EFFECT OF SOME NITROPHENOLS ON THE RESPIRATION OF MYROTHECIUM VERRUCARIA SPORES*

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The evidences as presented by Plantefol (1), Field, Martin, and Field (2, 3), Krahl and Clowes (4), Shoup and Kimler (5), Bodine and Boell (6), Tyler (7), Peiss and Field (8), and Newcomb (9), including the reviews by von Oettinger (10) and Clifton (11), indicate both the well established stimulatory effect, especially induced by low concentrations, as well as the lesser emphasized depressant effect nitrophenols may have on tissue respiration. The influence of alkyl-, cycloalkyl-, and aryldinitrophenols, including in particular their relative effectiveness, on respiration appears not to have been investigated for fungus spores. Data are presented to show that certain of the substituted dinitrophenols (DNP) have a potent inhibitory effect on the oxidative mechanism of Myrothecium verrucaria (USDA 1334.2, Metarrhizium glutinosum), an organism introduced as a mildew-resistant test organism for fabrics by Greathouse, Klemme, and Barker (12).

EXPERIMENTAL

In all cases a weighed amount of the compound, previously confirmed by melting point determination, was dissolved in methyl cellosolve\(^1\) and the solution made up to the desired volume with buffered nutrient solution (pH 6.5) composed of sucrose 5.0 gm., NH\(_4\)NO\(_3\) 3.0 gm., KH\(_2\)PO\(_4\) 2.59 gm., K\(_2\)HPO\(_4\) 2.21 gm., MgSO\(_4\)\(\cdot\)7H\(_2\)O 0.75 gm., and distilled water to make 1 liter.

Spore suspensions were prepared from 4 day-old cultures of the fungus incubated at 30° and grown in Petri dishes containing an agar-mineral salts medium overlaid with a circle of Whatman No. 5 filter paper, the salt and carbohydrate portion of the medium having the same composition as that used for preparing spore suspensions. Spores were harvested by washing the surface of the culture with nutrient solution and by using a glass rod to free the spores. This suspension was then filtered through

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1 Suitable as a solubilizing agent, since alone it showed no effect upon spore respiration at concentrations needed for solubilisation.

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cheese-cloth and subsequently adjusted to a population of $50 \times 10^6$ spores per ml.

Measurements of oxygen respiration were made at $30^\circ$ by the customary Warburg manometer technique with single side arm respirometers of approximately 15 ml. capacity. In the main cavity of the respirometer were placed 2 ml. of freshly prepared spore suspension; 0.5 ml. of nutrient solution containing the derivative was added to the side arm. A 2 cm. square of filter paper and 0.2 ml. of 10 per cent KOH were placed in the alkali well for absorption of the CO$_2$ evolved.

**DISCUSSION**

The relative capability of the various nitrated phenols to inhibit the oxidative mechanism of *M. verrucaria* spores is shown in Table I, where the concentration necessary to induce a 50 per cent. inhibition of the normal oxygen uptake rate is indicated. These values were ascertained by interpolation of plots of the detailed dosage-response data for each chemical.

The two cycloalkylated dinitrophenols (Compounds 12 and 13, Table I), which demonstrate a high order of inhibitory activity, were found by Field, Martín, and Field (13) to evoke essentially the same total stimulatory effect in yeast respiration as DNP; however, the effectiveness according to concentration indicated the cycloalkyl derivatives to be 28 to 40 times more efficient. The comparative inhibitory effectiveness shows these derivatives to be 21 to 22 times more active than DNP. A similar elevated order of biological response, determined on a larvicidal, insecticidal, and scalicidal basis by Kagy (14), was concluded for the cycloalkylated derivatives with still higher activity indicated for the n-hexyldinitrophenol.

The ortho introduction of a carboxyl, amino, or an additional nitro group into the active 2,4-DNP nucleus yielded acid derivatives with greatly reduced activity. The bacteriostatic potency at pH 6.5 of picric and picramic acids, when compared with DNP as determined by Cowles and Klotz (15), indicated a correlating low level of toxicity for these acids.

Replacement of the 4-nitro group in the inactive picric acid molecule with a cyclohexyl, diisobutyl, n-octyl, or chloro group resulted in 2,6-DNP derivatives with restored high inhibitory activity. Chlorine substitution in 2,6-DNP apparently does not induce the degree of toxicity resulting from alkyl or cycloalkyl substitution. Although introduction of the cyclohexyl group in the para position of 2,6-DNP provided the most potent compound of this group, it is to be noted that its isomer, 6-cyclohexyl-2,4-dinitrophenol was approximately twice as effective.

A rather striking parallelism exists between the ability of the various
nitrophenols reported by Cross, Taggart, Covo, and Green (16) to affect the cyclophorase system and their inhibitory effect on fungus spore respiration. At concentrations of $2.5 \times 10^{-4}$ M, Compounds 5, 6, 9, 13, 14, and 21, indicative of a high order of toxicity in this study, were found to inhibit phosphorylation completely, while at this one concentration Compounds 15 and 17, here very inactive, had no effect on phosphorylation and Com-

\begin{table}
\centering
\caption{Effect of Some Nitrophenols on Respiration of Myrothecium verrucaria Spores}
\begin{tabular}{|c|c|c|c|c|c|c|}
\hline
\textbf{Compound No.} & \textbf{Carbon position of phenol substituent} & \textbf{Concentration necessary to inhibit respiration 50 per cent} \\
\hline
2 & NO$_2$ & & & & $\times 10^{-4}$ M \\
3 & NO$_2$ & & & & 20 \\
4 & NO$_2$ & & & & 15 \\
5 & CH$_3$ & & & & 10 \\
6 & & & CH$_3$ & & 7 \\
7 & & & i-C$_5$H$_7$ & & 2.8 \\
8 & & & 2.8 \\
9 & & & s-C$_4$H$_9$ & & 1.5 \\
10 & & & n-C$_6$H$_{13}$ & & 3.2 \\
11 & & & C$_{6}$H$_{17}$ & & 0.95 \\
12 & & & C$_{6}$H$_{17}$ & & 0.25 \\
13 & & & C$_{6}$H$_{17}$ & & 0.7 \\
14 & & & C$_{6}$H$_{17}$ & & 0.32 \\
15 & & & COOH & & 0.33 \\
16 & & & NH$_2$ & & 1.05 \\
17 & & & NO$_2$ & & 0.8 \\
18 & & & n-C$_6$H$_{17}$ & & >80 \\
19 & & & C$_{6}$H$_{17}$ & & >40 \\
20 & & & Cl & & >100 \\
21 & & & Cl & & >100 \\
\hline
\textbf{Miscellaneous compounds} & & & & & \\
22 & 2,4-Dinitro-2-heptylphenol & & & & 0.7 \\
23 & 2,4-Dinitro-2-n-octylphenol & & & & 0.6 \\
24 & Dicyclohexylamine salt of 6-cyclohexyl-2,4-dinitrophenol & & & & 0.4 \\
\hline
\end{tabular}
\end{table}
pound 16, also inactive, caused a 50 per cent inhibition. The toxicological studies of Spencer, Rowe, Adams, and Irish (17) on five of the dinitrophenols (Compounds 5, 6, 9, 13, and 24) yielded markedly similar and correlating data for survival and lethal dose.

The various dinitrophenols were kindly supplied by Dr. F. B. Smith of The Dow Chemical Company and by Mr. W. E. Craig of the Rohm and Haas Company.

SUMMARY

An investigation was undertaken of the effectiveness of certain nitrophenols and some of the substituted dinitrophenol derivatives to interfere with the respiratory mechanism of spores of the fungus Myrothecium verrucaria. Of twenty-four compounds studied, the three most potent inhibitors of oxygen uptake were 6-cyclohexyl-, 6-cyclopentyl-, and 6-n-hexyl-2,4-dinitrophenol. Concentrations of the order of 2.5 to 3.3 \times 10^{-5} \text{M}, or 7 to 9 parts per million, respectively, were sufficient to inhibit respiration 50 per cent.

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