GROWTH REPRESSION OF HIGHER PLANTS BY 2-PYRIDINETHIOL, 1-OXIDE$^{1,2}$

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The compound 2-pyridinethiol, 1-oxide and its salts have been reported to have pronounced antibacterial and antifungal activity of a type that, were it a microbiological product, would cause it to be classed as a broad-spectrum antibiotic (5, 8). This property is shared by a number of other cyclic thiodydroxamic acids, variously substituted, though some are much more active than others. The high potency of this grouping, and the diversity of microorganisms to which compounds containing it are inhibitory, suggest interference with some essential metabolic process. Furthermore, certain antibiotics of comparable potency have been found to repress growth in some plant systems (1, 3, 4). Accordingly, the activity of 2-pyridinethiol, 1-oxide (PTO) and derivatives was tested on plants in various ways.$^3$

METHODS

Most of the procedures followed are well established in practice and not critical in detail. Those found of greatest use in quantitative comparisons were (1) a modification of the bioassay method of Ready and Grant (7) whereby the repression of elongation of the primary root of cucumber seedlings, var. Early Fortune, is determined at 25° C and 96 hours and (2) the determination of repression of weight increase in roots of young barley seedlings, var. Moore or Atlas 46, in aerated deep culture at 25° C for 6 days in the dark (2). This is essentially a study of the effect of the test compound on the conversion of endosperm to roots.

RESULTS AND DISCUSSION

Experimentation with these compounds has largely been confined to root presentation studies because it was established initially that neither droplet application of 50 or 100 μgm PTO to a unifoliolate leaf of a young bean plant nor 24-hour single leaf dipping experiments involving exposure of young plants to solutions of 10⁻⁴ or 10⁻³ M PTO caused any visible effect on subsequent top growth, growth habit or growth sequence. At the higher rates there was usually some contact injury to the tissue treated in summer temperatures. Later experiments involving exposure of the whole foliage of established seedlings did, however, result in development of some of the responses described below, but only at relatively high treatment rates.

Plant roots proved to be quite responsive to 2-pyridinethiol, 1-oxide presented as the sodium salt. Increase in root weight of barley seedlings was extensively inhibited by concentrations of only 3 to 5 μgm/ml (fig 1). The concentration effecting 50% inhibition in root growth of Atlas barley at 25° C in 6 days was only 2.7 μgm/ml (1.8 × 10⁻⁵ M). Root elongation was similarly repressed. With cucumber as the test seedling even lower concentrations caused inhibition. The concentration causing 50% reduction in length was only 1.4 μgm/ml or 9.5 × 10⁻⁶ M. These concentrations are in the same range as those found inhibitory to a number of pathogenic bacteria.

Eight analogs or derivatives of PTO prepared by the Squibb Institute were similarly subjected to the cucumber test. All had activity, but their potency

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$^2$ Paper No. 12 from the Plant Nutrition Laboratory, Michigan Memorial Phoenix Project No. 32 of the University of Michigan.
$^3$ These compounds were supplied by the Squibb Institute for Medical Research through the courtesy of Dr. A. F. Langlykke. The compound 2-pyridinethiol, 1-oxide is under test as a fungicide and has been given the name Omadine.

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**Fig. 1.** Repression of root growth of barley seedlings treated with 2-pyridinethiol, 1-oxide, Na salt. Seedlings grown for 6 days at 25° C in the dark.
varied by several orders of magnitude (fig 2). It is interesting and perhaps of some significance that the three having the most pronounced antibacterial and antifungal activity were also those most active in repressing root elongation (table I). Below this level, however, the correlation was not perfect, and the next two on the list, 2-benzylmercaptoptyridine, 1-oxide and 2-mercaptopisonicotinamide, 1-oxide, were relatively more inhibitory in the plant system than to microorganisms.

However, this does not weaken the general observation that the most active compounds of this type inhibit root growth and proliferation of microorganisms at approximately the same concentrations. No suggestion as to mode of action can yet be made; reversal studies have not been informative.

Numerous experiments were next carried out involving applications of PTO or dithiodipyridinedioxide (DTPO) to the roots of various plants. The plants were grown in vermiculite in quart plastic containers, and were supplied throughout with adequate nutrients. Applications of the chemicals were made in solution to well-established seedlings of various species, and their subsequent development carefully watched. The species included bushbean, soybean, tomato, sunflower, cucumber, corn, cress, flax, garden pea and petunia. In every case relatively low rates of application (5 to 15 mg/pot) resulted in the development of smaller or dwarfed plants. Higher rates were lethal, but the lethal thresholds were not determined. There were some differences between species in responsiveness to these compounds. Cucumbers, for example, were more sensitive than bushbeans or tomatoes. Representative series are shown in figure 3. Plants treated with sub-lethal levels did not exhibit any morphological abnormalities, nor in general was there any delay in flowering or alteration in flower development and seed set. The plants were smaller in height and leaf areas, with shorter internodes. Shortly after the application of PTO or DTPO and for some days thereafter, treated plants were observed to wilt if exposed to high light–high temperature–low humidity conditions, but if the soil moisture supply was adequate the wilt did not proceed to the point of irreversible leaf injury. Axillary bud development in decapitated plants was not affected by PTO application to the roots. Seeds harvested from bean plants dwarfed by application of PTO or DTPO in amounts up to 30 mg in the seedling stage germinated normally and in turn provided seedlings that were not smaller than the controls. It may be inferred that these com-

**Table I**

<table>
<thead>
<tr>
<th>COMPOUND</th>
<th>ANTIBACTERIAL ACTIVITY (MIC)*</th>
<th>ANTIFUNGAL ACTIVITY (MIC)*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Micrococcus variabilis</td>
<td>Klebsiella pneumoniae</td>
</tr>
<tr>
<td></td>
<td>F. var. aureus</td>
<td>F. moccus</td>
</tr>
<tr>
<td></td>
<td>Aspergillus fumigatus</td>
<td>Saccharomyces cerevisiae</td>
</tr>
<tr>
<td></td>
<td>Conc. for 50% Repression</td>
<td></td>
</tr>
<tr>
<td></td>
<td>M  µg/ml</td>
<td>µg/ml</td>
</tr>
<tr>
<td>MC 3772</td>
<td>2,2'-Dithiodipyridine-1, 1'-dioxide</td>
<td>3.4 x 10^-4</td>
</tr>
<tr>
<td>2589</td>
<td>2-Guanylmerecaptoptyridine, 1-oxide, hydrobromide</td>
<td>7.0 x 10^-4</td>
</tr>
<tr>
<td>3277</td>
<td>2-Pyridinemethyl, 1-oxide, sodium salt</td>
<td>9.5 x 10^-4</td>
</tr>
<tr>
<td>7415</td>
<td>2-Benzylmercaptoptyridine, 1-oxide</td>
<td>6.6 x 10^-4</td>
</tr>
<tr>
<td>3749</td>
<td>2-Mercaptopisonicotinamide, 1-oxide</td>
<td>3.0 x 10^-4</td>
</tr>
<tr>
<td>3830</td>
<td>4-Mercaptopurine, 1-oxide</td>
<td>1.2 x 10^-8</td>
</tr>
<tr>
<td>2588</td>
<td>2-Pyridinemethyl</td>
<td>1.4 x 10^-8</td>
</tr>
<tr>
<td>3832</td>
<td>2,2'-Thiodipyridine-1, 1'-dioxide</td>
<td>&gt; 1 x 10^-8</td>
</tr>
<tr>
<td>3863</td>
<td>(Pyridyl-1-oxide) mercaptoacetic acid</td>
<td>much &gt; 8 x 10^-8</td>
</tr>
</tbody>
</table>

* Code number and minimum inhibitory concentration (MIC) data supplied by the Squibb Institute.

**Fig. 2.** Repression of elongation of roots of cucumber seedlings by analogs or derivatives of 2-pyridine-thiol, 1-oxide. (For identification of the compounds, see table I.) Seeds incubated at 25°C for 96 hrs in the dark.
This raised the question of the synergistic effects of soil PTO, particularly in view of the antifungal and antibacterial activity of this compound. Known concentrations of PTO were added to soil samples (10 ml 1 x 10^{-2} M and downwards to 35 mg air dry soil), and incubated for various periods. The samples were transferred to a blender with 500 ml water and blended for 30 seconds. Alquots were centrifuged and subjected to bioassay using the cucumber root elongation test (7). It had previously been established that PTO concentrations of 1 μg/ml could be readily determined. However, neither immediately nor after incubation was it found possible to elute from soil sufficient PTO to cause any significant repression of root elongation even though the initial quantity applied to the soil was as high as 14.9 μg/ml soil. PTO, therefore, is quickly absorbed and firmly retained by soil colloids. DTPO was found to behave similarly.

When added to soil, and presumably absorbed, PTO is not completely inactivated with respect to roots present in the soil, but substantially larger quantities have to be added to obtain the same degree of plant response as is elicited in quarts sand or vermiculite. Even in vermiculite, however, some adsorption appears to take place. In a comparative experiment, using beans in quart containers approximately the same extent of growth repression or dwarfing was obtained by the addition of 10 mg PTO to quarts, 40 mg to vermiculite, and 100 mg to soil.

From these varied experiments it is clear that PTO and some related compounds are potent in affecting the growth of some higher plants, though they cannot be regarded as growth-regulators in the sense in which this term is applied to the substituted phenoxyacetic acids. It appears that their growth repressant effect is manifest mainly, if not exclusively, in root development and root function, and that the dwarfed tops are the resultant of the limitation of the root system.

**SUMMARY**

2-Pyridinethiol, 1-oxide and certain of its derivatives, which possess broad antibacterial and antifungal activity, have been found to be inhibitory also in certain plant systems. Root elongation and dry matter increase in cucumber and barley seedlings are repressed at low concentrations, but applications of similar concentrations to the tops of several species have not resulted in discernable responses in top growth or development. Root presentation, however, can cause a generalized dwarfing of the plant without the appearance of morphological abnormalities. Recently treated plants may wilt under high moisture stress. The normal developmental sequence is not significantly delayed. Seed from treated plants gives normal seedlings. Higher application rates are lethal. 2-Pyridinethiol, 1-oxide is strongly adsorbed by soil; the dwarfing response, which appears to arise from interference with root development and function, can best be seen in plants grown in sand or vermiculite.
The author wishes to acknowledge the able assistance of Mr. Leo Vander Beek in many of these experiments.

LITERATURE CITED

EFFECT OF CHEMICAL STRUCTURE ON THE GROWTH INHIBITION OF PLANTS WITH SOME ACID ANALOGS OF 2-MERCAPTOBENZIMIDAZOLE1,2,3,4
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Several substances structurally related to benzimidazole occur naturally in biological systems. A derivative of this compound, 5,6-dimethylbenzimidazole, constitutes part of the vitamin B12 molecule. Purines, an important component of nucleic acid structures and nucleotides, bear a structural similarity to benzimidazole. Nucleic acids play an important role in growth and appear to be associated with the synthesis of proteins by living material. Nucleotides are required for many of the metabolic processes of plants and animals. Therefore, it would appear logical that substances which might act as antagonists of purine compounds would also influence the growth of plants.

Several workers have indicated that benzimidazole is an antagonist of purine compounds. Woolley (5) noted that benzimidazole inhibited the growth of several yeasts and bacteria and observed that the inhibi-

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2 Journal Article No. 1934, Michigan Agricultural Experiment Station, East Lansing, Michigan.
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tion could be completely removed by aminopurines. Klots and Mellody (3) reported that yeast nucleic acid caused a reversal of the inhibitory effect of benzimidazole on the growth of the bacterium, Escherichia coli. Gillespie et al (2) demonstrated that 4-methoxy-6-methylbenzimidazole was an effective growth inhibitor of Tetrahymena gelii, a guanine-requiring protozoan, and also of developing embryos of the frog, Rana pipiens. By using peas as the test plant, Galston et al (1) found that benzimidazole was a metabolic antagonist of adenine and caused an inhibition of cell elongation. Recently Rebstock et al (4) demonstrated that several acid analogs of 2-mercapto benzimidazole inhibited the growth of Cranberry bean plants and root formation of cucumber seedlings.

In the present investigation the effect of chemical structure of a number of derivatives of 2-mercapto benzimidazole on the growth inhibition of Cranberry bean plants and root development in cucumber seedlings was studied. Different chemical groups were substituted in the benzene ring of the benzimidazole nucleus and in the side chain. These compounds were synthesized in this laboratory and their preparation will be reported elsewhere.

MATERIALS AND METHODS

Two methods of biological assay were employed for the evaluation of the inhibitory effects of the benzimidazole compounds upon the growth of plants. One of the assays was the bean leaf test. Seeds of the bean plant (Phaseolus vulgaris var. Cranberry) were selected for uniformity of size and planted in four-inch pots in the greenhouse. After germination, all but the uniform seedlings were removed from each