Inhibition of Very-Long-Chain Fatty Acid Formation by Indanofan, 2-[2-(3-Chlorophenyl)oxiran-2-ylmethyl]-2-ethylindan-1,3-dione, and Its Relatives

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Indanofan and its analogs inhibited the elongation of stearoyl- or arachidoyl-CoA by [2-^{14}C]-malonyl-CoA in leek microsomes from *Allium porrum*. Although the precise mode of interaction of indanofan at the *molecular* level is not completely clarified by the present study, it is concluded that indanofan and analogs act as inhibitor of the elongase enzyme involved in *de novo* biosynthesis of fatty acids with an alkyl chain longer than C_{18}, called very-long-chain fatty acids (VLCFAs). For a strong inhibition of VLCFA formation chloro substituents at the benzene ring and the oxirane group were necessary. Furthermore, the greenhouse test showed strong activity for indanofan and its analogs, and the scores coincided with cell-free elongation inhibition. The cell-free assay, however, failed to indicate any activity for an analog having a methylene instead of the oxirane group, while both *Digitaria ciliaris* and *Echinochloa oryzicola* were killed with 1 kg a.i./ha. This finding cannot be discussed because the applied use rate of 1 kg a.i./ha is too high to allow for a score differentiation. For high concentrations of this compound additional unknown inhibitory effects may be involved besides fatty acid elongation.

Introduction

Tridiphane, an oxirane compound, shows phytotoxic activity (Zorner *et al*., 1986). It conjugates with reduced glutathione (GSH) catalyzed by glutathione *S*-transferase (GST), and the conjugate inhibits the enzyme (Lamoureux and Rusness, 1986). A synergistic effect to atrazine is observed, since this herbicide cannot be detoxified anymore due to lack of GSH and inhibited GST. Tridiphane, however, includes five chlorines in the molecule which prompted researchers to develop oxiranes with less chlorine. Hosokawa *et al*. (2001) introduced indanofan, a herbicidal oxirane compound, with apparently more favorable environmental properties (for structures see Table I). Indanofan is phytotoxic against gramineous weeds in paddy rice. A 100% kill for *Echinochloa oryzicola* was found with 100 to 150 g a.i./ha.

In this paper, we report on a direct phytotoxic mode of action of both tridiphane and indanofan, namely the inhibition of very-long-chain fatty acid formation by these compounds and some analogs. The mode of action is similar to chloroacetamide herbicides (Matthes *et al.*, 1998).

Materials and Methods

The cell-free assays of this study are based on the incorporation of [2-^{14}C]malonyl-CoA into stearoyl-(C18:0)-CoA or arachidoyl-(C20:0)-CoA, as indicated in Table I. All labeled chemicals were purchased from Amersham, Braunschweig, Germany. Isolated microsomes from leek seedlings were used (for details see Schmalfuß *et al*., 2000; Takahashi *et al*., 2001). Indanofan and its analogs were prepared according to Hosokawa *et al*. (2001).

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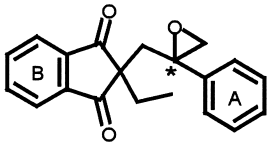
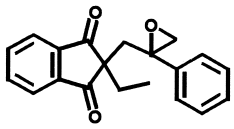
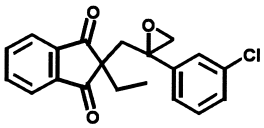
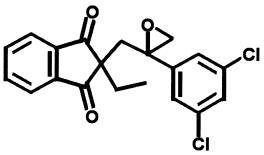
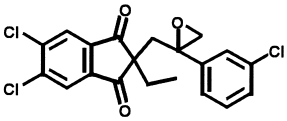
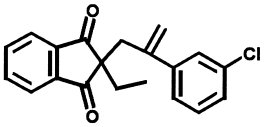
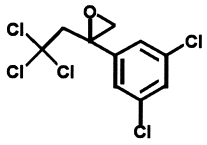
Biological test under paddy condition

Herbicidal activity under paddy condition was evaluated in greenhouse pot tests. At first *Digitaria ciliaris* and *Echinochloa oryzicola* were tested with 1.0 kg a.i./ha. Pots filled with light clay soil were watered to establish a paddy condition with 3 cm water depth. Weeds were seeded into the soil to less than 1 cm depth and rice plants were transplanted. The compounds were applied as flowable formulation to the water surface five days after seeding. Three weeks after treatment, herbicidal activities were evaluated and rated by scores 0 to 10 (0=no effect, 10=complete kill). In the second screening *Echinochloa oryzicola* was tested with a rate of 0.063 kg a.i./ha. The compounds were ap-

Table I. Inhibition of VLCFA elongation and herbicidal activity by indanofan and its relatives.



Compounds No.	Structure	Herbicidal activity *Digitaria ciliaris*	Herbicidal activity *Echinochloa oryzicola*		Inhibition of VLCFA elongation (% of control) C18:0 <upper row>, C20:0 <lower row> Leek (*Allium porrum*)		
		1.0 kg/ha	1.0 kg/ha	0.063 kg/ha	0.1 μM	1 μM	10 μM
1		8	9	8	– 9 (± 11)	– 43 (± 0)	– 81 (± 1)
2 (Indanofan)		10	10	10	31 (± 10) 49 (± 1)	44 (± 6) 83 (± 1)	58 (± 1) 95 (± 1)
3	Indanofan *S*-enantiomer	10	10	10	47 (± 2) –	57 (± 5) –	63 (± 6) –
4		9	10	8	– 44 (± 1)	– 83 (± 2)	– 94 (± 1)
5		5	10	8	– 46 (± 5)	– 86 (± 5)	– 93 (± 3)
6		10	10	5	– 0 (± 1)	– 2 (± 1)	– 7 (± 6)
7 (Tridiphane)		10	10	7	16 (± 9) 12 (± 9)	28 (± 18) 44 (± 19)	53 (± 1) 72 (± 3)

* Chiral carbon.

plied ten days after seeding. Four weeks after treatment phytotoxicity was evaluated by a 0 to 10 rating system (Hosokawa *et al.*, 2001).

Results and Discussion

Inhibition of acyl-CoA elongation using microsomes from leek seedlings

Compounds **1** and **7 (**tridiphane**)** inhibited C20:0 elongation with an approx. I_{50} value of 10^{-6} M (Table I). The I_{50} values of compounds **2** (indanofan), **4** and **5** were found around 10^{-7} M. Compounds **2** and **7** affected C20:0 elongation more strongly than C18:0 elongation, possibly indicating that these compounds inhibited an elongase II more strongly than elongase I (see Schmalfuß *et al.*, 2000, for the possible existence of two elongases in leek microsomes). It should also be noted that compound **3**, the *S*-enantiomer of indanofan, inhibits C18:0 elongation somewhat better than no. **2**, the racemic indanofan. Influence of chirality has also been found with chloroacetamides (Matthes *et al.*, 1998).

Chloro substituents at the benzene ring are important for strong inhibition as well as the oxirane group. Replacement of the latter by a methylene group (compound **6**) did not result in an active compound. An additional 4,5-dichlorination at the indandione moiety (compound **5**) did not improve activity further. The I_{50} value for arachidoyl-CoA elongation can be estimated as 10^{-7}M just like that for indanofan. Changing the trichloromethyl group of tridiphane to an indan-1,3-dione moiety increased inhibitory activity about 10-fold (compare compounds **7** and **4**). Compound **1** without any chlorine had about the same activity as compound **7** (tridiphane).

Herbicidal activity and elongation inhibition

The greenhouse test showed strong activity for compounds **1**–**5** (Table I). The scores coincided with cell-free elongation inhibition. This was particularly evident with *Echinochloa*, while the herbicidal effect of compound **5** was found somewhat less. Compound **6** is considered as an outlier. The cell-free assay failed to indicate any activity while both *Digitaria* and *Echinochloa* were completely killed with 1 kg a.i./ha. This finding cannot be discussed because the use rate of 1 kg a.i./ha is too high to allow for a score differentiation. Additional unknown inhibitory effects may be involved besides fatty acid elongation inhibition when applied at higher concentrations.

In the greenhouse test a higher phytotoxicity of the indanofan S-enantiomer *vs.* the racemic one (cpds. **3, 2**) could not be found.

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