# Hormaomycin, a New Peptide Lactone Antibiotic Effective in Inducing Cytodifferentiation and Antibiotic Biosynthesis in Some *Streptomyces* Species

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*Streptomyces,* Signal Metabolite, Hormaomycin, Cytodifferentiation, Antibiotic Overproduction, Antibiotic Activity

Hormaomycin is a novel signal metabolite from *Streptomyces griseoflavus* W-384 with aerial mycelium-inducing activity. The compound has been identified as an unusual peptide lactone. Hormaomycin displays three biological activities: First, it initiates the development of aerial mycelia in some *Streptomyces* strains. The mechanism responsible for this activity is unknown. Secondly, hormaomycin is effective in stimulating antibiotic production in different *Streptomyces* species. Thus, it is possible to get overproduction of a variety of antibiotics by the use of hormaomycin in fermentation processes. Thirdly, it inhibits the growth of some bacteria. The sensitive bacteria are restricted to coryneform taxa such as *Arthrobacter* and *Corynebacterium* which are closely related to *Streptomyces*.

## Introduction

Bacteria of the genus *Streptomyces* have evolved complicated differentiation mechanisms that include not only changes in metabolism but also changes in cellular structure. The streptomycete life cycle involves the formation of a substrate mycelium which, after a period of vegetative growth, produces an aerial mycelium. At the ends of the aerial hyphae, the cells differentiate into a chain of spores. The trigger for this morphogenetic change could be starvation; when the population begins to be depleted of an essential nutrient, formation of aerial mycelium and sporulation generally occurs. In some cases, the trigger may be build-up in the culture of some metabolite that, upon reaching a threshold level, induces morphogenesis [1]. The best studied signal metabolite is the A-factor [2-(6-methylheptanoyl)-3-hydroxymethyl-4-butanolide] from *Streptomyces griseus,* which triggers not only the formation of aerial mycelia but also the production of streptomycin [2, 3]. Additional signal molecules related in structure to the A-factor were isolated from *Streptomyces* species: Gräfe's factors [4, 5], virginiae butanolides [6, 7], and factor IM-2 [7]. Besides these butyrolactones, few other kinds of regulatory compounds were investigated. B-factor, a derivative of adenosine, stimulates rifamycin production in *Nocardia* sp. [8]. C-factor is a 34.5 kDa-protein that induces sporulation in *Streptomyces griseus* [9]. The macrodiolide pamamycin stimulates aerial mycelia formation in *Streptomyces alboniger* [10, 11].

Recently, we have isolated a novel signal metabolite from *Streptomyces griseoflavus* W-384 with aerial mycelium-inducing activity [12]. The isolated compound, named hormaomycin, has been identified as an unusual peptide lactone. The structure (Fig. 1) of hormaomycin has been elucidated [13]. The constituent amino acids are D-*allo*-threonine (1), L-isoleucine (1), L-*threo*-(3-methyl)-phenylalanine (2), and, for the first time identified from a natural source, 4-[(*Z*)-propenyl]proline (1) and 3-(2-nitrocyclopropyl)alanine (2).

Hormaomycin displays three distinct activities: The compound is effective in regulating both morphological differentiation and secondary metabolism. Additionally, it inhibits the growth of *Arthrobacter* strains. Here we describe the biological activities of hormaomycin.

## Materials and Methods

*Streptomyces* strains used in this study were taken from the culture collection of the Institut für Mikrobiologie, Tübingen. Stock cultures were maintained on SY agar by storage at 4 °C after growth at 27 °C for 5–10 days. SY agar (favoring

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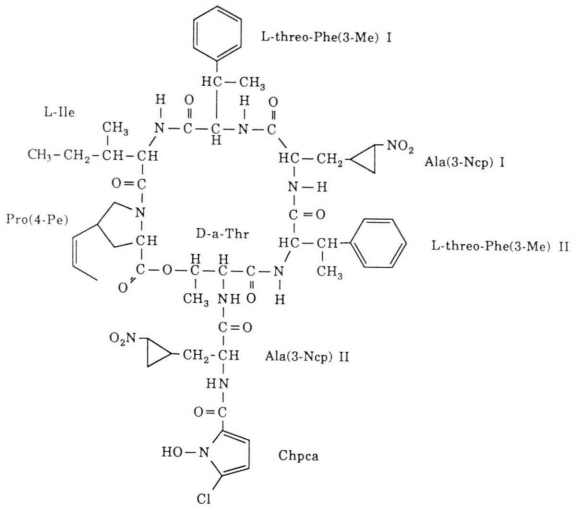


Fig. 1. Structure of hormaomycin. D-a-Thr, D-*allo*-threonine; L-Ile, L-isoleucine; L-threo-Phe(3-Me) I and II, L-*threo*-(3-methyl)phenylalanine; Pro(4-Pe), 4-[(*Z*)-propenyl]proline; Ala(3-Ncp) I and II, 3-(2-nitrocyclopropyl)alanine; Chpca, chlorine-containing N-hydroxypyrrole-2-carboxylic acid (from [13]).

aerial mycelium production) contained 2% soluble starch, 0.4% yeast extract, 2% agar; pH 7.0. SYC agar (suppressing aerial mycelium production) contained 2% soluble starch, 0.4% yeast extract, 1% Casamino acids, 2% agar; pH 7.0. Hormaomycin was isolated from mycelia of *Streptomyces griseoflavus* W-384 as described in [12].

*Sensitivity testing*

Minimal inhibitory concentrations (MICs) of hormaomycin were determined by serial dilution. The bacteria used are listed in Table I. Strains were grown under conditions and in media essentially as described [14]. The inoculum employed was approx. $10^5$ cells in a final volume of 1 ml. The lowest concentration that showed no visible growth after incubation was defined as the MIC.

*Assay for morphological activity*

For study of aerial mycelium formation, hormaomycin-impregnated paper disks were placed on SYC agar plates and inoculated with *Streptomyces* spores by streaking. After 2–5 days incubation at 30 °C, plates were examined and the powdery white zones around the disks indicating aerial mycelia were measured.

*Assay for antibiotic-inducing activity*

Selected *Streptomyces* strains were cultured in liquid SYC medium at 27 °C with shaking in the presence and absence of 0.01–1 µg/ml hormaomycin which was added to cultures with a delay of 24 h. During incubation the cultures were examined daily for growth and antibiotic production. Dry weight of cells was determined as described [15]. Antibiotic concentration was measured by a disk-diffusion test or by HPLC [12].

## Results

*Antimicrobial spectrum*

Table I lists the MIC values of hormaomycin against a series of bacteria obtained by serial dilution tests with standard bacterial inocula. Hormaomycin is very active against *Arthrobacter* strains. Other bacteria which also tend to show branching or to be irregular-shaped such as *Mycobacterium* and *Corynebacterium* species, are usually highly susceptible to this antibiotic. There is less activity against *Brevibacterium linens, Micrococcus luteus, Streptomyces cinnamomeus* and *Streptomyces collinus*. The activity against the remaining bacteria tested is very poor and most organisms are essentially resistant.

Hormaomycin was ineffective against 13 selected fungi belonging to the major classes of the zygomycetes, ascomycetes, basidiomycetes and deuteromycetes. The susceptibility was examined by the disk-diffusion test at a drug concentration up to 100 µg per disk (data not shown).

*Aerial mycelium-inducing activity*

All strains of *Streptomyces* examined were capable of producing aerial mycelium superimposed upon substrate mycelium. The aerial mycelium production, however, depended on the composition of the medium. On poor SY agar, the strains formed aerial mycelium abundantly within 2–4 days at 30 °C. On rich SYC agar (SY agar supplemented with 1% Casamino acids), aerial mycelium formation was scanty or completely suppressed during the 10 days of incubation. Under these suppressing conditions, the aerial mycelium-inducing activity of hormaomycin in these strains was assayed by the agar diffusion method at concentra-

Table I. Antibacterial spectrum of hormaomycin.

| Organism | MIC [μg/ml] |
|---|---|
| *Arthrobacter crystallopoietes* ATCC 15481 | 0.0001 |
| *Arthrobacter pascens* ATCC 13346 | 0.0001 |
| *Arthrobacter oxydans* ATCC 14358 | 0.0005 |
| *Corynebacterium insidiosum* ATCC 10253 | 0.1 |
| *Corynebacterium* spec. ATCC 23830 | 0.1 |
| *Corynebacterium rathayi* ATCC 13659 | >100 |
| *Mycobacterium "thamnopheos"* DSM 43293 | 0.1 |
| *Mycobacterium phlei* DSM 43237 | >100 |
| *Brevibacterium linens* ATCC 9172 | 10 |
| *Micrococcus luteus* ATCC 398 | 10 |
| *Streptomyces cinnamomeus* Tü 89 | 10 |
| *Streptomyces collinus* Tü 365 | 10 |
| *Streptomyces* spec. Tü 1306 | 100 |
| *Streptomyces ramocissimus* ATCC 27529 | >100 |
| *Bacillus subtilis* ATCC 6633 | 50 |
| *Bacillus megaterium* ATCC 13632 | 100 |
| *Salmonella typhimurium* ATCC 13311 | 100 |
| *Staphylococcus aureus* ATCC 11632 | 100 |
| *Aerobacter aerogenes* Tü 213 | >100 |
| *Alcaligenes faecalis* DSM 30030 | >100 |
| *Clostridium pasteurianum* ATCC 6013 | >100 |
| *Escherichia coli* Tü A 19-15 | >100 |
| *Escherichia coli* ATCC 11775 | >100 |
| *Escherichia coli* Tü pfU | >100 |
| *Erwinia amylovora* ATCC 15580 | >100 |
| *Lactobacillus casei* ATCC 7460 | >100 |
| *Propionibacterium acnes* DSM 1897 | >100 |
| *Proteus vulgaris* ATCC 13315 | >100 |
| *Pseudomonas fluorescens* ATCC 13525 | >100 |
| *Pseudomonas saccharophila* ATCC 15946 | >100 |

ATCC, American Type Culture Collection, Rockville, MD, U.S.A; DSM, Deutsche Sammlung von Mikroorganismen, Braunschweig, F.R.G.; Tü, Institut für Mikrobiologie I, Universität, Tübingen, F.R.G.

tions up to 10 μg per paper disk. It was found that 5 of 56 strains regained their full developmental capacity when exposed to small amounts of hormaomycin. A powdery white zone of aerial mycelium growth was thus created around the disk, the diameter was proportional to the amount of hormaomycin added to the disk. A typical example is shown in Fig. 2. Hormaomycin exhibits aerial mycelium-inducing activity in *Streptomyces* spec. Tü 1306 even at 0.1 μg/disk.

*Antibiotic-inducing activity*

The morphological differentiation in *Streptomyces* is often associated with the secondary metabolism [1]. In the preceding section hormaomycin was shown to induce the formation of aerial mycelium in five strains of *Streptomyces*. Consequently, one might expect that this substance would also interfere with the synthesis of secondary metabolites (*e.g.* antibiotics) in these organisms.

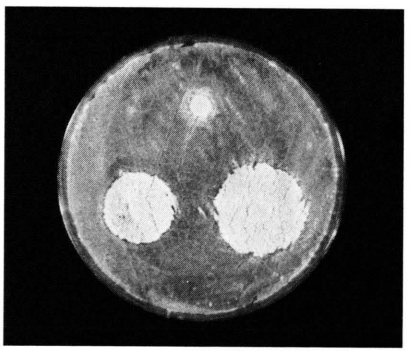

Fig. 2. Aerial mycelium-inducing activity of hormaomycin in *Streptomyces* spec. Tü 1306. The paper disks contained 0.01, 0.1 and 1 μg hormaomycin.

The five streptomycetes produce small amounts of antibacterial substances only when grown on SYC medium. Antibiotic production could be stimulated markedly in all five cases by addition of hormaomycin. An example is given in Fig. 3. *Streptomyces* spec. Tü 1306 produces the antibiotics tirandamycin A and B [16]. The concentration of hormaomycin in the medium required for stimulation ranged from 0.05 to 1 μg/ml. At 1 μg/ml, tirandamycin productivity was increased four times. Hormaomycin stimulated the antibiotic synthesis without affecting the growth rate or the yield of biomass (Fig. 4 and 5).

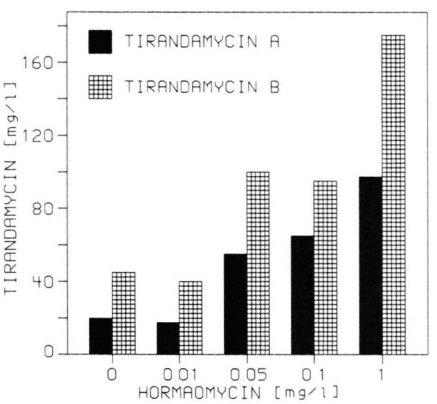


Fig. 3. Stimulation of tirandamycin A and B production by hormaomycin. *Streptomyces* spec. Tü 1306 was grown in SYC medium with or without hormaomycin. The antibiotic concentrations were determined by HPLC 160 h after inoculation.

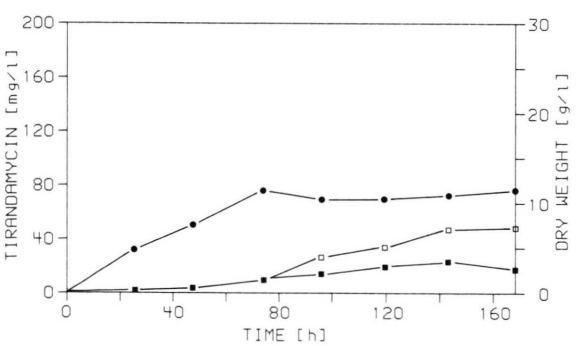


Fig. 4. Growth and tirandamycin production of *Streptomyces* in SYC medium. ●, Biomass; ■, tirandamycin A; □, tirandamycin B.

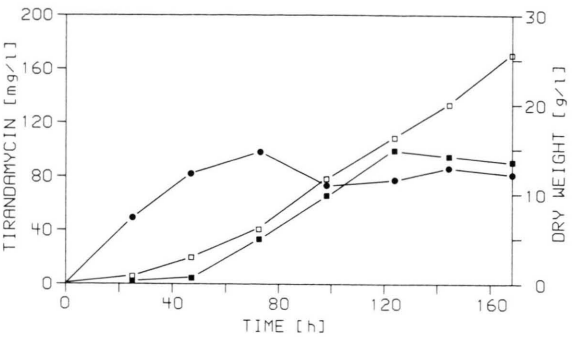


Fig. 5. Growth and tirandamycin production of *Streptomyces* in SYC medium. Addition of 1 μg/ml hormaomycin. ●, Biomass; ■, tirandamycin A; □, tirandamycin B.

## Discussion

Hormaomycin is a new signal metabolite produced by *Streptomyces griseoflavus* W-384. The compound has been identified as an unusual peptide lactone [12]. So far no compound having an approximately similar structure has been found to be a signal molecule in *Streptomyces* species. In addition, hormaomycin provides an excellent example for the structural diversity of signal molecules within these organisms.

Hormaomycin displays three biological activities. First, it initiates the development of aerial mycelia in some *Streptomyces* strains by an unknown mechanism. In the assay, aerial mycelium formation was suppressed by the addition of amino acids to a nutritionally poor medium (SY). An inhibition of the substantial uptake of amino acids into the cell provides a reasonable explanation for this morphological effect whereby re-suppression by hormaomycin may occur. Alternatively, it is interesting to speculate that hormaomycin can interfere more directly with the regulation of the morphological differentiation of sporulation.

Secondly, it stimulates the production of antibiotics in some *Streptomyces* strains. This finding is in agreement with the fact that there are common regulatory genes involved in both sporulation and secondary metabolism [1]. A-factor has also been shown to stimulate the two processes [2]. Unlike A-factor which acts species-specifically as an autoregulator, hormaomycin is effective in stimulating antibiotic production of different *Streptomyces* species. This non-specificity offers an unique possibility to get overproduction of a varie-

ty of antibiotics by the use of hormaomycin in fermentation processes.

Finally, it inhibits selectively the growth of some bacteria at extremely low concentrations. The sensitive bacteria are restricted to coryneform taxa such as *Arthrobacter* and *Corynebacterium* which are closely related to *Streptomyces* [17]. Considering the close relationships among these genera we suggest that a common target site may be responsible for the different activities of hormaomycin. Therefore, the study of the mechanism of inhibition in *Arthrobacter* which seems to be experimentally accessible can provide information that may be directly applicable to the effects of hormaomycin on the morphological and physiological differentiation in *Streptomyces*.

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