FEMS Microbiology Letters 96 (1992) 31–36


Published by Elsevier

FEMSLE 04996

# Influence of inhibitors of lipid biosynthesis on the production of avermectins in *Streptomyces avermitilis*

Tomáš Řezanka [a], Helena Míková [b] and Marie Jurková [a]

[a] *Institute of Microbiology, and* [b] *Institute of Chemical Technology, Prague, Czechoslovakia*

Received 18 March 1992
Revision received 27 May 1992
Accepted 27 May 1992

Key words: Lipid; Fatty acid; Avermectin; *Streptomyces avermitilis*

## 1. SUMMARY

*Streptomyces avermitilis* was grown in the presence of four inhibitors of fatty acid biosynthesis (*p*-chlorophenoxyacetic, DL-2-(*p*-chlorophenoxy)-propionic, 2-(*p*-chlorophenoxy)-2-methyl-propionic and *p*-fluorophenoxyacetic acids). The production of total lipids was inhibited and the cellular content of individual types of fatty acids was altered by these compounds. The anteiso-acid content decreased by up to one-half, whereas the content of iso-odd acids increased 10-fold in the presence of inhibitors. The decrease in anteiso-acids was accompanied by increased production of avermectins having a *s*-butyl group in the side chain (type 'a' avermectins). It was concluded that the common precursor, 2-methylbutyryl-CoA, was diverted from the biosynthetic pathway of fatty acids to that of avermectins.

*Correspondence to:* T. Řezanka, Institute of Microbiology, Vídeňská 1083, 14220 Prague, Czechoslovakia.

## 2. INTRODUCTION

Avermectins belong to a group of macrolides with anthelmintic and insecticidal effects [1]. Eight very similar compounds are produced by *Streptomyces avermitilis*, comprising four major ($A_{1a}$, $A_{2a}$, $B_{1a}$, $B_{2a}$) and four minor (5–10%) ($A_{1b}$, $A_{2b}$, $B_{1b}$, $B_{2b}$) components [2].

Avermectins are oligoketides and are synthesized from the same precursors as fatty acids. The similarity of their biosynthesis with that of fatty acids is illustrated by the inhibition of biosynthesis of oligoketide antibiotics by cerulenin [3]. Cane et al. [4] found that the aglycone of avermectins is formed from seven acetate, five propionate and one 2-methylbutyrate (avermectins of type 'a') or isobutyrate (type 'b') units.

Other compounds besides cerulenin interfere with lipid metabolism. They include hypolipidemic drugs, such as derivatives of halogen-phenoxy-alkanoic acids. Clofibric acid is used as a hypolipidemic agent in human medicine since it lowers the synthesis of triglycerides [5]. Compounds of this type exhibit, in addition to the

hypolipidemic action, a weak auxin effect and they are used as herbicides. They act on lipid metabolism and affect the permeability of plant cell walls [6].

Triglycerides formed by *S. avermitilis* greatly complicate the subsequent isolation and separation of avermectins. Maximum production of avermectins by *S. avermitilis* precedes maximum triglyceride production by three to four days [7]. Křen et al. [8] described how chloro-phenoxy-alkanoic acids inhibited the biosynthesis of lipids and ergot alkaloids. In the present study, similar inhibitors were used to determine the effects of decreasing triglyceride production on the production of avermectins.

## 3. MATERIALS AND METHODS

### *3.1. Strain and cultivation*

The mutant strain *Streptomyces avermitilis* C-18 obtained by genetic improvement of ATCC 31267 was used throughout this study. The strain was maintained on yeast–maltose agar. The inoculum was cultivated for 24 h in a complex medium containing: glucose, 0.5%; soyabean meal, 1.5%; yeast extract, 0.5%. Fermentation flasks were inoculated with a 2% inoculum (24 h old) and incubated for 10 days. The following synthetic production medium was used (%): glucose, 3; $CaCO_3$, 0.5; NaCl, 0.2; $K_2HPO_4$, 0.05; $FeSO_4 \cdot 7H_2O$, 0.005; $ZnSO_4 \cdot 7\ H_2O$, 0.005; $MnSO_4 \cdot 7\ H_2O$, 0.005; $MgSO_4 \cdot 7\ H_2O$, 0.01. Inhibitors were added to the synthetic production medium after sterilization in the concentration described in the text. Fermentations were performed in 500-ml round bottom flasks on a rotary shaker (2.8 Hz) at 28°C [9].

*p*-Chlorophenoxyacetic (CPAc), DL-2-(*p*-chlorophenoxy)-propionic (CPP), 2-(*p*-chlorophenoxy)-2-methyl-propionic (CPMP), (Aldrich, FRG) and *p*-fluorophenoxyacetic (FPAc) acids (Sigma, FRG) were added as filter-sterilized aqueous solutions of their sodium salts.

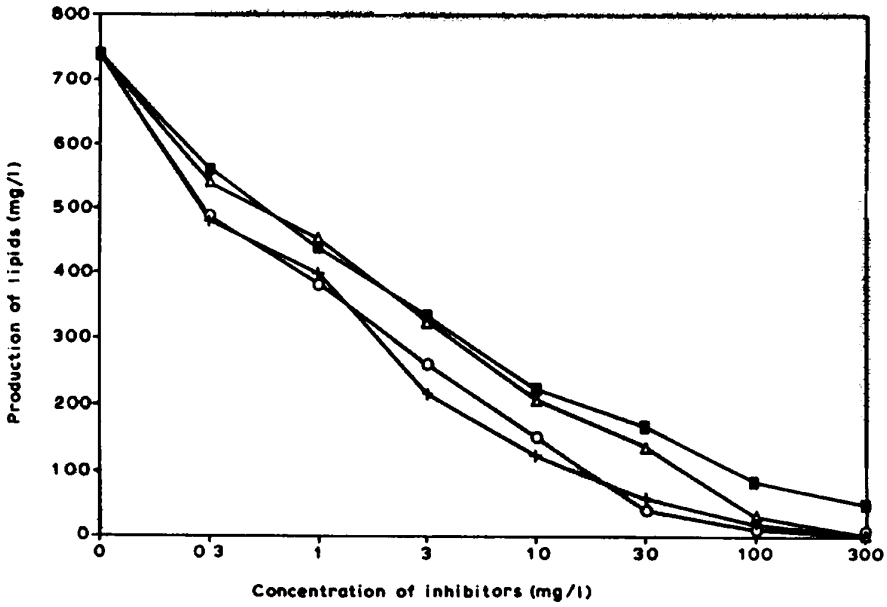


Fig. 1. Influence of inhibitors on total lipid production by *S. avermitilis* (■, *p*-chlorophenoxyacetic acid; +, DL-2-(*p*-chlorophenoxy)-propionic acid; ◇, 2-(*p*-chlorophenoxy)-2-methyl-propionic acid; △, *p*-fluorophenoxyacetic acid).

*3.2. Analytical methods*

Fatty acids composition was determined after alkaline hydrolysis and methyl-esters were analysed by GC-MS (QP 1000, Shimadzu) [10].

Production of total avermectins was determined by TLC according to Cimbůrková et al. [19]. Individual avermectins were determined by reverse phase HPLC [11].

## 4. RESULTS AND DISCUSSION

Increasing concentrations of all four inhibitors caused decreases in lipid production (Fig. 1) and parallel decreases in total avermectin production (Fig. 2). The similarity of the curves is consistent with the structures of the different inhibitors. CPAc and FPAc have an unbranched side chain whereas CPP and CPMP contain one or two methyl groups bound to the $\alpha$-carbon. These methyl groups bring about, probably for steric reasons or because of their different catabolism, different effects on fatty acid biosynthesis.

Very low concentrations of the inhibitors (of the order of mg/l) caused a decline in the production of total avermectins but a faster drop in the production of total lipids. Under circumstances where excess lipids complicate the isolation of avermectins, the inhibitors could be used to suppress lipid production, albeit with a partial lowering of avermectin production. The concentration of bacterial biomass was constant (approx. 9 g/l) up to 30–100 mg/l of the inhibitors.

The addition of any one of the inhibitors caused changes in the content of individual types of fatty acids (Table 1). The content of anteiso-acids, which arise from the precursors 2-methyl-butyryl CoA or Ile, fell by about one-half after the addition of any of the four inhibitors. Iso-even acids (precursor iso-butyryl CoA or Val) decreased as well, but by much less. The content of even-chain acids arising from acetyl CoA decreased, whereas that of odd-chain acids from propionyl CoA increased. The greatest effect, however, was the increase in the content of iso-odd acids, which rose up to 10-fold. Even the

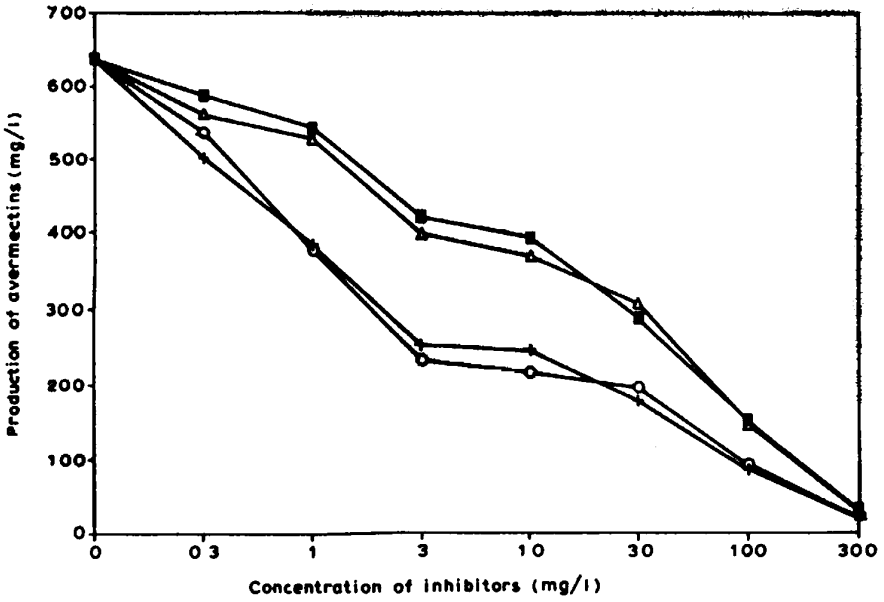


Fig. 2. Influence of inhibitors on total avermectin production by *S. avermitilis* (■, *p*-chlorophenoxyacetic acid; +, DL-2-(*p*-chlorophenoxy)-propionic acid; ◇, 2-(*p*-chlorophenoxy)-2-methyl-propionic acid; △, *p*-fluorophenoxyacetic acid).

Table 1

Content (weight %) of individual classes of fatty acids from *S. avermitilis* grown in the presence of different inhibitors

| Fatty acids | Concentration of inhibitors (mg/l) | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | 0 | 0.3 | 1 | 3 | 10 | 30 | 100 | 300 |
| **CPAC** | | | | | | | | |
| Σ iso-even | 33.4 [a] | 32.6 | 33.4 | 32.2 | 31.4 | 30.6 | 30.1 | 29.8 |
| Σ iso-odd | 1.7 | 4.4 | 6.5 | 8.8 | 10.1 | 12.4 | 17.2 | 23.6 |
| Σ anteiso | 33.9 | 32.0 | 30.3 | 29.4 | 28.0 | 25.4 | 21.1 | 16.5 |
| Σ even | 28.6 | 27.1 | 24.7 | 23.7 | 24.2 | 23.9 | 22.5 | 18.8 |
| Σ odd | 3.2 | 3.9 | 5.1 | 5.9 | 6.3 | 7.7 | 9.1 | 11.3 |
| Σ monoenoic | 9.9 | 9.8 | 9.7 | 9.5 | 9.0 | 9.8 | 9.6 | 10.0 |
| **CPP** | | | | | | | | |
| Σ iso-even | 33.4 | 31.3 | 31.3 | 30.9 | 29.7 | 28.2 | 28.1 | 25.9 |
| Σ iso-odd | 1.7 | 5.5 | 7.7 | 10.0 | 10.9 | 15.7 | 17.7 | 23.8 |
| Σ anteiso | 33.9 | 33.4 | 33.1 | 31.9 | 29.0 | 25.6 | 23.3 | 19.4 |
| Σ even | 28.6 | 22.4 | 20.8 | 20.2 | 21.1 | 19.2 | 18.0 | 16.4 |
| Σ odd | 3.2 | 3.4 | 6.1 | 7.0 | 9.3 | 11.3 | 12.9 | 14.5 |
| Σ monoenoic | 9.9 | 9.7 | 9.8 | 10.2 | 11.3 | 11.8 | 10.8 | 11.4 |
| **CPMP** | | | | | | | | |
| Σ iso-even | 33.4 | 24.7 | 23.3 | 20.2 | 19.9 | 20.0 | 20.6 | 19.8 |
| Σ iso-odd | 1.7 | 4.5 | 11.7 | 19.6 | 24.8 | 26.6 | 27.6 | 29.2 |
| Σ anteiso | 33.9 | 33.2 | 32.8 | 27.4 | 24.4 | 22.9 | 19.6 | 17.2 |
| Σ even | 28.6 | 25.2 | 25.7 | 25.0 | 21.5 | 19.8 | 20.2 | 20.4 |
| Σ odd | 3.2 | 4.4 | 6.5 | 7.8 | 9.4 | 10.7 | 12.0 | 13.4 |
| Σ monoenoic | 9.9 | 9.4 | 9.4 | 8.8 | 9.3 | 9.3 | 10.1 | 10.1 |
| **FPAC** | | | | | | | | |
| Σ iso-even | 33.4 | 26.2 | 25.4 | 25.3 | 23.0 | 22.9 | 22.8 | 22.0 |
| Σ iso-odd | 1.7 | 7.4 | 9.6 | 11.1 | 15.1 | 15.6 | 19.3 | 22.2 |
| Σ anteiso | 33.9 | 30.8 | 29.6 | 27.2 | 26.1 | 25.4 | 19.8 | 16.7 |
| Σ even | 28.6 | 28.6 | 28.2 | 27.5 | 26.4 | 26.2 | 23.5 | 22.4 |
| Σ odd | 3.2 | 7.0 | 7.2 | 8.9 | 9.4 | 9.9 | 14.6 | 16.7 |
| Σ monoenoic | 9.9 | 9.9 | 10.1 | 9.4 | 8.5 | 9.6 | 9.0 | 9.2 |

[a] Each value represents the mean from three analyses.

highest concentration of inhibitors had no effect on the content of monoenoic acids. It should be noted that the sum of all six types of acids given in Table 1 apparently exceeds 100% because monoenoic acids can be both odd and even.

Some mutants of *S. avermitilis* produce only component A (with OH on $C_5$) or 'a' (with sec-butyl on $C_{25}$, precursor Ile) [12]. The 'a' to 'b' ratio was altered by adding inhibitors of fatty acid biosynthesis (Table 2). Type 'a' avermectins decreased and type 'b' avermectins increased in the presence of inhibitors.

A study published last year [13] reported on the preparation of mutants lacking the branched-chain 2-oxo acid dehydrogenase activity. A sequel to this paper [14] concerned the preparation of 'unnatural' avermectins through the use of carboxylic acids or their precursors. The avermectins denoted 'a' ($A_{1a}$, $A_{2a}$, $B_{1a}$ and $B_{2a}$) have 2-methyl-butyryl-CoA from Ile as precursor. 'b'-

Table 2

Production (weight %) of avermectins types 'a' and 'b' by *S. avermitilis* C-18 in the presence of different inhibitors (30 mg/l)

| | Control | CPAc | CPP | CPMP | FPAc |
|---|---|---|---|---|---|
| Σ'a' | 82.5±0.8 [a] | 91.3±0.7 | 89.4±0.8 | 88.7±1.0 | 92.1±0.5 |
| Σ'b' | 17.5±1.1 | 8.7±1.2 | 10.6±0.8 | 11.5±1.2 | 7.9±0.9 |

[a] Each value represents the mean ± SD from six analyses.

Type avermectins ($A_{1b}$, $A_{2b}$, $B_{1b}$, $B_{2b}$) use isobutyryl-CoA from Val as precursor. These results allow us to conclude that the basic building unit 2-methyl-butyryl-CoA, for which the primary metabolic pathway of fatty acid synthase and the secondary metabolic oligoketide synthase (which synthesizes the aglycone of avermectins) compete, is diverted into the secondary metabolism under conditions of inhibition by halogen-phenoxy-alkanoic acids.

The reasons for the decrease in avermectin production and the influence of the inhibitors on the ratio of anteiso and iso acids are not clear. The inhibitors could influence the biosynthesis of the enzymes, the activity of the enzymes or the production (biosynthesis) of acyl-CoA precursors.

## REFERENCES


[1] Egerton, J.R., Ostlind, D.A., Blair, L.S., Eary, C.H., Suhayda, D., Ciffeli, S., Riek, R.F. and Campbell, W.C. (1979) Antimicrob. Agents Chemother. 15, 372–378.

[2] Albers-Schönberg, G., Arison, B.H., Chabala, J.C., Douglas, A.W., Eskola, P., Fisher, M.H., Lusi, A., Mrozik, H., Smith, J.L. and Tolman, R.L. (1981) J. Am. Chem. Soc. 103, 4216–4221.

[3] Omura, S. (1976) Bacteriol. Rev. 40, 681–697.

[4] Cane, D.E., Liang, T.C., Kaplan, L., Nallin, M.K., Schulman, M.D., Hensens, O.D., Douglas, A.W. and Albers-Schönberg, G. (1983) J. Am. Chem. Soc. 105, 4110–4112.

[5] Schacht, E. (1977) Top. Curr. Chem. 72, 99–123.

[6] Upchurch, R.P., (2972) Herbicides and plant growth regulators. In: Organic Chemicals in the Soil Environment (Goring, C.A.I. and Hamaker, J.W., Eds.), pp. 443–512. Marcel Dekker, New York, NY.

[7] Novák, J., Řezanka, T., Koza, T. and Vaněk, Z. (1990) FEMS Microbiol. Lett. 70, 291–294.

[8] Křen, V., Pažoutová, S., Řezanka, T., Víden, I., Amler, E. and Sajdl, P. (1990) Biochem. Physiol. Pflanzen 186, 99–108.

[9] Cimburková, E., Zima, J., Novák, J. and Vaněk, Z. (1988) J.basic Microbiol. 28, 491–499.

[10] Řezanka, T., Reichelová, J. and Kopecký, J. (1991) FEMS Microbiol. Lett. 84, 33–36.

[11] Čurdová, E., Jechová, V., Zima, J. and Vaněk, Z. (1989) J. Basic Microbiol. 29, 341–346.

[12] Omura, S., Ikeda, H. and Tanaka, H. (1991) J. Antibiot. 44, 560–563.

[13] Hafner, E.W., Holley, B.W., Holdom, K.S., Lee, S.E., Wax, R.G., Beck, D., MacArthur, H.A.I. and Wernau, W.C. (1991) J. Antibiot. 44, 349–356.

[14] Dutton, C.J., Gibson, S.P., Goudie, A.C., Holdom, K.S., Pacey, M.S., Ruddock, J.C., Bu'Lock, J.D., Richards, M.K., (1991) J. Antibiotics 44, 357–365.