GROWTH REGULATION BY ORGANIC COMPOUNDS

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(WITH THREE FIGURES)

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Introduction

A large and diverse group of organic compounds is reported to "regulate" plant growth. Many of the determinations of growth regulation, however, do not measure the accelerating effects on growth but the inhibition of growth or toxicity effects. Such evidence does not suffice to identify the substances as growth regulators which may either accelerate or inhibit the growth process depending upon their concentration.

Many techniques exist for the quantitative determination of the acceleration of plant growth by organic compounds (**4, 9, 10, 11, 16, 17,**). Of these the straight growth of Avena coleoptile sections has distinct advantages. This test gives quantitative data on growth response, it enables the study of accessory factors involved in the growth process, it is sensitive to the effects of substances which do not cause an Avena curvature response, and it does not depend on the complicated series of reactions of the pea test.

This study was made to determine the similarity of the effects of several organic compounds and the plant auxin, indoleacetic acid (**20**), on the straight growth of Avena coleoptile sections. Most of the compounds have been studied by other investigators but their results cannot be correlated satisfactorily because of the different techniques employed in the measurement of the growth effects.

Materials and methods

Test plants were grown according to the procedure described by BONNER (**4**). After 96 hours the apical 2 mm. of the coleoptile were removed and two hours later a single section 3 mm. in length was cut from the apex with the primary leaf included within the coleoptile. The cutter used in these experiments gave sections with a standard deviation of initial length of $\pm$ 0.03 mm. Fifteen sections were floated on the surface of 25 ml. of the solution in a Petri dish and placed in an incubator at 28° C for 24 hours. The sections were not mounted on glass rod or combs (**4, 10**) as curvature during the growth period occurred very infrequently. Growth measurements were made with an ocular micrometer mounted in a binocular dissecting microscope at a magnification of eight diameters. Most of the compounds which were tested were freshly prepared. A few were the products of commercial laboratories recrystallized several times before use. Melting points of all compounds were determined to check their purity.

Experimental results

The residual auxin in the coleoptile section allows for an elongation of the section floating on distilled water. This elongation varies considerably depending on the conditions under which the test plants are grown. In these experiments it was usually 8 per cent. of the original length. With each experiment the growth in distilled water was determined and the data are expressed as the percentage elongation of sections in a given solution of the chemical minus the percentage elongation in distilled water. Differences of 1 or 2 per cent. are recorded as zero. At high concentrations of the organic compounds an inhibitory effect on elongation becomes apparent

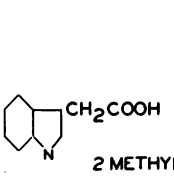
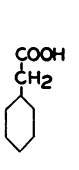
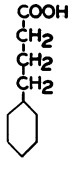
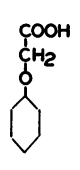
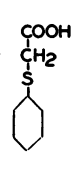
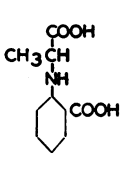
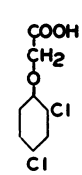
MOLAR CONC. X 10^{-4}	 2 METHYL IAA						
10.0	−12						
5.0	− 6	−12	− 4	−23	−11	−14	−10	−13
2.0	+23	+10	+25	−11	+ 6	− 3	+15	− 8
1.0	+38	+32	+30	+13	+10	+15	+16	+10
0.5	+36	+29	+31	+16	+ 6	+ 9	+ 8	+28
0.1	+36	+16	+19	+13	+ 3	0	0	+30
0.05	+29	+ 8	+14	+ 8	0			+31
0.01	+25	0	+ 8		0		0	+24
0.005		0				0	0	+21
0.001	0				0			+ 7

FIG. 1. Comparison of the effects on cell elongation of several organic compounds and the plant auxin (indoleacetic acid). Values are the differences between percentage elongation in solutions of the chemicals and percentage elongation in water.

(negative values in figures). Death of the tissues generally occurs at a concentration of 2×10^{-4}M or higher, and a marked shrinkage in length results from a loss of turgor. The amount of food material available and the amount of growth regulating substance together determine the maximum elongation (**10**). Thus the relative activity of different chemicals is indicated by the molecular concentration sufficient to bring about maximum elongation as well as by the relative elongation occurring at any particular concentration of the chemicals.

Many recent investigations present evidence of the growth regulating properties of the selective herbicide, 2,4-dichlorophenoxyacetic acid (re-

ferred to as 2,4-D hereafter). AKAMINE (1) reports the initiation of root development on cotyledons by 2,4-D, ZIMMERMAN (22) reports it to be active in causing stem and petiole curvature and parthenocarpy, and FULTS and PAYNE (5) report it active in the pea test. However, in the Avena deseeded test 2,4-D has only 0.1 per cent. of the activity of indoleacetic acid (IAA) according to AVERY, BURGER and SHALUCHA (2). The data in figure 1 show that 2,4-D is a growth regulating substance of equal or greater effect on cell elgonation than IAA!

Tests of compounds related to 2,4-D were made to determine, if possible,

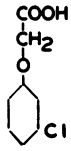
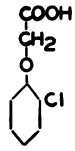
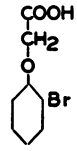
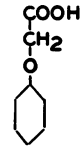
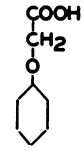
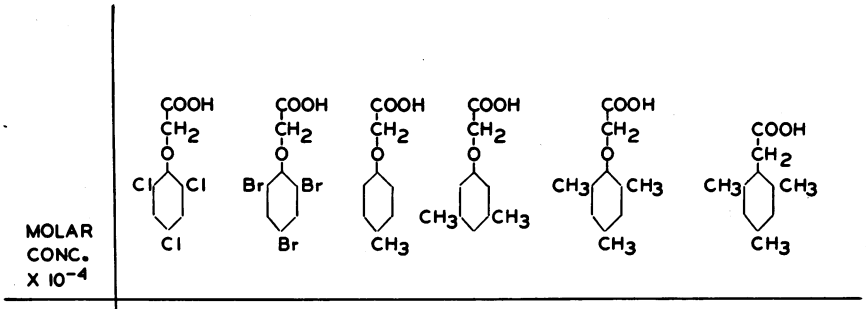
MOLAR CONC. X 10^{-4}					
5,0	−16	− 6	−11	−13	−14
3,0	−11	− 4	− 8		
2,0	− 6	+11	0	− 8	−11
1,0	+21	+13	+20	+ 9	+13
0,5	+25	+ 9	+ 9	+19	+21
0,1	+11	+ 3	0	+14	+14
0,05	+ 9	0	0	+13	
0,01	+ 4		0	+ 9	+ 3
0,005	0		0	0	
0,001	0		0	0	

FIG. 2. Effect of monohalogen derivatives of phenoxyacetic acid on cell elongation. Values are the differences between percentage elongation in solutions of the chemicals and percentage elongation in water.

the structural features responsible for its activity. Phenylacetic acid (PAA) was reported to have little activity in the regulation of cell elongation until THIMANN and SCHNEIDER (13) found it to have a maximum elongation effect which was about half that of IAA. BONNER (3) reports that if corrections for greater dissociation are made, the activity of PAA approaches that of IAA. The results of tests given in figure 1 show that PAA has an activity which approaches that of IAA at high concentrations but not at low concentrations. THIMANN and SCHNEIDER (13) also found that γ-phenylbutyric acid (PBA) directly accelerated cell elongation although

other investigators had ruled it inactive. In the tests reported here the elongation induced by PBA is approximately half that induced by PAA. The data for phenoxyacetic acid (POAA), S-phenylglycolic acid, and o-(carboxyphenylamino) propionic acid in which an oxygen atom, a sulfur atom and a nitrogen atom respectively are introduced between the acetic acid group and the benzene ring show that they have less activity than PBA in which two carbon atoms are introduced similarly. The activity of 2,4-D is not a consequence of the introduced oxygen atom alone.

The contributions of the substituted halogen atoms to the growth activity



MOLAR CONC. X 10^{-4}						
5.0			−12	−11	−12	−10
2.0			+ 8	− 5	− 4	
1.0	−11	−11	+17	+ 9	+ 9	+ 3
0.5	+ 4	− 3	+ 6	+11	+ 7	+ 3
0.1	0	0	0	+ 5	+ 4	0
0.05	0	0			0	0
0.01	0	0	0	+ 3	0	0
0.001	0	0				

FIG. 3. Comparison of the effects of several organic compounds on cell elongation. Values are the differences between percentage elongation in solutions of the chemicals and percentage elongation in water.

of 2,4-D were investigated by testing first, the effects of a single chlorine or bromine atom in the ortho, meta or para positions of POAA and second, the effects of substitution of chlorine or bromine in the 2,4 and 6 positions. The data given in figure 2 indicate that the substitution of a single halogen atom in the ortho, meta or para position increases the growth activity of the POAA structure. Substitution in the para or meta position has a greater effect than substitution in the ortho position but does not equal the effect of 2,4-D which has chlorine atoms at both the ortho and para positions.

In figure 3 the results of tests of the 2,4,6-trichlorophenoxyacetic acid

and the similar bromine compound show them to be inactive, the chlorine in the 6 position nullifies both the 2,4-D activity and that associated with the POAA structure. Similar effects have been noted by ZIMMERMAN (21) and SYNERHOLM and ZIMMERMAN (11) in their growth tests. They also report that 2,6-dichlorophenoxyacetic acid, 2,3,4,6-tetrachlorophenoxyacetic acid and the pentachlorophenoxyacetic acid are all inactive but the 2,3,4- and 2,4,5-trichlorophenoxyacetic acids are active. Incomplete data are available which indicate that 2,4,5-trichlorophenoxyacetic acid has an effect on cell elongation which approaches that of 2,4-D.

It was of interest to determine the effects of substituted methyl groups and compare them with the substituted halogens. The data in figure 3 show the growth activities of para methylphenoxyacetic acid, 3,5-dimethyl- and 2,4,6-trimethylphenoxyacetic acids which differ very little from that of POAA. Substitution of a methyl group does not have the augmenting effect on growth activity that substitution of a halogen has, nor is the activity of the POAA structure completely nullified by the substitution of methyl groups at the 2,4 and 6 positions as was the case for the similar halogen substituted compounds. Substitution of methyl groups at the 2,4 and 6 positions of PAA however, does nullify the growth activity. Similar diminution of the growth activity of IAA occurs upon substitution of a methyl group in the 2 position (fig. 1). This effect was reported by KÖGL and KOSTERMANS (8) who found also that substitution of an ethyl group at this position caused complete loss of growth activity.

Discussion

KOEPFLI, THIMANN and WENT (7) propose that an active growth regulator has an unsaturated ring system with a side chain carrying a carboxyl group at least one carbon atom removed from the ring. WENT (19) states that the side chain must be adjacent to the ring double bond. The chemical constitution of the phenoxyacetic acids meets all of these structural requirements for plant growth activity. Blocking of both positions ortho to the side chain carrying the carboxyl group, however, destroys the growth promoting activity. Therefore, in addition to the other structural requirements, the active phenoxyacetic acid compound must have a free, or potentially free ortho position.

In addition to these structural requirements, other properties have been used to account for the differences in growth regulating activity. According to BONNER (3) differences in the degree of ionization of the acids may be used to explain relative growth promoting activities of similar compounds. Attempts to apply this theory to some of the compounds tested in this investigation have been unsuccessful. The dissociation constants of the acids as reported by HAYES and BRANCH (6) are given in table I. Phenoxyacetic acid is less ionized than any of the halogen acids, yet the latter all have greater growth activity. Similarly the ortho and para halogen compounds have about the same ionization constants yet the para compounds are much more active. VELDSTRA (15) shows that differences in

dissociation of the acids derived from naphthalene also do not explain their relative growth activities.

VELDSTRA (**14**) proposes that the properties of active growth regulators are associated with an adsorption of the active substance at a boundary surface. A more common interpretation of the activity of growth regulators involves a reaction with a substrate (**12, 18**). The lack of activity of compounds substituted in both ortho positions is best explained by the latter theory. Thus growth regulators may be considered as reacting with a substrate at two points, first by means of the carboxyl group, and second at a position ortho to the attachment of the carboxyl group. This would explain the striking lack of growth activity of 2,4,6-trichloro- and tribromophenoxyacetic acids compared to the great activity of 2,4-D and 2,4,5-trichlorophenoxyacetic acid. Substantiating evidence may be adduced from the lack of activity of 2,4,6-trimethylphenylacetic acid with substituents in both ortho positions whereas phenylacetic acid is almost as active as IAA. Since

TABLE I

DISSOCIATION CONSTANTS OF COMPOUNDS TESTED FOR GROWTH ACTIVITY

COMPOUND	K
Phenylacetic acid	4.88×10^{-5}
Phenoxyacetic acid	6.75×10^{-4}
p-chlorophenoxyacetic acid	7.89×10^{-4}
p-bromophenoxyacetic acid	7.37×10^{-4}
p-methylphenoxyacetic acid	6.09×10^{-4}
o-chlorophenoxyacetic acid	8.90×10^{-4}
o-bromophenoxyacetic acid	7.53×10^{-4}
m-chlorophenoxyacetic acid	8.51×10^{-4}

the activity of 2,4,6-trimethylphenoxyacetic acid is not less than that of POAA, it is possible that the reaction with the substrate may take place through a methyl group or by removal of a methyl group. Experiments are in progress to test these hypotheses more rigorously.

Summary

IAA and 2,4-D have equal growth activity as measured by cell elongation. Other substituted phenoxyacetic acids have growth activities which vary from small values to values equalling that of 2,4-D. The growth activity of 2,4-D and other phenoxyacetic acids can be related to chemical structure by the supposition that the position on the benzene ring adjacent to the point of attachment of the side chain is directly involved in the growth reaction.

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