Effect of Different Cyclohexane-1,3-dione Derivatives on the *de novo* Fatty-Acid Biosynthesis in Isolated Oat Chloroplasts

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In the test system of isolated oat chloroplasts various structurally different cyclohexane-1,3-dione derivatives were investigated for their inhibitory effect on *de novo* fatty-acid biosynthesis. Cycloxydim proved to be the most efficient inhibitor in the group of the tested cyclohexane-1,3-diones. The alkoxyimino side-chain appears to be essential for the herbicidal activity. Compounds with variations in other substituents of the cyclohexanedione structure were less inhibitory. The I_{50}-values of most of the applied substances for a 50% inhibition of *de novo* fatty-acid biosynthesis were in the range of 0.15 µM to 100 µM. Some compounds, however, showed no inhibitory effect.

Introduction

In the early observations on the effect of the cyclohexane-1,3-dione derivative sethoxydim on plants it was found to inhibit biogenesis, development and replication of chloroplasts including accumulation of photosynthetic pigments [1]. Sethoxydim did not block the biosynthesis of prenyllipids (*e.g.* chlorophylls, carotenoids, sterols) but only their accumulation [2]. This observation pointed to a sethoxydim target outside the prenyllipid metabolism. The first hint that the target was the plant's lipid and fatty-acid metabolism came from findings in 1984 that sethoxydim inhibits the accumulation of plant glycerolipids in sensitive maize seedlings [3]. This inhibition was then shown to be due to a particular block of the *de novo* fatty-acid and glycerolipid biosynthesis in chloroplasts [2, 4]. In the higher plants, chloroplasts are known to be the only site for fatty-acid biosynthesis in the plant cell [5] and its inhibition must also affect glycerolipid biosynthesis and accumulation.

The exact target of these herbicides, the acetyl-CoA carboxylase (ACC) of grasses, was detected in 1987 in an independent work in four laboratories [6–9]. The particular sensitivity of many members of the Poaceae family towards cyclohexanedione derivatives, whereby other monocotyledonous and the dicotyledonous plants are resistant, was pointed out in a more detailed work with whole plants and isolated chloroplasts [10].

The few cyclohexanedione derivatives tested hitherto exhibit differential inhibitory activities. In order to obtain more information on the relations between chemical structure and herbicidal activity we screened several structurally related cyclohexanedione derivatives with respect to their inhibition effect on *de novo* fatty-acid biosynthesis (*via* incorporation of [1-^{14}C]acetate into the total fatty-acid fraction) of isolated oat chloroplasts.

Materials and Methods

Oat (*Avena sativa* L. var. Flämingsnova) seedlings were cultivated on a mineral containing peat (TKS II, Fa. Floratorf) in the light (light intensity: $1500 \cdot \mu mol \cdot m^{-2} \cdot s^{-1}$) in a 14/10 h day/night rhythm. Chloroplasts were isolated from primary leaves of 7 d old plants with an isolation medium, described by Kobek *et al.* 1988 [10]. The incubation with [1-^{14}C]acetate was carried out as described in [10], except that the final incubation volume was 0.5 ml. After a 20 min incubation period during which the incorporation rate of acetate into fatty acids was linear, the lipids were saponified with KOH for 90 min at 80 °C, then acidified with H_2SO_4. The fatty acids and non-acyllipids were extracted with light petrol (b.p. 50–70 °C). After evaporation of the light petrol, the radioactivity of the fatty-acid fraction was measured in a liquid scintillation counter. Separation of the lipid fraction by TLC revealed that more than 97% of the

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radioactivity taken up into the lipid fraction was incorporated into the fatty-acid fraction [10].

Results and Discussion

The inhibitory effect of the investigated cyclohexanedione derivatives on fatty-acid biosynthesis of isolated oat chloroplasts varied strongly depending on small variations in the chemical structure. The I_{50}-values for inhibition of *de novo* fatty-acid biosynthesis of isolated oat chloroplasts varied between 0.15 µM (cycloxydim) and > 100 µM (compound D 1). Some substances had no significant inhibition effect even at high concentrations of 100 or 150 µM (Table I). The most effective substances as inhibitors of *de novo* fatty-acid biosynthesis were cycloxydim and clethodim (Fig. 1 and 2), which were selected for commercial development by BASF AG [11] and Chevron Chemical Company respectively. They were more efficient in their inhibitory effect than the early cyclohexane-1,3-dione grass herbicides alloxydim and sethoxydim. In a screening experiment with whole plants of several cyclohexane-1,3-diones also cycloxydim was proved to be the substance with the best herbicidal activity [12]. This was confirmed by us for clethodim also. Thus the herbicidal effects on the whole plant and on the chloroplast level are identical.

Table I. I_{50}-values of different cyclohexane-1,3-dione derivatives for the inhibition of *de novo* fatty-acid biosynthesis of isolated oat chloroplasts arranged according to decreasing inhibitory efficiency. Mean of at least 6 determinations from two chloroplast isolations. The percentage standard deviation (SD in %) is indicated. * In these cases the I_{50}-values and SDs are based upon 12 repetitions from 4 chloroplast isolations. (n.i. = no significant inhibition at a concentration of ⩾ 100 µM.)

Substance	I_{50}-value [µM]	SD [%]
Cycloxydim*	0.15	(4)
Clethodim*	0.15	(4)
B 2 (BAS 202378)	0.2	(7)
A 1 (BAS 215032)	0.5	(12)
Sethoxydim*	0.5	(5)
E 1 (BAS 216991)	0.9	(8)
C 2 (BAS 154272)	1.0	(7)
A 2 (BAS 215040)	1.1	(18)
Alloxydim*	2.9	(5)
C 1 (BAS 211725)	2.9	(7)
E 2 (BAS 216996)	5.2	(16)
B 1 (BAS 215003)	8.0	(21)
C 3 (BAS 212348)	10.0	(14)
D 1 (BAS 213757)	>100	–
D 2 (BAS 213756)	n.i.	–
M 1-S	n.i.	–
F 1	n.i.	–
F 2	n.i.	–
G 1	n.i.	–
G 2	n.i.	–

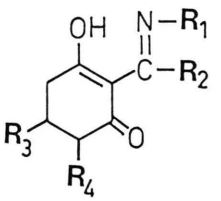
With respect to sethoxydim the ring formation of the substituent R_3 (for nomenclature see Fig. 1) as in cycloxydim improved the herbicidal activity. Replacement of the sulphur by an oxygen atom in the R_3-substituent of cycloxydim diminished the efficiency (product C 2). Other ring-shaped R_3-substituents (C 1, C 3) were even less efficient than the compound C 2. Another cyclohexanedione (compound D 2), which differs from cycloxydim in position R_3 and R_4, had absolutely no effect on fatty-acid biosynthesis.

With respect to cycloxydim a prolongation of the R_1 side chain as found in substance B 2 did not substantially change the efficacy of the substance, but branching of this R_1 side chain (compound B 1) significantly diminished the inhibitory effect on *de novo* fatty-acid formation. The importance of the R_1 side chain for the inhibitory effect on *de novo* fatty-acid biosynthesis was furthermore demonstrated by varying the structure of this side chain at the sethoxydim molecule. A prolongation of the R_1-substituent as seen in compound A 1 had no significant effect on the herbicidal activity of the product. This is also shown in a comparison of cycloxydim with substance B 2. Replacement of the end-standing methyl group of compound A 1 by a chloride atom (both in *trans*-position) as *e.g.* in clethodim enhanced the inhibitory effect on *de novo* fatty-acid formation. In the case of clethodim and the compounds A 1 and B 2 the *trans*-configuration of the R_1 side chain was applied. The corresponding *cis*-isomers seem to have much less herbicidal activity and were not tested here. Insertion of an aromatic ring system into the side chain at position R_1 (compound A 2), however, diminished the inhibitory efficiency. Elimination of the ethoxy group in the R_1 side chain led to an absolute loss of herbicidal activity (substance M 1-S). Replacement of the R_3 side chain of clethodim by aliphatic ring systems as in compounds E 1 and E 2 (see Fig. 2) diminished the herbicidal effect. The side chain at position R_2 of all the tested compounds was either an ethyl- or propyl rest, but this variation did not

seem to have much influence on the herbicidal efficacy of the substances.

Alloxydim was the first commercially available grass herbicide of the cyclohexanedione type [13], but its I_{50}-value for inhibition of fatty-acid biosynthesis in the oat chloroplast test system was relatively high (2.9 μM, see Table I). Substances with variations in R_4 (compound D 1) or in R_1 and R_4 (compound D 2) as compared to alloxydim had very low (D 1) or not effect (D 2) on fatty-acid biosynthesis. Also other alloxydim-like cyclohexanediones without substituents at R_2 and R_4 (F 1, F 2, see Fig. 2) had no effect on *de novo* fatty-acid biosynthesis.

Finally, substances containing an oxygen atom and an additional double bond instead of a C-atom in the cyclohexanedione ring (G 1, G 2, Fig. 2) did not affect *de novo* fatty-acid biosynthesis. The substances F 1, F 2 as well as G 1 and G 2 are known to be rather weak inhibitors of the photosynthetic electron transport [14], with no effect, however, on *de novo* fatty-acid formation as shown here. Within the 20 min incubation period of [^{14}C]acetate incorporation into fatty acids these



	R_1	R_2	R_3	R_4
sethoxydim	$-O-C_2H_5$	$-C_3H_7$	$H_5C_2-S-\underset{\displaystyle CH_3}{CH}-CH_2-$	$-H$
clethodim	$-O-CH_2-CH=CH-Cl$ (t)	$-C_2H_5$	"	$-H$
A 1	$-O-CH_2-CH=CH-CH_3$ (t)	$-C_2H_5$	"	$-H$
A 2	$-O-CH_2-C_6H_4-Cl$	$-C_2H_5$	"	$-H$
M1-S	$-H$	$-C_3H_7$	"	$-H$
cycloxydim	$-O-C_2H_5$	$-C_3H_7$		$-H$
B 1	$-O-\underset{\displaystyle CH_3}{CH}-C_2H_5$	$-C_3H_7$	"	$-H$
B 2	$-O-CH_2-CH=CH-CH_3$ (t)	$-C_2H_5$	"	$-H$
C 1	$-O-C_2H_5$	$-C_3H_7$		$-H$
C 2	$-O-C_2H_5$	$-C_3H_7$		$-H$
C 3	$-O-C_2H_5$	$-C_2H_5$		$-H$
alloxydim	$-O-CH_2-CH=CH_2$	$-C_3H_7$	$-CH_3$ *	$O=\underset{\displaystyle |}{C}-O-CH_3$
D 1	$-O-CH_2-CH=CH_2$	$-C_2H_5$	$-CH_3$ *	$CH_3-\underset{\displaystyle |}{C}=N-O-CH_3$
D 2	$-O-C_2H_5$	$-C_2H_5$	$-CH_3$ *	$CH_3-\underset{\displaystyle |}{C}=N-O-CH_3$

Fig. 1. Chemical structure of different cyclohexanedione derivatives, classified according to corresponding side chains and substituents. * In the case of alloxydim and the compounds D 1 and D 2 there are two methyl groups at the position 3 of the cyclohexanedione ring. t = *trans*-configuration.

four compounds neither significantly lowered fatty-acid biosynthesis nor the photosynthetic rates at the applied concentrations (up to 100 μM). Fatty-acid biosynthesis in isolated chloroplasts is dependent on the photosynthetic production of ATP and NADPH [10], which can be decreased by inhibitors of the photosynthetic electron transport chain. For this reason we also tested the effect of the substances F1, F2, G1, G2 in our isolated etioplast test system [15], where *de novo* fatty-acid biosynthesis is independent of the light and the photosynthetically produced ATP and NADPH. In the etioplast test system NADPH apparently derives from the oxidative pentosephosphate path-

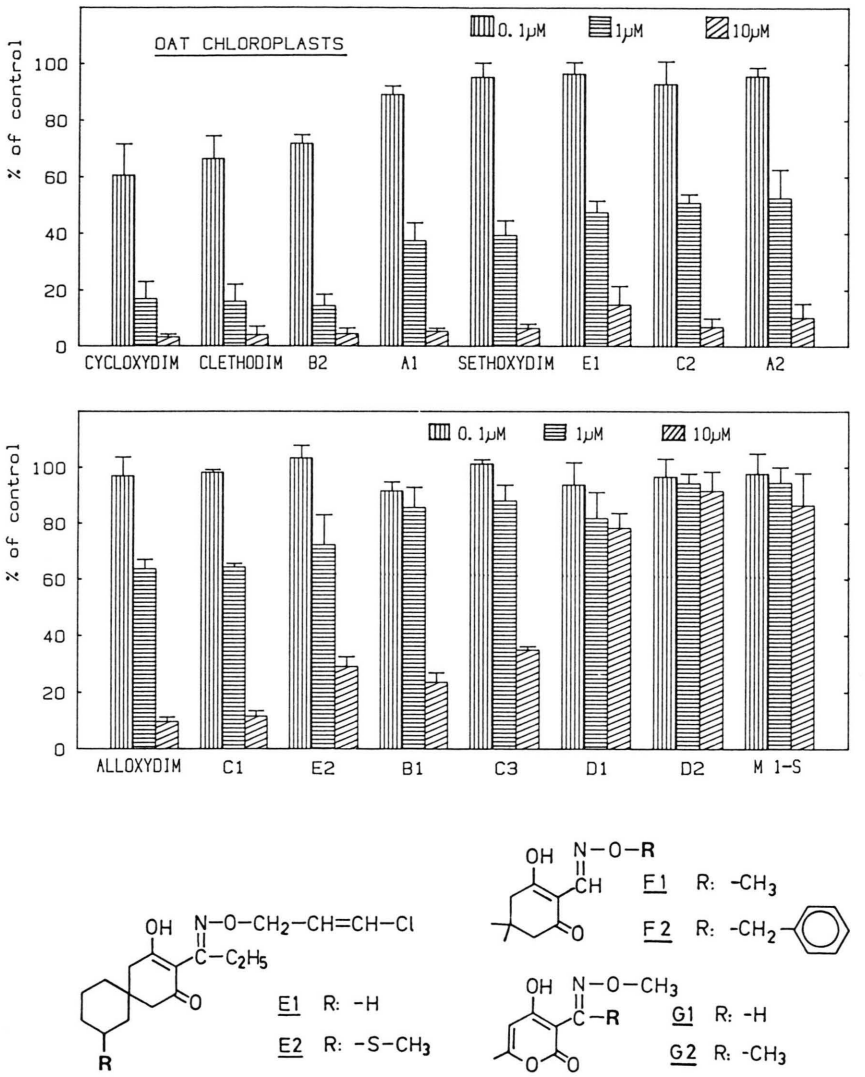


Fig. 2. *Upper part:* Effect of some cyclohexane-1,3-dione derivatives on the *de novo* fatty-acid biosynthesis of isolated oat chloroplasts. Applied were 37 kBq [^{14}C]acetate (= 35 μM acetate) per sample. The chlorophyll content amounted to 40–60 μg a + b per sample. Incorporation rates of controls (without inhibitor) were 12 ± 2.5 kBq per mg a + b in the 20 min linear incubation period. Mean values of 6 determinations from two chloroplast isolations with standard deviations. *Lower part:* Chemical structure of the cyclohexanediones E1, E2, F1, F2, G1 and G2. * In the case of E1 and E2 the chlorine atom is situated in the *trans*-configuration.

way and ATP from substrate-bound phosphorylation which proceed within the plastid. Also there we found no inhibition of fatty biosynthesis by the compounds F1, F2, G1 and G2. This indicates that structurally related herbicidal substances may have quite different targets such as photosynthetic electron transport or *de novo* fatty-acid biosynthesis.

The data presented in this work shows that many cyclohexane-1,3-dione derivatives are very strong inhibitors of *de novo* fatty-acid biosynthesis. The I_{50}-values for inhibition of *de novo* fatty-acid biosynthesis are low and amount to only 0.15 μM for the most active substances (cycloxydim, clethodim). The side chains which appear to be most important for the efficiency of cyclohexanediones are those at positions R_1 and R_3. The aminoalkoxy group in R_1 seems to be essential for herbicidal efficacy, since M 1-S, a product similar to sethoxydim, but without the ethoxy group, is absolutely inactive.

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