New Peroxidizing Herbicides: Activity Compared with X-Ray Structure

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The crystal structure of 3-(4-chloro-5-cyclopentyloxy-2-fluorophenyl)-5-isopropylideneoxazolidine-2,4-dione (BW-4), chlorophthalim and oxyfluorfen were determined by X-ray analysis. The molecular size of these compounds was almost the same, but the angle between the oxazolidine and the phenyl ring (BW-4), the imide and phenyl ring (chlorophthalim) and between the two phenyl rings (oxyfluorfen) differed.

The phytotoxic activities, determined by growth inhibition of *Scenedesmus* cells, loss of chlorophyll, and peroxidative destruction of photosynthetic pigments, were different among these compounds. The angle and length of BW-3, BW-4 and oxyfluorfen showing best phytotoxic activity were found approximately 65–95° and 11–13 Å, respectively. These preliminary findings indicate that both angle and length of the molecule have some bearing on peroxidative activity.

Introduction

Several 3-aryl-5-isopropylideneoxazolidine-2,4-diones are already known as light-dependent peroxidizing herbicides [1].

Some of these compounds, *e.g.* 3-{4-chloro-2-fluoro-5-((1-methyl-2-propynyl)oxy)phenyl}-5-isopropylideneoxazolidine-2,4-dione (BW-3), and 3-(4-chloro-5-cyclopentyloxy-2-fluorophenyl)-5-isopropylideneoxazolidine-2,4-dione (BW-4), attracted our attention as a possible future type of peroxidizing herbicides due to their rapid action and remarkably high phytotoxicity. BW-4 is a useful herbicide, because it has especially high phytotoxicity as a paddy field herbicide [2, 3].

In this study, we report a relation between X-ray crystal structure and phytotoxic activity of oxazolidinedione type herbicides compared with chlorophthalim and oxyfluorfen.

Materials and Methods

Chemicals

The synthetic route of oxazolidinedione derivatives (**1a,** BW-3; **1b,** BW-4) is presented in Fig. 1 [4]. 4-Chloro-2-fluoro-5-alkyloxyphenylisocyanates (**3**) were synthesized by phosgenation of the corresponding anilines (**2**) using conventional methods. 2-Hydroxy-3-butenoate (**4**) was synthesized by ring-opening isomerization of β,β-dimethylglycidic esters. Reaction of arylisocyanate (**3**) with hydroxybutenoate (**4**) in the presence of base gave new oxazolidinedione derivatives (**1a,** BW-3; **1b,** BW-4).

Chlorophthalim, N-(4-chlorophenyl)-3,4,5,6-tetrahydrophthalimide, was prepared by the condensation reaction of 3,4,5,6-tetrahydrophthalic anhydride and 4-chloroaniline, according the method given elsewhere [5].

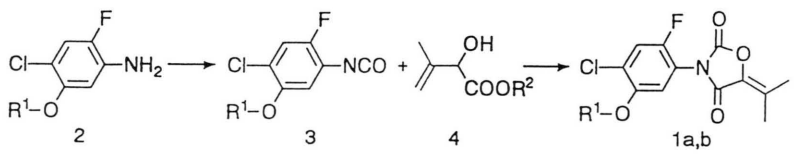

Fig. 1. Synthetic route of oxazolidinedione derivatives: BW-3 (**1a;** R^1 = 1-methyl-2-propynyl); BW-4 (**1b;** R^1 = cyclopentyl).

Oxyfluorfen, 2-chloro-4-trifluoromethylphenyl-3′-ethoxy-4′-nitrophenyl ether, was prepared by treating 1,3-bis-2-chloro-4-(trifluoromethyl)phenoxy-4-nitrobenzene, synthesized from resorcinol, with ethanolic KOH [6].

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Compounds prepared were purified by recrystallization and column chromatography, and their structures were confirmed by IR, ^{1}H-NMR spectrography and elementary analysis for C, H, N and halogen.

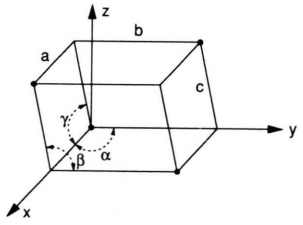
X-ray analysis

The single crystals of BW-4, chlorophthalim, and oxyfluorfen employed for X-ray analysis were recrystallized from a mixture of ethyl acetate and hexane, ethanol, and hexane, respectively. Crystal and intensity data were collected on a Mac Science MXC[18] automated four-circle difractometer using a graphite-monochromated Cu Kα radiation (λ = 1.54178 Å). The structure was solved by the direct method (MONTECARLO MULTAN) [7, 8], and refined by full-matrix least-square methods [9]. Crystallographic data and details of the refinement are summarized in Table I.

Scenedesmus cultivation and determination of phytotoxic activity

Scenedesmus acutus was grown autotrophically in sterile culture by gassing with air enriched with 4% (v/v) of CO_2 in a mineral medium according to [10]. The cultures were kept in a water bath at 22 °C under continuous illumination by fluorescent light (approx. 6000 lux). Each herbicide dissolved in methanol was applied at the required concentration to a 24 h old algal cell suspension (with a density of 2 µl packed cell volume (pcv) per ml of cell suspension). Herbicide treatment was performed in gas-tight 9 ml vials. 2 ml algae suspension was shaken in a Warburg apparatus for 16 h at 22 °C in the light (17,000 lux). After incubation cell growth, chlorophyll content and ethane formation were determined. Cell growth was expressed as pcv per ml of algal culture volume. Total chlorophyll content was determined

Table I. Crystallographic data and experimental details. Unit cell dimensions: *a, b* and *c* indicate the length of the unit cell; α, β and γ are the angles of the unit cell (see figure below). *Z*, number of molecules in a unit cell; D_{obs}, density observed; D_{calc}, density calculated; µ(Cu Kα) [cm^{-1}], absorption coefficient of X-rays.



Compound	BW-4	Chlorophthalim	Oxyfluorfen
Formula	$C_{17}H_{17}ClFNO_4$	$C_{28}H_{24}Cl_2N_2O_4/2$	$C_{15}H_{11}ClF_3NO_4$
Formula weight	353.80	523.40/2	361.70
Crystal size [mm]	0.30 × 0.35 × 0.20	0.40 × 0.20 × 0.20	0.30 × 0.35 × 0.20
Unit cell dimensions:			
a [Å]	20.131(4)	11.955(8)	29.151(8)
b [Å]	5.551(1)	14.82(1)	7.650(2)
c [Å]	15.002(3)	7.137(5)	14.534(5)
α°		–	
β°	96.18(2)	–	102.16(2)
γ°		–	
Volume of unit cell [Å^3]	1666.6(6)	1265(1)	3168(2)
Crystal system	monoclinic	triclinic	monoclinic
Space group	$P2_1/c$	P-1	$C2/c$
Z	4	4	8
D [g/cm^3]: D_{obs}; D_{calc}	1.47; 1.41	1.38; 1.37	1.60; 1.52
µ(Cu*K*α) [cm^{-1}]	21.97	49.98	25.07
Max sin θ/λ	0.584	0.585	0.584
No. of unique reflections	2793	4165	2638
No. of reflections used	2646	3775	2239
Residuals: *R*; R_w	0.047; 0.086	0.058; 0.078	0.136; 0.174

spectrophotometrically in methanolic extracts. Determination of ethane formation was performed as described previously [11]. The I_{50} values for chlorophyll content was calculated by adapted Dixon plots according to Lambert *et al.* [12, 13]. The "activity value" for ethane formation was estimated through double-reciprocal plots similar to K_m determinations (K_{a50} values).

Results

X-ray analysis (crystal structure of peroxidizing herbicides)

X-ray crystal structures of peroxidizing herbicides are shown in Fig. 2. The oxazolidine ring (B) and the cyclopentyl ring of BW-4 were approximately planar. Noteworthy that the planes of the oxazolidine ring (B) and phenyl ring (A) are not coplanar to each other; the flat oxazolidine ring is tilted by 75.9° with respect to the phenyl ring to the axis of the C–N bond. The angle of chlorophthalim between the imide ring (B) and phenyl ring was found 137.4°, and the angle between the two phenyl rings of oxyfluorfen was 68.3°. Furthermore, the longest length of BW-4, chlorophthalim and oxyfluorfen was measured as 12.56 Å, 11.37 Å and 12.31 Å, respectively. That means the molecular sizes of these peroxidizing herbicides have almost the same dimension (Fig. 2).

Phytotoxic activity of peroxidizing herbicides

The effects of two oxazolidinedione compounds (BW-3 and BW-4) on cell growth, chlorophyll degradation and ethane formation of *Scenedesmus* cells for a 10^{-5} M concentration of these compounds during an incubation period of 16 h are shown in Table II. These compounds severely inhibited cell growth, degraded chlorophyll of the cells, and exhibited a high ethane formation. Obviously, BW-3 and BW-4 are peroxidizing herbicides.

The phytotoxic activity indicated by pK_{a50} values of ethane formation and decrease of chlorophyll content per packed cell volume (pI_{50} values) are shown in Fig. 2. The phytotoxic activity was different by these peroxidizing herbicides. The order the pK_{50} values (ethane) was chlorophthalim < BW-4 < oxyfluorfen ≈ BW-3 (racemic).

Discussion

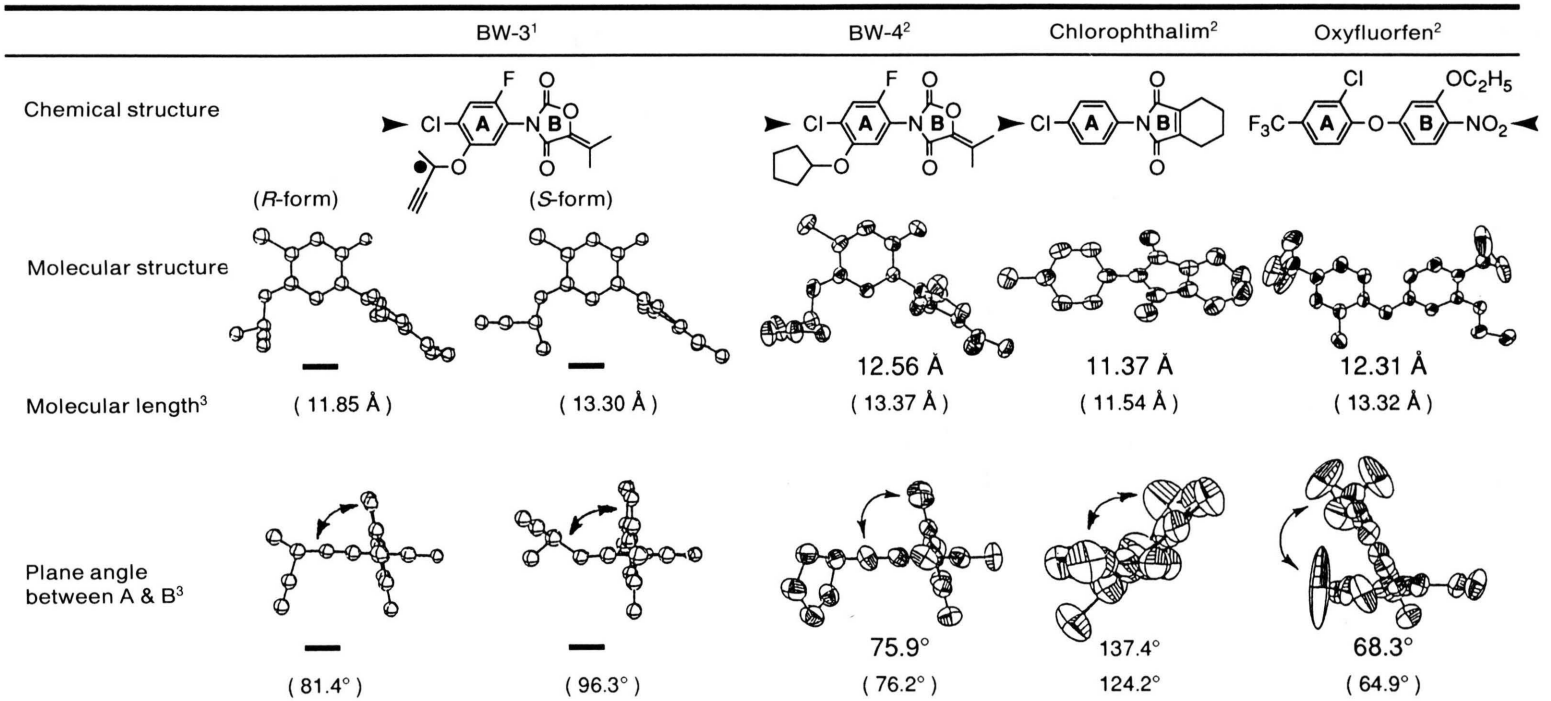
Acifluorfen (AF) is known to act as a competitive inhibitor of the enzyme protoporphyrinogen oxidase (Protox) by binding to the same active site as the substrate protoporphyrinogen IX (Protogen) [14, 15]. Recently, Nandihalli *et al.* reported that the molecular similarities between Protogen and AF by comparing their molecular and electronic properties as derived from semiempirical MO calculations [16]. These authors stated that the maximum length (y-axis: 12.29 Å) and width (x-axis: 5.53 Å) of AF matched closely with the full length and one-half width of the Protogen molecule. In addition, the torsion angle of AF at the ether oxygen matched with the angle at the methylene bridge between two neighboring pyrrole rings.

In our crystallographic analyses, the longest lengths of BW-4, chlorophthalim and oxyfluorfen were found as 12.56 Å, 11.37 Å, and 12.31 Å, respectively.

Moreover, the lowest energy most stable conformations of the compounds listed in Fig. 2 were calculated using MOPAC Manual, Version 6.01,

Table II. Influence of peroxidizing herbicides on cell growth, chlorophyll content and ethane formation in autotrophic *Scenedesmus* cells (determined at 10^{-5} M concentration) after a 16-h incubation in the light.

Compounds	Growth [µl pcv/ml]	Chlorophyll content [mg/ml pcv]	Ethane formation [nmol/ml pcv]
Control	2.8	7.45	0.20
BW-3	2.0	3.06	17.65
BW-4	2.0	3.19	12.31
Chlorophthalim	2.6	4.90	12.02
Oxyfluorfen (10^{-6} M)	2.2	2.92	10.75



Phytotoxic activity	(racemic)				
Ethane, pK_{a50}	7.10		6.20	6.04	7.00
Chlorophyll, pI_{50}	7.34		6.88	7.10	8.15

[1] MO-calculated perspective drawing.
[2] ORTEP drawing of X-ray crystal structure.
[3] Data in parentheses were calculated using MOPAC Version 6.01 [17].
The chiral center of BW-3 is indicated by (●). At present, BW-3 is only available as an racemic mixture, so that no crystals of the *S*- and *R*-forms could be separated.

Fig. 2. Molecular structures and phytotoxic activity of peroxidizing herbicides.

and then the longest lengths and torsion angles of the molecules were estimated (see Fig. 2). The calculated values of the longest length were a little bigger (0.2–1.0 Å) than the values from X-ray analysis. Any values from X-ray analysis or MO calculation indicate that the molecular sizes of these peroxidizing herbicides have about the same size and match closely with the full length of the Protogen, although these peroxidizing herbicides have different chemical structures. These values (*ca.* 11–13 Å) may indicate the suitable size for the receptor size of Protox. On the other hand, the torsion angle between A ring and B ring indicated in Fig. 2 was found quite different among BW-3, BW-4, chlorophthalim and oxyfluorfen. The angle of BW-3, BW-4 and oxyfluorfen, which showed best phytotoxic activity, were *ca.* 65–95° in X-ray analysis and MO calculation. The angles determined experimentally were found close to the calculated ones.

It appears that the angle of the molecules has some bearing on phytotoxic activity and may contribute as a steric factor to a fit against Protox. Of course more X-ray data from crystals of analogs of different activity are needed to support this view.

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[1] M. Hori, K. Hirai, H. Kohno, Y. Sato, and K. Wakabayashi, Abstract 17th Ann. Meeting Pesticide Science Society of Japan, p. 49 (1992).
[2] S. Ugai, S. Nagato, T. Yoshimura, T. Taguchi, K. Hirai, T. Yano, E. Ejiri, H. Hirose, A. Fugita, and K. Sato, *ibid.*, p. 47 (1992).
[3] T. Yoshimura, S. Ugai, S. Nagato, M. Hori, K. Hirai, T. Yano, and E. Ejiri, *ibid.*, p. 48 (1992).
[4] H. Sato, A. Fujita, and K. Hirai, Abstract of the 56th Ann. Meeting Chemical Society of Japan, p. 1365 (1988).
[5] H. Ohta, S. Suzuki, H. Watanabe, T. Jikihara, K. Matsuya, and K. Wakabayashi, Agr. Biol. Chem. **40,** 745–751 (1976).
[6] R. H. Yih and C. Swithenbank, J. Agric. Food Chem. **23,** 592–593 (1975).
[7] A. Furusaki, Acta Cryst. **A 35,** 220 (1979).
[8] C. Katayama *et al.*, Acta Cryst. **A 42,** 19 (1986).
[9] P. Coppens and W. C. Hamilton, Acta Cryst. **A 26,** 71 (1970).
[10] G. Sandmann and P. Böger, Z. Naturforsch. **43c,** 699–704 (1988).
[11] B. Nicolaus, G. Sandmann, H. Watanabe, K. Wakabayashi, and P. Böger, Pestic. Biochem. Physiol. **35,** 192–201 (1989).
[12] R. Lambert and P. Böger, Pestic. Biochem. Physiol. **20,** 183 (1983).
[13] R. Lambert, G. Sandmann, and P. Böger, Pestic. Biochem. Physiol. **19,** 309–320 (1983).
[14] J. M. Camadro, M. Matringe, N. Magnin, R. Scalla, and P. Labbe, Biochem. J. **277,** 17 (1991).
[15] R. Varsano, M. Matringe, N. Magnin, R. Mornet, and R. Scalla, FEBS Lett. **272,** 106 (1990).
[16] U. B. Nandihalli, M. V. Duke, and S. O. Duke, Pestic. Biochem. Physiol. **43,** 193–211 (1992).
[17] J. J. P. Stewart, MOPAC Manual, Version 6.01, Chemical Program Exchange Organization, Japan (1990).