freeze-dried powder. Its properties have been examined.

2. The enzyme belongs to the group of α -amylases, but differs from either salivary or malt α -amylase in that its action on potato amylose and starch ceases abruptly when 65.5 and 66.5% apparent maltose respectively have been liberated.

3. Some observations on the maltase secretion of the organism have been made.

The authors wish to express their thanks to Prof. S. Peat, F.R.S., for his advice and encouragement and to Mr F. Baker whose pioneer researches in this field made possible this investigation.

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The Relationship Between the Constitution and the Effect of Chemical Compounds on Plant Growth

3. CHLORINATED BENZALDEHYDES AND BENZOIC ACIDS

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(Received 30 September 1950)

In the course of a systematic investigation of the phytotoxic properties of different chemical types, we have turned our attention to aromatic aldehydes and ketones and some of the carboxylic acids corresponding thereto. Interest in aldehydes and ketones was stimulated by the finding of Gray & Bonner (1948*a, b*) that leaves of the shrub *Encelia farinosa* contained 2-methoxy-5-acetylbenzaldehyde and that this is toxic to tomato seedlings at concentrations as low as 50 p.p.m. This compound, containing both aldehyde and ketone groups, was synthesized by Mr N. Greenhalgh in these laboratories and tested against germinating rape and wheat using our standard technique. It was very toxic to both species at 50 p.p.m., less so at 10 p.p.m. and showed no selective effect. The examination of a number of simple substituted benzaldehydes and acetophenones suggested that the former grouping was perhaps of

greater interest, and since the aldehydes are more readily accessible than the ketones, our investigation has been concentrated upon the aldehydes. At the same time the corresponding carboxylic acids were examined, since although auxin-like activity is generally considered to demand some type of bridge linking the carboxylic acid group to the aromatic nucleus, several substituted benzoic acids show marked formative effects when applied to plants (Zimmerman & Hitchcock, 1941).

METHODS

Preparation of compounds. Nearly all the compounds used in this investigation are known ones, and many were already available in these laboratories. Attention is called, however, to the compounds substituted in the 2:3:4- and 2:3:6-positions (aldehydes, and acids) since the literature contains serious errors. The preparation and characterization of these

compounds will be described in full elsewhere, but in order that the actual substances used may be defined, the following physical constants are mentioned here.

	m.p. (°)
2:3:4-Trichlorobenzaldehyde	86
2:3:4-Trichlorobenzoic acid	188
2:3:6-Trichlorobenzaldehyde	87
2:3:6-Trichlorobenzoic acid	124-126
2:3:6-Trichlorobenzyl alcohol	70-73
2:3:6-Trichlorobenzylamine	43-44

Biological testing. The technique, using rape and wheat seeds germinating on agar, is described in detail in the first paper of this series (Jones, Metcalfe & Sexton, 1949). In the tables which follow, two plus signs indicate the highest measure of activity (at least 50% reduction of root length in more than half the seeds, the control seeds being grown on agar alone).

RESULTS

Table 1 gives the results with miscellaneous aromatic aldehydes and ketones, excluding the chlorinated compounds which are considered together in later tables.

Table 1. *Activity of miscellaneous aromatic aldehydes and ketones against germinating rape and wheat*

Compound	Concentration (p.p.m.)	Rape	Wheat
Benzaldehyde	50	++	-
	10	+	.
	1	-	.
<i>p</i> -Fluorobenzaldehyde	50	++	+
	10	-	-
<i>p</i> -Chlorobenzaldehyde	50	++	-
	10	+	.
<i>m</i> -Aminobenzaldehyde	50	-	-
<i>o</i> -Anisaldehyde	50	++	-
	10	-	.
2:3-Dimethoxybenzaldehyde	50	++	+
	10	+	.
3:4:5-Trimethoxybenzaldehyde	50	-	-
α -Naphthaldehyde	50	++	+
	10	++	-
	1	++	.
β -Naphthaldehyde	50	++	+
	10	-	-
Acetophenone	50	-	-
<i>p</i> -Fluoroacetophenone	50	-	-
<i>p</i> -Bromoacetophenone	50	+	+
4-Chloro-2-methyl-acetophenone	50	+	-
α -Acetonaphthone	100	-	+
β -Acetonaphthone	10	++	+
	1	++	.

From the known effects of chlorination in raising the herbicidal activity in the phenoxyacetic acid series, it was decided to examine the effect of

chlorination in the aldehydes. Early results showed large differences in the activities of the first few compounds examined and it was therefore necessary to test all the possible isomers. The results are given in Table 2.

Table 2. *Activity of chlorinated benzaldehydes against germinating rape and wheat*

	Isomer	Concen- tration (p.p.m.)	Rape	Wheat
Unsubstituted		50	+ +	+
		10	+	+
		1	-	.
Monochlorobenz- aldehydes	2	50	+ +	+
		10	+	+
		1	+	.
	3	50	+ +	+
		10	+ +	+
		1	-	.
	4	50	+ +	+
		10	+	-
	Dichlorobenz- aldehydes	2:3	50	+ +
10			+ +	+
1			+	.
2:4		10	+ +	-
		1	-	.
2:5		50	+ +	+ +
		10	+ +	+
		1	+	.
2:6		10	+ +	+ +
		1	+	.
3:4		50	+ +	+ +
		10	+ +	+
		1	+	.
3:5		50	+ +	+
		1	+	-
Trichlorobenz- aldehydes	2:3:4	10	+ +	+ +
		1	+ +	-
	2:3:5	10	+ +	+
		1	-	.
	2:3:6	10	+ +	+ +
		1	+ +	+
	2:4:5	10	+ +	+
		1	-	.
	2:4:6	50	+ +	+
		10	+	-
	3:4:5	10	+ +	+
		1	-	.

The chlorinated benzoic acids were next tested. For convenience of comparison the results are set out side by side with those of the aldehydes, in Table 3. Only the results with rape are given since wheat is less susceptible. Of the compounds showing marked activity at 1 p.p.m., 2:3:6-trichlorobenzaldehyde was found the most potent when experiments were made at greater dilutions. Further results on the 2:3:6-substituted compounds are given in Table 4.

These were obtained by our colleague Dr W. G. Templeman at Jealotts Hill Research Station, and they include results with 4-chloro-2-methylphenoxy-acetic acid ('methoxone'), one of the most active of the known inhibitors of rape growth (Templeman &

Sexton, 1946). In the first experiment recorded in the table a non-toxic wetting agent was employed as an aid to solubility.

Finally, results are given in Table 5 of tests on miscellaneous substances mentioned in the discussion, including esters of 2:3:6-trichlorobenzoic acid and certain substances which are known to exert formative effects on plants.

Table 3. *Comparison of activities of chlorinated benzaldehydes and benzoic acids as inhibitors of germinating rape*

Isomer	Aldehyde		Acid	
	10 p.p.m.	1 p.p.m.	10 p.p.m.	1 p.p.m.
2:3	++	+	++	++
2:4	++	-	+	-
2:5	++	+	++	+
2:6	++	+	+	-
3:4	++	+	++	+
3:5	+	-	+	-
2:3:4	++	++	++	-
2:3:5	++	-	++	+
2:3:6	++	++	++	+
2:4:5	++	-	++	-
2:4:6	+	-	+	-
3:4:5	++	-	+	-

DISCUSSION

2:3:6-Trichlorobenzaldehyde is the most active compound emerging from these studies. It shows the same specificity in regard to rape and wheat as do the phenoxyacetic acids, but it is much less active than the best of these. Applied to growing plants it showed formative responses resembling those produced by auxin-like substances, and the question arises whether it should be regarded as an auxin or possibly as a hemi-auxin having what Went (1949) describes as 'preparatory activity'. The structural requirements for primary activity of the auxin type

Table 4. *Comparison of activities of 2:3:6-trichlorobenzaldehyde and 2:3:6-trichlorobenzoic acid and 4-chloro-2-methylphenoxyacetic acid ('methoxone') as inhibitors of germinating rape*

Exp. no.	Treatment	Mean root length (mm.) per seedling at various concentrations (p.p.m.)					
		0	0.01	0.05	0.1	0.5	1.0
1	'Methoxone'	42.5	20.5	7.9	4.8	4.2	2.9
	Aldehyde + wetter	34.2	42.1	35.1	45.5	21.6	10.9
	Wetter alone	38.9	48.9	32.6	38.8	44.7	38.7
2	Aldehyde	42.7	.	.	21.6	.	10.9
	Acid	47.3	.	.	41.5	.	41.2

Table 5. *Activities of miscellaneous substances against germinating rape and wheat*

Compound	Concentration (p.p.m.)	Rape	Wheat
2-Chloro-5-nitrobenzoic acid	50	-	-
2-Chloro-3-nitrobenzoic acid	50	++	-
	10	+	-
2:3:5-Triiodobenzoic acid	10	++	++
	1	+	-
2:3:6-Trichlorobenzyl alcohol	10	++	+
	1	+	-
2:3:6-Trichlorobenzylamine	50	+	-
	10	-	.
Methyl 2:3:6-trichlorobenzoate	10	+	++
	1	-	+
Ethyl 2:3:6-trichlorobenzoate	10	+	++
	1	-	+
<i>n</i> -Propyl 2:3:6-trichlorobenzoate	10	++	++
	1	-	+
2:3:6-Trichlorobenzoic acid	10	++	++
	1	+	-
2:3:6-Trichlorobenzaldehyde	10	++	++
	1	++	+

have previously been considered to be (i) an acidic group, e.g. COOH, (ii) a cyclic nucleus, (iii) a group linking (i) and (ii). For preparatory activity, two of the structural features are considered necessary by Went. If 2:3:6-trichlorobenzaldehyde does indeed exhibit true auxin-like activity then Went's structural analysis will require modification.

At our suggestion, Miss J. A. Bentley of Manchester University has examined the effect of several trichlorobenzaldehydes and of 2:3:6-trichlorobenzoic acid on the growth of *Avena* coleoptiles (Bentley, 1950). Of the aldehydes, the 2:3:6-isomer alone showed activity and this was greater than the activity of β -indolylacetic acid at 1 p.p.m. 2:3:6-Trichlorobenzoic acid was also active, though less so than the aldehyde. There is therefore evidence that 2:3:6-trichlorobenzaldehyde and the corresponding acid have an auxin-like activity, and their phytotoxic effect may well be associated with this property. The phytotoxic effect of the 2:4:6-, 2:4:5- and 3:4:5-trichloroaldehydes, which is of a lower order, may be due to a different mechanism, since Miss Bentley found these to be inactive on the *Avena* growth test.

It is necessary to consider, however, whether the

aldehyde acts after conversion to the acid, either by oxidation or perhaps by the action of an aldehyde mutase, giving rise to alcohol and acid. It is known that germinating peas contain an aldehyde mutase and that it will act on aromatic aldehydes (Mikhlin & Borodina, 1940) and it is perhaps noteworthy that 2:3:6-trichlorobenzyl alcohol (Table 5) has activity about equal to that of the acid. 2:3:6-Trichlorobenzoic acid shows the same specificity of action as the aldehyde, though it is less active. It should be stated, however, that this aldehyde is much more resistant than most aromatic aldehydes to oxidation, and we were unable to bring about a Cannizzaro conversion to acid and alcohol by means of aqueous or alcoholic alkali. If the activity of the aldehyde is due to conversion to the acid by the plant it is possible that the higher activity of the aldehyde might be due to better cell permeability through its higher lipid-water partition and for this reason we examined the esters (Table 5). These, however, showed quite a different pattern of activity from the acid, and this result suggests that the plant is not capable of bringing about their hydrolysis, which is surprising in view of the known high activity of esters of phenoxyacetic acids (Templeman & Sexton, 1946). Thus while there is no evidence to show that the aldehyde is acting through conversion to the acid, this possibility cannot be rigidly excluded.

It is of interest to compare the results (Table 5) obtained with three substituted benzoic acids which are recorded in the literature as being active in causing modification of plant organs (Zimmerman & Hitchcock, 1941). 2-Chloro-3-nitrobenzoic acid shows a similar selective effect to 2:3:6-trichlorobenzoic acid in the seed germination test, though it is less

active. 2-Chloro-5-nitrobenzoic acid was inactive at the highest strength tested, while triiodobenzoic acid was almost as toxic to wheat as to rape.

On the effect of positional substitution, little can be said. The superiority of the 2:3:6-isomers may possibly be due to physical factors such as cell permeability. From the relatively low activity of the 2:6-dichloro and 2:4:6-trichloro compounds (Table 3) it is clear that the placing of chlorine atoms on either side of the CHO or COOH group is not the significant feature. The fact that in 2:3:6-trichlorobenzaldehyde the position of the substituents reveals a steric similarity to pyridoxal is interesting, but probably fortuitous, since we were unable to demonstrate any antagonism of its action by pyridoxine or pyridoxal, themselves non-toxic to rape at 50 and 10 p.p.m. respectively. The low activity of 2:3:6-trichlorobenzylamine (Table 5) is also of interest in connexion with this point.

SUMMARY

1. The effect on germinating rape and wheat of mono-, di- and tri-chlorobenzaldehydes and the corresponding di- and tri-substituted benzoic acids has been studied.
2. The highest activity against rape is shown by 2:3:6-trichlorobenzaldehyde, the corresponding acid being somewhat less active. Activity against wheat is also less.
3. The mode of action is discussed, particularly in relation to the possibilities that (a) 2:3:6-trichlorobenzaldehyde may have an auxin-like action, and (b) that the aldehyde may owe its activity to conversion in the plant tissue to the acid.

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