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GOUGEROTIN—AN INHIBITOR OF PROTEIN SYNTHESIS AND AUXIN-INDUCED ELONGATION IN PLANTS

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Abstract—Gougerotin, an aminoacyl nucleoside antibacterial substance, has been shown to be an effective inhibitor of auxin-induced elongation and protein synthesis in *Avena* coleoptiles.

GOUGEROTIN,‡ an aminoacyl nucleoside antibiotic isolated from *Streptomyces gougerotii*,[1] is a specific inhibitor of protein synthesis in cell-free systems from *Escherichia coli*,[2] mouse liver tissue[3] and rabbit reticulocytes.[4] Several investigators have suggested that gougerotin[4] and a number of other aminoacyl nucleosides such as puromycin,[5,6] amicetin,[7] blasticidin S[8] and homocitrullylamino adenosine[9] act by inhibiting the transfer of amino acids from aminoacyl-soluble RNA to the growing peptide chain.

In a study of the mechanism of action, Casjens and Morris[4] observed that gougerotin inhibited selectively the incorporation of amino acids into protein without affecting the release of completed protein chains from the ribosomes. They suggested that the locus of action of gougerotin was an enzyme which formed the peptide bond during the polymerization of amino acids into protein; and that the antibiotic, a structural analog of aminoacyl-soluble RNA, interreacted with the enzyme to prevent further synthesis of the peptide chain. Since a primary amino group is absent in gougerotin, no peptide bond could be formed between it and the growing peptide chain as is the case with puromycin.[5,6]

The biological activity of many antibiotics, including actinomycin D[10] chloramphenicol,[11] cycloheximide,[12] and puromycin[10,12] has been reported in plants. These antibacterial substances are of considerable value in assessing the role of protein synthesis in auxin-induced elongation and other plant responses. The mode of action of gougerotin and its specific actions in other systems is known in some detail. Consequently, this antibiotic would be a valuable compound for making additional studies of biological responses in

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plants. In this paper, the effect of gougerotin on protein synthesis and cell elongation in *Avena* coleoptiles is presented.

The *Avena* coleoptiles were prepared as described by Nitsch and Nitsch[13] using *Avena sativa* (cv. Torch). The compounds for assay, gougerotin and 10^{-5} M indole-3-acetic acid, were dissolved in a pH 5 citrate phosphate buffer[13] containing 2 per cent sucrose and 0·1 per cent Tween 80. In the elongation studies, ten coleoptiles, 4·5 mm in length, were placed in each test tube with 1·8 ml of the designated solution and incubated for the appropriate time in a revolving drum (1 rpm) at 26° in the dark and the sections were then removed and measured to the nearest 0·1 mm using a photographic enlarger.[14]

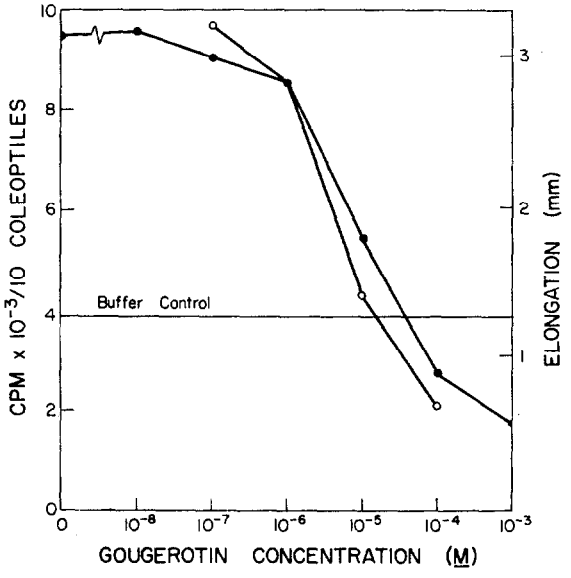

FIG. 1. EFFECT OF GOUGEROTIN ON AUXIN-INDUCED ELONGATION AND ^{14}C-LEUCINE INCORPORATION INTO THE PROTEIN FRACTION OF *Avena* COLEOPTILES.

●——● Elongation after 22 hr, o——o ^{14}C-leucine incorporation after 4 hr incubation. The buffer control elongation is also illustrated.

Protein synthesis was determined by incubating fifteen coleoptiles in the appropriate solution along with ^{14}C-leucine. After incubation the coleoptiles were rinsed with water and partially dried on several sheets of paper towels. Five coleoptiles, which were used to determine total uptake, were placed in 15 ml of scintillation fluid[15] and disrupted by sonication (Bronson Sonic Power Sonifier equipped with a micro tip) and the radiation determined. The ten remaining coleoptiles were employed to estimate the incorporation of ^{14}C-leucine into the protein fraction. In the preliminary experiments, the proteins were precipitated by trichloroacetic acid and purified by successive washings.[4] In most of the studies, a membrane filter method was utilized. The coleoptiles were sonicated in 3 ml water and the insoluble cell-wall material was removed by centrifugation. The precipitated materials were resuspended in 3 ml of water and again centrifuged. To the combined supernatants, 4 ml of 25 per cent trichloroacetic acid was added and the solution was allowed to set for 5 min. A

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white flocculent precipitate developed. The solution was heated for 15 min at 90°. The hot solution was filtered on a Millipore glass fiber filter and the precipitate was washed on the filter with 5 ml of ethanol:ether (1:1) and then with 5 ml of ether. After drying, the pad was placed in a scintillation vial, and 5 ml of scintillation fluid added. This procedure proved much less meticulous and gave results nearly identical to the previous procedure.[4]

Gougerotin was an effective inhibitor of auxin-induced elongation in *Avena* coleoptiles (Fig. 1). A concentration of 10^{-5} M gougerotin was required for 50 per cent inhibition of elongation. The time course for inhibition with a 10^{-4} M and 5×10^{-6} M solution is shown in Fig. 2. Within 1 hr, repression of elongation by 10^{-4} M gougerotin was observed, and in 2 hr the inhibition was 45 per cent and in 4 hr 57 per cent. After the final time course of 22 hr

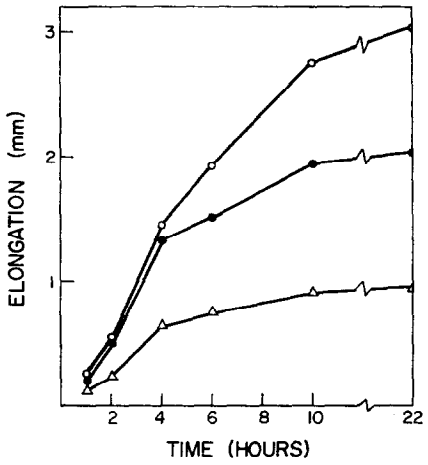

FIG. 2. EFFECT OF 5×10^{-6} M AND 10^{-4} M GOUGEROTIN ON THE KINETICS OF AUXIN-INDUCED ELONGATION OF THE *Avena* COLEOPTILE.

All incubations were run in the presence of 10^{-5} M indole-3-acetic acid. o——o Control, ●——● 5×10^{-6} M gougerotin, △——△ 10^{-4} M gougerotin.

there was 72 per cent inhibition of elongation. After an initial lag period, 5×10^{-6} M gougerotin was an effective inhibitor or elongation. The same concentrations also inhibited incorporation (Fig. 1) of ^{14}C-leucine into the protein fraction. After a 4 hr incubation period, incorporation of ^{14}C-leucine was inhibited 78 per cent by 10^{-4} M gougerotin. The ^{14}C-leucine uptake was also inhibited but to a much lesser extent than incorporation at all concentrations studied. A concentration of 10^{-5} M gougerotin was required for 50 per cent inhibition of protein synthesis.

These studies suggest that gougerotin does repress elongation and protein synthesis in plant tissue. The concentration required for inhibition was about 10 times greater than those reported for a cell-free system from *E. coli*[2] but was comparable to those reported for animal systems.[3, 4] The data are further[10] evidence that protein synthesis is an essential requirement for auxin-induced cell elongation.

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