Note

Respiratory Inhibition by the Antifungal Benzoquinone, 2-Hydroxy-6-methoxy-3,5-dimethyl-1,4-benzoquinone

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One of the authors previously reported 2-hydroxy-6-methoxy-3,5-dimethyl-1,4-benzoquinone (**1**), isolated from the culture broth of *Phoma wasabiae* YOKOGI, to be a new natural benzoquinone.¹⁾ However, later studies on the metabolites of this mold disclosed that **1** is not a true fermentation product but an oxidation product of wasabdienone-A, a tautomeric mixture of (6*R*)-5-hydroxy-3-methoxy-2,6-dimethyl-6-[(2*R*)-2-methylbutyryloxy]-2,4-cyclohexadien-1-one and 5-hydroxy-3-methoxy-4,6-dimethyl-6-(2-methylbutyryloxy)-2,4-cyclohexadien-1-one, during isolation.^{2,3)} We found that **1** is

moderately antifungal. Some benzoquinone derivatives inhibit mitochondrial functions through various mechanisms.^{4,5)} This report deals with the mode of action of this antifungal benzoquinone, **1**.

The minimum inhibitory concentrations (MIC) of **1** against a variety of microorganisms are presented in Table I. Compound **1** was active against filamentous fungi and

TABLE I. ANTIMICROBIAL ACTIVITY OF BENZOQUINONE (**1**)

Microorganisms tested	MIC (μg/ml) ^a
<i>Staphylococcus aureus</i> NCTC 8530	> 100
<i>Bacillus subtilis</i> K-49	> 100
<i>Micrococcus lysodeikticus</i> IFO 3333	> 100
<i>Escherichia coli</i> IFO 3545	> 100
<i>Proteus vulgaris</i> IAM 12003	> 100
<i>Pseudomonas aeruginosa</i> IAM 1007	> 100
<i>Saccharomyces cerevisiae</i> IFO 0203	> 100
<i>Candida utilis</i> ATCC 42402	25
<i>Schizosaccharomyces pombe</i> IFO 0342	> 100
<i>Hansenula anomala</i> IFO 0136	> 100
<i>Mucor mucedo</i> IFO 7684	25
<i>Rhizopus chinensis</i> IFO 4745	12.5
<i>Aspergillus niger</i> ATCC 6275	50
<i>Penicillium crustosum</i> Thom	100

^a Measured by a two-fold serial broth dilution method.⁶⁾

TABLE II. EFFECTS OF BENZOQUINONE (**1**) ON SOME ENERGY-LINKED FUNCTIONS IN MITOCHONDRIA ISOLATED FROM RAT LIVER

Rat liver mitochondria were prepared by the method of Schneider and Hogeboom.⁸⁾ Oxygen consumption was measured polarographically as described previously.⁹⁾ The measurement was repeated five times. Oxidative phosphorylation activity was calculated from the oxygen electrode tracing and expressed as a respiratory control ratio and an ADP/O ratio.

Respiratory substrate	Concentration of 1 (M)	Respiratory rate (nanoatoms O/mg protein/min)			Respiratory control ratio	ADP/O
		State				
		3	4	4U ^a		
		3	4	4U ^a		
α-Ketoglutarate	0	79.0 ± 3.2 ^b	17.9 ± 1.7 ^b	88.2 ± 4.2 ^b	4.42	2.42 ± 0.07 ^b
	10 ⁻⁵	59.3 ± 2.7	18.6 ± 2.6	73.0 ± 2.2	3.19	2.17 ± 0.11
	10 ⁻⁴	53.1 ± 2.3	19.5 ± 2.4	63.8 ± 2.5	2.72	2.28 ± 0.14
	10 ⁻³	33.5 ± 1.4	19.8 ± 1.9	42.2 ± 2.8	1.69	2.25 ± 0.06
Succinate	0	93.9 ± 3.1	17.7 ± 1.7	99.2 ± 4.6	5.30	1.60 ± 0.19
	10 ⁻⁵	74.9 ± 3.2	18.8 ± 1.6	85.2 ± 3.9	3.98	1.62 ± 0.15
	10 ⁻⁴	39.3 ± 2.8	17.7 ± 1.8	35.4 ± 2.6	2.22	1.51 ± 0.05
	10 ⁻³	18.6 ± 2.0	18.6 ± 2.0	18.6 ± 2.0	1.00	—

^a In the presence of 2,4-dinitrophenol at 40 μM.

^b Mean ± S.E.

TABLE III. INHIBITION OF NADH- AND SUCCINATE-CYTOCHROME *c* REDUCTASE OF RAT LIVER MITOCHONDRIA BY BENZOQUINONE (1)

Concentration of 1 (M)	Activity ^a (ΔA_{550} /mg protein/min)	
	NADH-cytochrome <i>c</i> reductase	Succinate-cytochrome <i>c</i> reductase
0	0.47	0.75
10^{-5}	0.26	0.61
10^{-4}	0.14	0.36
10^{-3}	0.05	0.01

^a Assayed by measuring the increase in the absorbancy at 550 nm resulting from the reduction of horse heart cytochrome *c*.^{10,11)}

inactive against both Gram-positive and Gram-negative bacteria. The activity against yeasts differed from species to species. Interestingly, *Candida utilis* which was susceptible to 1 assimilates a main carbon source in the assay medium, maltose, oxidatively, but not to ferment it.⁷⁾ On the other hand, other insusceptible yeasts, *Saccharomyces cerevisiae*, *Schizosaccharomyces pombe*, and *Hansenula anomala*, both assimilate and ferment maltose. Thus, the antifungal activity of 1 may be attributed to inhibition of mitochondrial functions. Indeed, respiratory inhibition by 1 was observed polarographically in cell suspensions of *Candida utilis* and in mycelial suspensions of *Rhizopus chinensis* (data not shown).

To find more precisely the site of action on mitochondria, the effects of 1 on some energy-linked functions in isolated mitochondria were investigated. Instead of fungi, rat liver was used as a source of mitochondria because rat liver mitochondria were relatively stable during isolation procedures and had a higher activity of oxidative phosphorylation. As shown in Table II, 1 at 10^{-5} – 10^{-3} M lowered the respiratory control ratio but did not affect the ADP/O ratio when α -ketoglutarate or succinate was used as the respiratory substrate. It significantly depressed the rate of respiration in state 3 and in 2,4-dinitrophenol uncoupled mitochondria (state 4U), though it hardly affected that in state 4. Thus, 1 seems to act neither as an uncoupler nor as an inhibitor of energy transfer. Uncouplers usually stimulate the respiratory rate in state 4, while inhibitors of energy transfer inhibit only the respiratory rate in state 3 without effect in state 4U. This benzoquinone seems to have an action on some respiratory chain enzymes. As shown in Table III, 1 significantly inhibited NADH- and succinate-cytochrome *c* reductase at 10^{-5} – 10^{-3} M. This again suggested that 1 blocks electron transport primarily by affecting some site preceding cytochrome *c*. As shown in Fig. 1, when dichlorophenolindophenol (DPIP) was used as the terminal ac-

FIG. 1. Inhibition of Succinate-DPIP Reductase of Rat Liver Mitochondria by Benzoquinone (1).

The reductase activity was assayed by a decrease in absorbancy at 600 nm resulting from reduction of DPIP.¹²⁾ ○, without 1; ◐, 10^{-5} M 1; ⊗, 10^{-4} M 1; ●, 10^{-3} M 1.

ceptor of electrons transferred from the oxidation of succinate, 1 was very effective in inhibiting succinate-DPIP reductase. DPIP is known to be reduced before the antimycin-sensitive site.¹³⁾

Considering these observations, the site of respiratory inhibition by the antifungal benzoquinone 1 appears to be near coenzyme Q of the electron transport system, although further studies on the molecular mechanism of the action are needed.

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