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# Antibiotic Properties of Malformin[1]

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Malformations of various plants and severe curvatures of corn roots after treatment with malformin, a metabolic product of *Aspergillus niger* van Tiegh., have been described (R. W. Curtis, Plant Physiol. 36:37, 1961). The structure cyclo - L - isoleucyl - D - cysteinyl - L - valyl - D - cysteinyl-D-leucyl was proposed for malformin A (S. Marumo and R. W. Curtis, Phytochem. 1:245, 1961). Because a number of antibiotics are also cyclic peptides, we examined malformin A for antibiotic properties.

A 10-mg amount of malformin A was dissolved in 2 ml of glacial acetic acid, diluted with 8 ml of water, and frozen until needed. Liquid media were supplemented with portions of the malformin solution, adjusted to *p*H 7.0, and were sterilized by filtration. Unamended media and media containing acetic acid in the same concentration as the malformin-containing media served as controls. The media employed were as follows: (1) glucose, 5.0 g; $(NH_4)_2HPO_4$, 1.0 g; $KH_2PO_4$, 0.5 g; $MgSO_4 \cdot 7H_2O$, 0.1 g; NaCl, 2.5 g; and water, 1,000 ml; (2) same as medium 1 except containing 2.0 g of sodium glutamate instead of $(NH_4)_2HPO_4$; (3) nutrient broth; and (4) nutrient broth plus 2% glycerine. Fungi were grown on Czapek solution, and yeast were grown on the synthetic medium of J. Lodder and N. J. W. Kreger-van Rij (*The Yeasts*, p. 25, North-Holland Publishing Co., Amsterdam, 1952).

Treated and control media were inoculated with a variety of bacteria and fungi, incubated at temperatures suitable for development of the test organism, and observed visually for growth. Concentrations of malformin above 10 mg per liter were not employed. Results are expressed as the minimal concentration of malformin required for complete inhibition of visible growth, as shown in Table 1.

Antibacterial properties of malformin were unrelated to Gram-staining properties of the bacteria. *Bacillus subtilis* and *Xanthomonas stewartii* were most sensitive to malformin, and two species of *Mycobacterium* were fairly sensitive. At sub-bacteriostatic concentrations of malformin, we occasionally observed aggregation of bacteria into clumps. Although antibacterial properties might be utilized as a rapid assay for malformin, the corn root curvature test is considerably more sensitive and probably more specific.

Malformin caused no reduction at any concentration in the growth of *Mucor mucedo* (L.) Fres., *Neurospora tetrasperma* Shear and Dodge, *Penicillium chrysogenum* Thom, *Rhizopus nigricans* Ehr., and *Saccharomyces cerevisiae* Meyen. *A. niger*, which produces malformin, is apparently quite tolerant of the compound, because culture filtrates have contained up to 80 mg per liter. Moreover, thoroughly washed mycelium from 8-day-old cultures and mature conidiospores

TABLE 1. *Antibiotic activity of malformin*

| Organism | Medium | Minimal concn for complete inhibition |
|---|---|---|
| | | *mg/liter* |
| Gram-positive bacteria | | |
| *Arthrobacter tumescens* | 3 | 10.0 |
| *Bacillus cereus* | 2 | 5.0 |
| *B. subtilis* | 1 | 2.5 |
| *Micrococcus luteus* | 3 | >10.0 |
| *M. roseus* | 3 | >10.0 |
| *Mycobacterium phlei* | 4 | 5.0 |
| *M. smegmatis* | 4 | 5.0 |
| *Nocardia corallina* | 4 | 5.0 |
| *N. rubra* | 4 | 5.0 |
| *Sarcina lutea* | 3 | >10.0 |
| *Staphylococcus aureus* | 3 | >10.0 |
| *Streptomyces griseus* | 4 | 5.0 |
| *S. venezuelae* | 4 | 5.0 |
| Gram-negative bacteria | | |
| *Achromobacter liquefaciens* | 2 | 5.0 |
| *Aerobacter aerogenes* | 2 | 5.0 |
| *Agrobacterium radiobacter* | 2 | 5.0 |
| *Escherichia coli* | 1 | 5.0 |
| *Proteus vulgaris* | 3 | >10.0 |
| *Pseudomonas aeruginosa* | 3 | >10.0 |
| *P. andropogonis* | 2 | 5.0 |
| *P. fluorescens* | 3 | >10.0 |
| *Salmonella typhosa* | 3 | >10.0 |
| *Serratia plymuthica* | 3 | >10.0 |
| *Xanthomonas stewartii* | 2 | 2.5 |
| Gram-variable bacteria | | |
| *Arthrobacter globiformis* | 2 | 5.0 |
| *Flavobacterium suaveolens* | 3 | >10.0 |

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were estimated to contain 0.06 and 0.1 mg/g (dry weight), respectively.

Because the activity of malformin in higher plants was inhibited by a number of reducing agents, thiol compounds, or SH-reagents, we suggested that malformin might prevent the action of thiol compounds essential to growth and possibly linked with auxin metabolism (S. Suda and R. W. Curtis, Plant Physiol. **39**:904, 1964). To examine inhibition of malformin as an antibiotic agent, we used *B. subtilis* because of its ability to grow in chemically defined media. The activity of malformin was completely inhibited in the presence of $10^{-2}$ M cysteine, $10^{-2}$ M glutathione, $10^{-2}$ M ascorbic acid, $10^{-4}$ M BAL (2,3-dimercapto-1-propanol), and $5 \times 10^{-2}$ M maleic hydrazide.

In general, the antibacterial activity of malformin was not high, although a few species of bacteria were fairly sensitive. The mechanism of growth inhibition in bacteria may result from an aberration in a metabolic pathway involving thiol groups but not necessarily related to auxin metabolism.

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