Antifungal Activity of Substituted Nitrobenzenes and Anilines

HERMAN GERSHON, MAYNARD W. McNEIL, RAULO PARMEGIANI, AND PATRICIA K. GODFREY

Boyce Thompson Institute for Plant Research, Yonkers, New York 10701

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Series of 1,3-dihalogeno-5-nitrobenzenes, 3- and 3,5-halogenoanilines, and 2,6-dihalogeno-4-nitroanilines were tested for fungitoxicity against Aspergillus niger, A. oryzae, Trichoderma viride, Myrothecium verrucaria, and Trichophyton mentagrophytes in shaken culture by using Sabouraud dextrose broth enriched with yeast extract as the test medium. 1,3-Dichloro-5-nitrobenzene, 1,3-dibromo-5-nitrobenzene, 3-iodoaniline, 3,5-dichloroaniline, and 3,5-dibromoaniline were found to possess sufficient activity, compared with 8-quinolinol, to warrant further study.

As the result of the preparation of a series of quinolines for another study, a number of 1,3-disubstituted-5-nitrobenzenes, 3,5-disubstituted anilines, and 2,6-disubstituted-4-nitroanilines became available. A search of the literature revealed that, although many chloronitrobenzenes (3) had been examined as antifungal agents, the 1,3-disubstituted isomers were not included. In the same way, it was reported that nitrobenzene showed very little activity against Rhizoctonia solani and Pythium ultimum. An examination of a large number of nitro compounds, as potential antifungal agents, showed that 1,3-dibromo-5-nitrobenzene was weakly inhibitory toward seven fungi among which was Aspergillus niger (9). The 3- and 3,5-substituted anilines have received very little attention. On the other hand, 2,6-dichloro-4-nitroaniline was found to be highly effective against Botrytis cinerea and is used commercially (1). The dibromo and diiodo analogues were somewhat less effective against this organism. In another study (9), the dibromo and diiodonitroanilines showed poor inhibition against seven fungi, whereas the parent compound, 4-nitroaniline, was nearly devoid of activity.

Since few data are available on the antifungal activity of these compounds, and 2,6-dichloro-4-nitroaniline is in commercial use as an agricultural fungicide, it was of interest to examine these compounds for fungitoxicity.

MATERIALS AND METHODS

Compounds. Of the compounds studied which are listed in Table 1, nitrobenzene, 1,3-dichloro-5-nitrobenzene, aniline, 3-fluoroaniline, 3-chloroaniline, 3-bromoaniline, 3-iodoaniline, 3,5-dichloroaniline, 3,5-dimethoxyaniline, 4-nitroaniline, 2,6-dichloro-4-nitroaniline, and 2,6-diido-4-nitroaniline were commercially available. The remaining compounds were prepared by methods found in the literature as follows: 1,3-difluoro-5-nitrobenzene (4), 1,3-dibromo-5-nitrobenzene (7), 1,3-diido-5-nitrobenzene (8), 3,5-difluoroaniline (4), 3,5-diidoaniline (8), 2-fluoro-4-nitroaniline (2), and 2,6-dibromo-4-nitroaniline (6).

Organisms. The five fungi employed in this test system included A. niger, A. oryzae, Trichoderma viride, Myrothecium verrucaria and Trichophyton mentagrophytes.

All of the compounds were screened against the spores of the five organisms in Sabouraud dextrose broth (Difco) shaken culture enriched with 0.05% yeast extract (Difco) by published methods (5). Minimal levels of fungistatic and fungicidal action of the compounds were recorded and are summarized in Table 1.

RESULTS

Nitrobenzene was inhibitory to M. verrucaria and T. mentagrophytes at high concentrations of compound. Both 1,3-difluoro- and 1,3-dichloro-5-nitrobenzenes inhibited all five fungi and were fungicidal to three, and 1,3-dibromo- and 1,3-diido-5-nitrobenzenes were inhibitory to three and two organisms, respectively. The difluoro and dichloro analogues possessed the broadest spectra of toxicity with 1,3-dichloro-5-nitrobenzene being the most fungitoxic of the group. However, 1,3-dibromo-5-nitrobenzene was the most fungistatic against T. viride, M. verrucaria, and T. mentagrophytes. Aniline, 3-fluoroaniline, and 3,5-dimethoxyaniline were inactive under these test conditions. The 3-chloro, 3-bromo, and 3-iodo analogues of aniline were moderately fungistatic.
to all five fungi in the following order \( I > Br > Cl \). The 3,5-dihalogenoanilines were all more fungitoxic than the corresponding 3-halogenoanilines, except 3,5-diiodoaniline which was somewhat less inhibitory to \( A. niger \) and \( A. oryzae \). It should be mentioned that the activity of most of the halogenoanilines was fungistatic with the exception of the dichloro and dibromoanilines which were also fungicidal at fairly low levels of compound. 4-Nitroaniline and its 2-fluoro analogue were fungistatic to all five organisms, but the dichloro, dibromo, and diiodo analogues were completely inactive in this test system.

**DISCUSSION**

A comparison of antifungal data obtained on compounds in different test systems where there may be differences in test organisms, media, and other conditions is invalid. To overcome this problem, 8-quinolinol, a well-known antifungal agent, was included in the study for comparison. A number of the more active compounds such as 3,5-dichloro- and 3,5-dibromoanilines and 1,3-dichloro-5-nitrobenzene possess fungitoxic activity within the same order of magnitude as that of 8-quinolinol. Of further interest is the fact that the dichloro and dibromoanilines are fungicidal against \( A. niger \) and \( A. oryzae \), whereas 8-quinolinol is fungistatic against these organisms, under the conditions of this test system. It is evident from these data that several of the compounds (1,3-dichloro-5-nitrobenzene, 1,3-dibromo-5-nitrobenzene, 3-iodoaniline, 3,5-dichloroaniline, and 3,5-dibromoaniline) merit further study.

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**Table 1. Minimal antifungal activity (mMoles/liter) of substituted nitrobenzenes and anilines**

| Compound                | Aspergillus niger s | C | A. oryzae | s | C | Trichoderma viride | s | C | Myrothecium verrucaria | s | C | Trichophyton mentagrophytes s |
|-------------------------|---------------------|---|-----------|---|---|-------------------|---|---|------------------------|---|---|----------------------------|
| Nitrobenzene            | NA                  |   | NA        |   |   | 7.3               |   |   | 3.7                    |   |   | 1.9                       |
| 1,3-Difluoro-5-nitrobenzene | 4.4 NA             | 5.0 NA | 2.5 3.8 | 3.1 4.4 | 0.26 1.3 | 0.26 0.52 | 0.16 1.3 |
| 1,3-Dichloro-5-nitrobenzene | 1.0 NA             | 0.78 NA | 0.11 NA | 0.14 NA | 0.14 2.7 |
| 3,5-Dihaloaniline       | NA                  |   | NA        |   |   | 1.9 2.7           |   |   | 1.1 1.3 |
| 3-Chloroaniline         | NA                  |   | NA        |   |   | 3.9 2.7           |   |   | 2.7 NA |
| 3-Bromoaaniline         | NA                  |   | NA        |   |   | 3.9 2.0           |   |   | 2.0 3.5 |
| 3-Iodoaniline           | NA                  |   | NA        |   |   | 3.9 2.0           |   |   | 3.9 2.0 |
| 3,5-Difluoroaniline     | 7.7 NA              | 7.7 NA | 4.7 NA | 3.9 NA | 1.2 3.8 | 1.1 NA | 1.1 2.1 |
| 3,5-Dichloroaniline     | 0.92 3.1 0.92 2.5 | 0.31 1.8 | 0.37 1.5 | 0.31 0.62 |
| 3,5-Dibromoaniline      | 0.36 1.0 0.60 1.0 | 0.16 0.80 | 0.16 0.60 | 0.16 0.24 |
| 3,5-Diiodoaniline       | 1.0 NA              |   | NA        |   |   | 0.17 NA           |   |   | 0.12 NA | 0.23 0.29 |
| 3,5-Dimethoxyaniline    | NA                  |   | NA        |   |   | 0.02 0.62 | NA | 0.28 NA | 0.034 |
| 4-Nitroaniline          | 3.6 NA              | 3.6 NA | 2.2 NA | 3.6 NA | 2.9 5.4 |
| 2-Fluoro-4-nitroaniline | 2.9 NA              | 2.9 NA | 2.6 NA | 3.2 6.4 | 2.9 6.4 |
| 2,6-Dichloro-4-nitroaniline | NA               | NA | NA | NA | NA |
| 2,6-Dibromo-4-nitroaniline | NA               | NA | NA | NA | NA |
| 2,6-Diiodo-4-nitroaniline | NA               | NA | NA | NA | NA |
| 8-Quinolinol            | 0.14 NA             | 0.16 NA | 0.021 0.62 | 0.028 0.034 | 0.021 0.021 |

* Values expressed as millimoles per liter. Compound tested after 6 days of 28 C in Sabouraud dextrose broth shaken culture.
* s = fungistatic; C = fungicidal.
* NA = not active below 1,000 \( \mu g/ml \), highest level tested.
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