Inhibition of *de novo* Fatty-Acid Biosynthesis in Isolated Chloroplasts by Different Antibiotics and Herbicides

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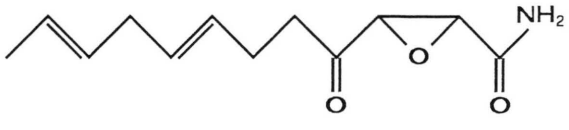
Cerulenin, *de novo* Fatty-Acid Biosynthesis, Isolated Chloroplasts, Thiolactomycin

Two natural antibiotics, cerulenin and thiolactomycin, were tested for their inhibitory efficacy on *de novo* fatty-acid biosynthesis of chloroplasts isolated from oat and spinach seedlings and compared with that of known herbicides. With both antibiotics a strong dose-dependent inhibition of the incorporation of [1-^{14}C]acetate into the fatty-acid fraction of the isolated plastids was detected. The I_{50}-values for the inhibition of acetate incorporation into fatty acids are about 4 μM in the case of thiolactomycin and about 50 μM in the case of cerulenin for both mono- and dicotyledonous plants. These values are much higher than those of the particular graminicides cycloxydim and diclofop (0.15 and 0.1 μM), which were developed to control grass weeds in dicotyledonous crop cultures.

Introduction

Cerulenin and thiolactomycin (for chemical structure see Fig. 1) are the only antibiotics hitherto known to inhibit fatty-acid biosynthesis. Cerulenin isolated from the culture filtrate of *Cephalosporium caerulens* [1] blocks 3-oxoacyl-ACP synthase from different organisms like some bacteria, yeast, *Euglena,* rat liver and higher plants [2–4]. Thiolactomycin, an antibiotic isolated from *Nocardia* sp. [5], was found to inhibit ACP-acetyl transferase as well as 3-oxoacyl-ACP synthase in *E. coli* [6]. An inhibition of *de novo* fatty-acid biosynthesis was also described in higher plants [7]. The inhibition of fatty-acid biosynthesis seems to be reversible in the case of thiolactomycin [6], whereas cerulenin is supposed to bind covalently to the enzyme [1]. In order to elucidate the effective concentrations and I_{50}-values of these antibiotics as inhibitors of *de novo* fatty-acid biosynthesis we studied their effect on [^{14}C]acetate incorporation into the total fatty-acid fraction of isolated oat and spinach chloroplasts. These results were compared with those obtained with cycloxydim and diclofop, which are known to selectively block acetyl-CoA carboxylase at low concentrations [8, 9].

CERULENIN



THIOLACTOMYCIN

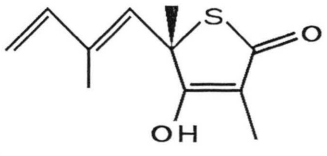


Fig. 1. Chemical structure of the antibiotics cerulenin and thiolactomycin.

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Materials and Methods

Seedlings of *Avena sativa* L. var. *flämmingnova* and *Spinacia oleracea* L. var. *matador* were cultivated and intact chloroplasts were isolated as previously described [9]. The incubation with [1-^{14}C]acetate was carried out for 20 min in an iso-osmotic buffer system containing 300 mM sorbitol, 50 mM tricine, 50 mM phosphate (pH 7.9), 30 mM $NaHCO_3$, 2.5 mM DTE, 2 mM ATP, 0.5 mM CoA, 0.5 mM $MgCl_2$, 35 μM [1-^{14}C]acetate (37 kBq per sample) and chloroplasts with a chlorophyll content of *ca.* 100 μg per ml. Light intensity was 1400 $\mu mol \cdot m^{-2} \cdot s^{-1}$. Saponification, acidification, extraction of fatty acids and the measurement of the incorporated radioactivity have been described before [9].

Results and Discussion

The antibiotics cerulenin and thiolactomycin inhibited the *de novo* fatty-acid biosynthesis (incorpo-

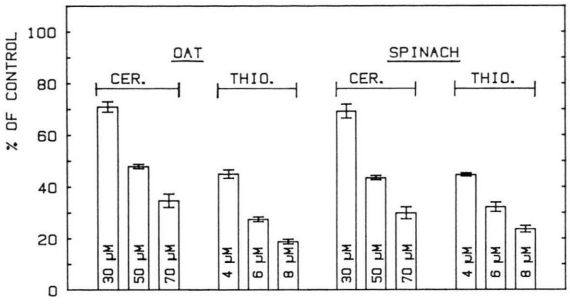

Fig. 2. The dose-dependent inhibition by cerulenin (Cer.) and thiolactomycin (Thio.) of *de novo* fatty-acid biosynthesis of isolated oat and spinach chloroplasts.

ration of [^{14}C]acetate into the total fatty-acid fraction) of chloroplasts isolated from oat and spinach seedlings in a dose-dependent manner (Fig. 2). The extent of inhibition by thiolactomycin was stronger (I_{50}-value: ~ 4 µM) than by cerulenin (I_{50}-value: ~ 50 µM). This is in close agreement with results obtained by other authors with higher plants [1, 7] as well as with *E. coli* [6]. Preincubation of the isolated chloroplasts with cerulenin resulted in an enhanced inhibitory effect of this antibiotic (Fig. 3). In isolated oat and spinach chloroplasts (Fig. 4), the I_{50}-value for the inhibition of *de novo* fatty-acid biosynthesis by cerulenin after a preincubation of 30 min decreased

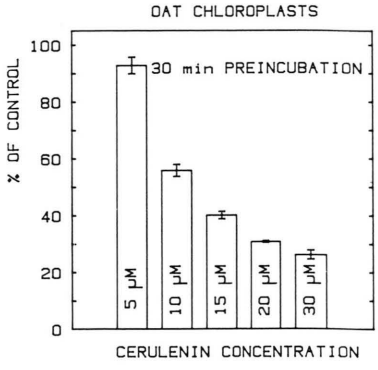

Fig. 4. Inhibitory effect of different cerulenin concentrations at a constant preincubation period (30 min) on [^{14}C]acetate incorporation into fatty acids of isolated oat chloroplasts.

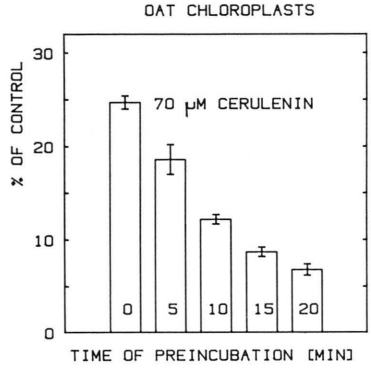

Fig. 3. Effect of cerulenin on [^{14}C]acetate incorporation into fatty acids of isolated oat chloroplasts after different times of chloroplast preincubation with cerulenin.

Table I: I_{50}-values for the inhibition by various antibiotics and herbicides of [^{14}C]acetate incorporation into the fatty-acid fraction of isolated chloroplasts of a dicotyledonous (spinach) and a monocotyledonous plant (oat).

	I_{50}-value [µM]		Target
	Spinach	Oat	
Antibiotics			
Thiolactomycin	4	4	ACP-acetyl transferase; 3-Oxoacyl-ACP synthase [6]
Cerulenin	50	50	3-Oxoacyl-ACP synthase [2–4]
Herbicides			
Cyclohexanedione derivatives			
Cycloxydim	no inhibition*	0.15	Acetyl-CoA carboxylase [8, 9]
Clethodim	no inhibition*	0.15	Acetyl-CoA carboxylase [9]
Sethoxydim	no inhibition*	0.5	Acetyl-CoA carboxylase [8, 9]
Alloxydim	no inhibition*	2.0	Acetyl-CoA carboxylase [11]
Diphenoxy-propionic acid derivatives			
Diclofop	>100	0.1	Acetyl-CoA carboxylase [10]
Fenoxaprop	>100	0.1	Acetyl-CoA carboxylase [10]
Haloxyfop	>100	0.3	Acetyl-CoA carboxylase [10]
Fluazifop	>100	3.0	Acetyl-CoA carboxylase [10]

* At a concentration of 200 µM.

to 10 μM. This time-dependent inhibition was expected since cerulenin seems to bind covalently and irreversibly to the enzyme 3-oxoacyl-ACP synthase, a process which takes some time to be completed [1, 3]. In contrast, preincubation of the plastids with thiolactomycin had no effect on the extent of inhibition, since thiolactomycin binds reversibly to the presumable targets ACP-acetyl transferase and 3-oxoacyl-ACP synthase [6]. In contrast, the selective grass herbicides of the cyclohexanedione type, *e.g.* cycloxydim, and the diphenoxy-propionic acid type, *e.g.* diclofop, specifically developed to control grass weeds in dicotyledonous crop plants, inhibit *de novo* fatty-acid biosynthesis by affecting the acetyl-CoA carboxylase, the key enzyme of *de novo* fatty-acid biosynthesis [8, 9]. These herbicides selectively inhibit the *de novo* fatty-acid biosynthesis of grasses (*e.g.* oat) with much lower I_{50}-values for inhibition of fatty-acid biosynthesis of about 0.15 and 0.1 μM (Table I). From our results it appears that the inhibitors of the starting enzymes of a biosynthetic pathway (here ACC) possess lower I_{50}-values than inhibitors of enzymes of the fatty-acid synthetase complex.

Acknowledgements

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