)
Candida albicans	3200 (*>3200*)	25 (*25*)	>400 (*>400*)
Aspergillus niger	>3200 (*>3200*)	50 (*100*)	>400 (*>400*)

[a]μg/mL. Numbers in italic type in parentheses are MBC or MFC.

functioning conformations. While H^+-ATPase is the most abundant plasma membrane protein, constituting over 20% of the total membrane protein in *S. cerevisiae*, other plasma membrane proteins may also be disrupted by octyl gallate. The data obtained are consistent with an effect on the bulk membrane rather than a direct interaction of H^+-ATPase. In brief, the primary fungicidal activity of octyl gallate comes from its ability to act as a nonionic surfactant, though it cannot be inferred that membrane damage is the only cause of the lethal effect.

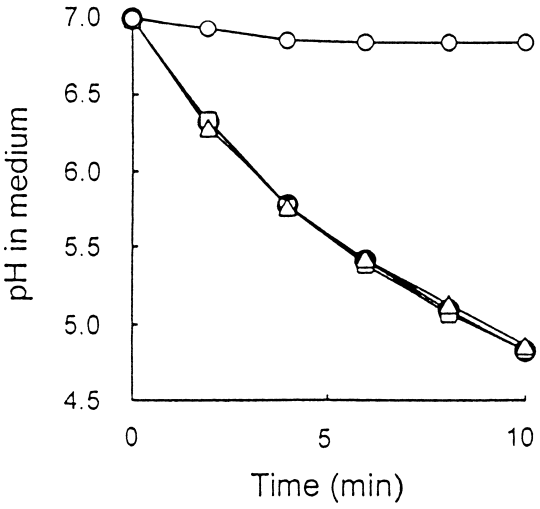


Figure 2. Inhibition of glucose-induced medium acidification by octyl gallate in *S. cerevisiae*. Each yeast cell suspension (2.4×10^8 cells/mL) was preincubated with control (●), 2.5 mM octyl gallate (○), 2.5 mM propyl gallate (□), or 2.5 mM dodecyl gallate (△) at 30 °C for 5 min. After preincubation, 2% glucose (final concn) was added and then medium acidification was started.

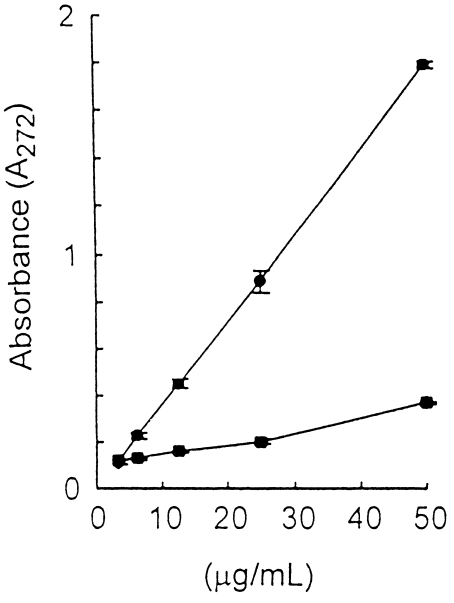


Figure 3. Binding of octyl gallate to *S. cerevisiae* cells. After octyl gallate was mixed with (■) or (●) without yeast cells (10^8 cells/mL), the suspension was vortexed for 5 s. Absorbance of the supernatant obtained by centrifugation for 2 min was measured.

Further support for the surfactant concept was obtained in an additional experiment[8] that indicates antifungal octyl gallate rapidly binds with *S. cerevisiae* cells as shown in Figure 3. Its hydrophilic pyrogallol moiety binds with an intermolecular hydrogen bond like a 'hook' by attaching itself to the hydrophilic portion of the membrane surface. The binding site of octyl gallate is unknown, though this is the first step of antifungal action as a surfactant. It should be noted that dodecyl gallate did not bind with *S. cerevisiae* cells. Most of the dodecyl gallate molecule seems to remain in the water based medium, probably in the form of an insoluble monolayer or spread film.[9] It appears that the length of the alkyl chain is not a major contributor but plays an important role in eliciting the activity. The rationale for the role of hydrophobic alkyl portion is still poorly understood and widely debated. It appears that octyl gallate has the potential as an antifungal agent since it very likely targets the extracytoplasmic region and thus does not need to enter the cells, thereby avoiding most cellular pump-based resistance mechanisms.

Lastly, it is worthwhile to add that both octyl and dodecyl gallates also exhibited antibacterial activity against *Salmonella choleraesuis* with the minimum bactericidal concentrations (MBCs) of 12.5 μg/mL (44 μM) and 50 μg/mL (148 μM), respectively. The MBC of octyl gallate against this food born bacterium is noted to be comparable with that of gentamycin. *S. choleraesuis* is one of the rare Gram-negative bacteria susceptible to gallates. All the gallates tested, regardless of their carbon chain length, showed potent scavenging activity on the 1,1-diphenyl-2-*p*-picryhydrazyl (DPPH) radical.[10] In addition to potent antioxidant activity, broad antimicrobial activity of octyl gallate would appear to be of great overall value.

Acknowledgements

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References and Notes

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3. Among the primary alkanols tested, undecanol was found to be the most potent fungicide against *S. cerevisiae* with an MFC of 25 μg/mL (150 μM).
4. To a solution of the corresponding phenolic acid (2.00 mM) and alcohol (2.00 mM) in THF (6 mL) cooled at 0 °C was added a solution of *N,N′*-dicyclohexylcarbodiimide (DCC) (4.2 mM) in THF (6 mL). After the solution had been allowed to stir for 20 h, the solvent was removed under reduced pressure. The residue was extracted with ethyl acetate several times and filtered. The filtrate was washed successively with dilute aqueous citric acid solution, saturated aqueous sodium

hydrogen carbonate solution and water, dried over $MgSO_4$, and evaporated. The crude products were purified by chromatography (SiO_2; elution with $CHCl_3$:MeOH, 98:2). Structures of the synthesized esters were established by spectroscopic methods (UV, IR, MS, and NMR). Their analogues described in this communication were also synthesized by the same manner.

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8. The test strain was cultured with shaking in YPD broth overnight at 30 °C and washed twice with 50 mM MOPS buffer (pH 6.0). After each octyl gallate was mixed with or without yeast cells (10^8 cells/mL) in the above buffer at 30 °C, the suspension was vortexed for 5 s. Absorbance of the supernatants obtained by centrifugation for 2 min was measured at 272 nm.
9. Jones, M. N.; Chapman, D. *Micelles, Monolayers and Biomembranes*; Wiley-Liss: New York, 1994.
10. Kubo, I. *Chemtech*. **1999**, *29*, 37.
11. The procedures used for antimicrobial assay were the same as previously described.[2]
12. The test strains used for this study were purchased from the American Type Culture Collection (Rockville, MD) the same as previously described.[3]