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Plant Growth Regulation with Triazoles of the Dioxanyl Type

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Abstract. The plant growth retarding activities of several dioxanylalkyl and dioxanylalkenyl triazoles were determined in seedlings of barley, rice, and oilseed rape. Out of these groups some substances proved to be among the most efficient growth retardants known. The compound 1-(4-trifluormethyl)-2-(1,2,4-triazolyl-(1))-3-(5-methyl-1,3-dioxan-5-yl)-propen-3-ol was investigated more closely. Shoot growth is reduced more intensively than root growth by this compound. At lower dosages root growth may even be stimulated. The action of this retardant can be antagonized by gibberellin A_3 and by *ent*-kaurenoic acid. It is suggested that its main biochemical action is to block the reactions that lead from *ent*-kaurene to *ent*-kaurenoic acid in the course of gibberellin biosynthesis.

Plant growth regulators are widely used in modern agriculture. Compounds like chlormequat chloride, mepiquat chloride, and ethephon can be applied successfully to solve the problem of lodging in intensive wheat and barley growing. In addition, mepiquat chloride is used in cotton, where it reduces unwanted vegetative growth and leads to more uniform ripening. In the future, appropriate growth retardants may be applied profitably in crops like rice, soybeans, maize, and oilseed rape or in perennials like fruit trees or grapes. Therefore, efforts are being made to find new and better compounds for these fields of application.

The plant growth retarding activity of triazole compounds was first described for the fungicides triadimefon and triadimenol (Buchenauer and Grossmann 1977, Buchenauer and Röhner 1981). Later, other types of triazoles were chemically optimized to reduce longitudinal growth in plants: compounds coded LAB 117682,129409, and 130827 (Rademacher and Jung 1981), PP333 (paclobutrazol) (Lever et al. 1982), RSW 0411 (triapenthenol) (Hildebrandt 1982, Lembrich et al. 1984), and S-3307 (Izumi et al. 1984).

In model experiments, LAB 117682, 129409, and 130827 clearly show greater biological activities when compared with compounds such as chlormequat chloride, mepiquat chloride, daminozide, or chlorphonium (Rademacher and Jung 1981, Rademacher et al. 1984). The reduction of shoot growth resulting from a treatment with a triazole-type retardant is accompanied by an enhanced pigment content per unit of leaf area, which is a typical effect of many retardants. Root growth is not usually inhibited. In many cases even longer and thicker roots can be observed (Rademacher et al. 1984).

The chemical stability of many of the triazole retardants appears to be very high, which might lead to long-lasting effects (for paclobutrazol see Lever et al. 1982, Froggatt et al. 1982).

In this contribution dioxanyl-type triazoles will be introduced as a new group of highly potent plant growth retardants. Starting from a lead structure, several derivatives have been synthetized within this group. To do so, the suggestions for drug design by Topliss (1972, 1977) were roughly followed in order to obtain the most potent structures in a minimum of time. The biological activities of the different structures will be compared here. In addition, some general effects of one of the most powerful compounds of this series will be demonstrated, and the results of some experiments to find out its principal mode of action will be presented.

Materials and Methods

Assays with Seedlings of Barley and Oilseed Rape

Eighty seedlings of barley (cv. Aramir) and 100 seedlings of oilseed rape (cv. Petranova) were each grown on a peat substrate in a round plastic pot with a volume of 500 ml under greenhouse conditions. Immediately after seeding, 25 ml of the particular aqueous test solution was applied uniformly to the substrate's surface. Further watering was done via the bottom of the pots. The shoot length of the plants was determined after approximately 14 days (barley) or 20 days (oilseed rape).

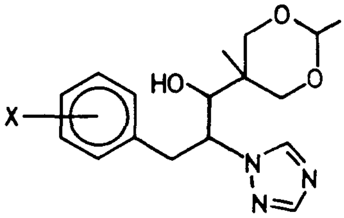
Rice Seedling Assay

Eight young seedlings of rice (cv. Girona) were each transferred into 100-ml cylinders containing defined concentrations of the test compound in 3 ml of a nutrient solution. After 6 days of growth under continuous light (Osram Universal White neon tubes with approximately 55 $\mu E \cdot m^{-2} \cdot sec^{-1}$ (400–750 nm)) at 25°C, the length of the second leaf sheaths was determined. Isoactive molar concentrations causing a 50% reduction ($= KI_{50}$) were calculated from these data (for details see Rademacher and Jung 1981, 1983). Experiments to antagonize the action of retardants were performed with the same technique.

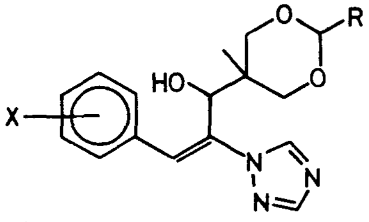
Tests with Hydroponically Cultivated Plants

Seedlings of maize (cv. Inrakorn) and sunflowers (cv. Sorex) were raised in vermiculite. At a shoot length of approximately 12 cm, uniformly grown plants

Table 1. Chemical structures of the tested chemicals.



Alkyl type



Alkenyl type

No.	X	R	No.	X	R
I	4-CH_3	H	XVI	H	H
II	4-CH_3	CH_2CH_3	XVII	4-CF_3	H
III	4-CF_3	H	XVIII	4-CF_3	CH_2CH_3
IV	4-CF_3	H	XIX	3-CF_3	H
V	4-Cl	H	XX	2,4-Cl	H
VI	4-Cl	CH_2CH_3			
VII	4-Cl	$(CH_2)_2CH_3$			
VIII	4-Cl	$CH(CH_3)_2$			
IX	4-Cl	$(CH_2)_3CH_3$			
X	2,4-Cl	CH_2CH_3			
XI	2,4-Cl	$CH(CH_3)_2$			
XII	2,4-Cl	$(CH_2)_3CH_3$			
XIII	3,4-Cl	CH_2CH_3			
XIV	4-Br	H			
XV	4-Br	CH_2CH_3			

were transferred to hydroponic vessels containing 4 l of a nutrient solution according to Scharrer and Jung (1956) (iron applied as Fe-NaEDTA). Four milliliters of a retardant solution in acetone was added to each vessel.

Plants were cultivated in a growth chamber. Artificial light (Radium HLRV lamps with about 250 $\mu E \cdot m^{-2} \cdot sec^{-1}$ (400–750 nm)) was used to establish a day length of 14 h. The day temperature was kept at 25°C and the night temperature at 20°C. Dry matter of shoots and roots was determined after drying for 48 h at 105°C.

Plant Growth Retardants Applied

The chemical structures of the dioxanylalkyl and dioxanylalkenyl triazoles used are given in Table 1. All compounds showed sharp melting points. Their identity was confirmed by spectral analysis. For further information regarding the chemical synthesis see Rentzea et al. (1980). Enantiomers were separated by chromatographic techniques. Purities were better than 90%.

Results and Discussion

All higher plant species hitherto tested respond to treatments with most of the dioxanylalkyl and dioxanylalkenyl triazoles used in this investigation. The

Table 2. The effect of structural variations on seedling growth of barley and oilseed rape (shoot length in % of control) and rice (KI_{50} value).

Compound No.	Barley		Oilseed rape		Rice
	3 mg/pot	12 mg/pot	3 mg/pot	12 mg/pot	KI_{50} (M)
I	94	87	73	54	7.5×10^{-6}
II	85	47	40	11	1.4×10^{-7}
III	98	89	48	30	4.7×10^{-6}
IV	96	87	90	78	1.2×10^{-5}
V	99	87	61	24	1.6×10^{-5}
VI	92	81	40	18	1.2×10^{-7}
VII	96	89	57	26	n.d.
VIII	73	57	30	6	1.6×10^{-6}
IX	96	88	76	39	2.2×10^{-6}
X	98	94	66	40	3.3×10^{-6}
XI	93	81	39	18	n.d.
XII	100	101	84	74	n.d.
XIII	100	99	71	34	2.2×10^{-6}
XIV	95	92	70	54	5.8×10^{-6}
XV	91	73	27	9	4.2×10^{-7}
XVI	80	61	89	72	8.9×10^{-5}
XVII	55	33	17	13	1.2×10^{-7}
XVIII	50	35	23	10	1.6×10^{-7}
XIX	97	77	93	73	4.3×10^{-6}
XX	94	81	88	70	n.d.

highest activities are found when basal plant parts are treated with these chemicals. Soil treatments, feeding via nutrient solutions in hydroponic cultures, or seed treatments lead to clear reductions of shoot growth, whereas leaf applications are generally less effective (data not shown). Most probably this phenomenon is due to the fact that triazole-type retardants are primarily transported acropetally in the xylem, as has been reported for paclobutrazol (Froggat et al. 1982, Lever et al. 1982).

The activities of the compounds under investigation greatly depend on the nature of the substituents present. As an example, the influence of the different functional groups X and R (Table 1) on shoot growth of barley, oilseed rape, and rice is illustrated in Table 2. With these plants the highest activities are found for compounds XVII, XVIII, VIII, II, XV, and VI; XII, IV, XIII, and XIX are the least active compounds. From these findings it may be assumed that high biological activity is linked to a substitution of the aromatic ring at position 4 only. No significant distinction can be made between the different substituents X employed here. An ethyl function seems to be the optimum for the moiety R. However, compounds XVII (R = H) and VIII ($R = CH(CH_3)_2$) do not fully fit into this scheme. Alkenyls appear to be more active than alkyl compounds, although a direct comparison can only be made between substances III and XVII. With KI_{50} values for rice in the range of 10^{-7} M, the most powerful triazoles of this series are equally as active as ancymidol and tetcyclacis (Rademacher and Jung 1981) or the triazole S-3307 (Izumi et al. 1984).

Table 3. Activities of different isomers of compound XVII in the rice seedling assay.

Substance	KI_{50} (M)
E-isomer (racemic)	3.2×10^{-7}
Z-isomer (racemic)	1.4×10^{-6}
E-isomer ((+)-enantiomer)	3.3×10^{-7}
E-isomer ((−)-enantiomer)	1.0×10^{-6}

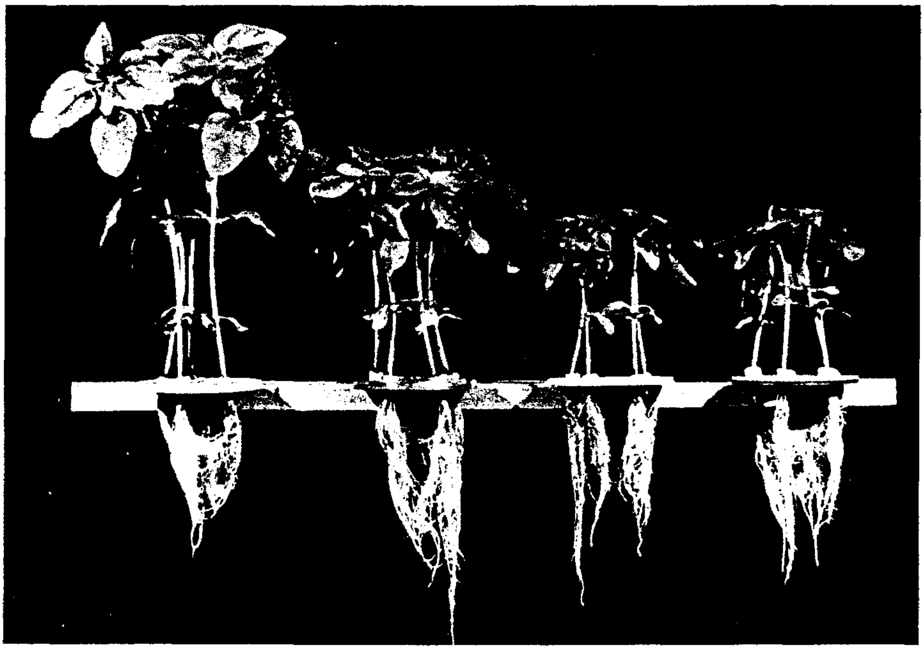


Fig. 1. Influence of different concentrations of compound XVII on shoot and root growth of hydroponically cultivated sunflowers (from left to right: control, 10^{-7} M, 10^{-6} M, 10^{-5} M applied via the medium).

In further experiments work was concentrated on compound XVII[1] as one of the most powerful structures. Different geometrical and optical isomers were tested with rice seedlings. The results shown in Table 3 indicate that the E−(+) enantiomer is the most active isomer. Both the Z (racemic) and the E−(−) isomers are significantly less active. This finding seems to be in good agreement with corresponding data for S-3307, of which the E-S isomer is the most active one (Izumi et al. 1985).

The growth behavior of plants over a longer period of time in general is affected as severely as that of young seedlings. Figure 1 shows the effect of compound XVII on the growth of sunflowers. No toxic effects can be observed

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[1] Chemical name: 1-(4-trifluormethyl)-2-(1,2,4-triazolyl-(1))-3-(5-methyl-1,3-dioxan-5-yl)- propen-3-ol. BASF code No.: LAB 150978.

Table 4. Effects of compound XVII on different parameters of growth in hydroponically cultivated maize (results taken 16 days after application of retardant).

Concentration (M)	Shoot length (±SE) (cm)	Dry matter per shoot (g)	Dry matter per root (g)	Shoot:root ratio
0	74.6 ± 4.3	2.44	0.50	4.88
10^{-8}	68.0 ± 2.7	2.40	0.55	4.36
10^{-7}	49.5 ± 2.2	2.02	0.47	4.30
10^{-6}	28.5 ± 1.4	1.21	0.40	3.03
10^{-5}	22.0 ± 1.3	0.66	0.25	2.64

Table 5. Influence of combinations of compound XVII with gibberellin A_3 on the growth of rice seedlings (length of the second leaf sheath in percent of control; 100% = 7.2 cm; SE < 5%).

Compound XVII (M)	Gibberellin A_3 (M)			
	0	3×10^{-8}	3×10^{-7}	3×10^{-6}
0	100	104	137	183
3×10^{-9}	95	95	134	170
3×10^{-8}	73	90	122	170
3×10^{-7}	45	64	97	171
3×10^{-6}	30	33	76	106
3×10^{-5}	17	17	43	107

even at the highest dosage. In contrast to shoot development, root growth appears to be less affected.

In a similar experiment with maize plants, the distribution of dry matter between shoot and root was determined. Again it emerged that root growth is far less influenced than shoot growth (Table 4). By increasing the concentration of compound XVII in the nutrient solution the shoot:root ratio is altered in favor of the root. In fact, the enhanced root growth found in this trial can often be observed at concentrations that only moderately reduce shoot height. Thicker main roots are formed while less adventitious roots are present. Similar results have been published for chlormequat chloride (Sturm and Jung 1964), ancymidol (Shive and Sisler 1976), tetcyclacis and certain triazole-type retardants (Rademacher et al. 1983), and paclobutrazol (Buchenauer et al. 1984).

The effect of compound XVII on shoot growth can be fully compensated by a simultaneous application of gibberellin A_3. The data from the rice seedling assay given in Table 5 indicate that even the effects of relatively high concentrations can be overcome by appropriate doses of gibberellin A_3.

Beside gibberellin A_3, *ent*-kaurenoic acid was tested for its ability to antagonize the effect of compound XVII on rice seedlings. As can be seen from Table 6, reversion is possible at least to some degree with this compound.

It has been shown previously that certain triazoles block the biosynthetic steps from *ent*-kaurene to *ent*-kaurenoic acid (Hildebrandt 1982, Graebe 1982, Goldsmith et al. 1983, Hedden and Graebe 1985), thereby reducing the level

Table 6. Influence of combinations of compound XVII with *ent*-kaurenoic acid on the growth of rice seedlings (length of the second leaf sheath in percent of control; 100% = 7.1 cm; SE < 5%).

Compound XVII (M)	*ent*-Kaurenoic acid (M)			
	0	3×10^{-6}	3×10^{-5}	3×10^{-4}
0	100	100	102	99
3×10^{-7}	46	65	80	90
3×10^{-6}	36	57	67	75
3×10^{-5}	16	25	57	68

of endogenous gibberellins (Rademacher et al. 1983 and 1984, Izumi et al. 1984). The results from Tables 5 and 6 indicate that compound XVII has the same mode of action. This suggestion is supported by findings with enzyme preparations of immature pumpkin endosperm. Here, a concentration of 10^{-6} M of compound XVII totally blocks the conversion of *ent*-kaurene to products that would normally follow in the sequential steps of gibberellin biosynthesis (Schwenen and Graebe, unpublished).

As an additional mode of action, an influence on sterol biosynthesis should not be left out of consideration, especially since structurally related compounds are known to inhibit fungal ergosterol biosynthesis (Schwinn 1983).

Some of the dioxanyl triazoles described here are shown to be among the most active plant growth retardants known. However, before any of these compounds can be used agronomically, more information about their performance under practical conditions is required. Examinations have to be carried out to see whether foliar uptake is sufficient or whether it can be enhanced, e.g., by using appropriate detergents. Furthermore, the persistency of these compounds should be investigated in more detail. Highly stable substances may be especially suitable for perennials or ornamentals, whereas their application in arable crops might be restricted owing to carryover risks.

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