

have locally slowed the forward advance of the delta. These channels have little in common with the rock gorge type of submarine canyons but resemble the outer shallow valleys that form an extension of the submarine canyons.

6. Virtually all the arguments now being used for subaerial origin are based on the hypothesis of great lowering of sea level during the ice ages, an idea that was abandoned more than two years ago.

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## Maleic Hydrazide as an Antiauxin in Plants<sup>1</sup>

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In the first report of the action of maleic hydrazide on plants, Schoene and Hoffmann (1) demonstrated that this compound inhibited stem elongation and overcame the normal apical dominance in tomato plants. The same responses have subsequently been observed in many other plant species.

Growth inhibitors in general do not cause lateral buds to develop. However, one agent that does inhibit stem elongation and that breaks apical dominance is x-irradiation (2), and, interestingly enough, x-irradiation also causes the destruction of indoleacetic acid or auxin (3). Auxin is essential for growth and is apparently the controlling factor in apical dominance. The possibility suggests itself, then, that maleic hydrazide may act within the plant in opposition to auxin, i.e., as an antiauxin.

If maleic hydrazide does act as an antiauxin, it would be logical to expect it to inhibit growth where auxin is limiting, and that this inhibition should disappear when auxin is not limiting. This has indeed been found to be the case.

Using the standard slit pea test (4), initially inhibitory levels of maleic hydrazide were added to serial concentrations of auxin. The results were collected using the stem-reference technique of Thimann and Schneider (5). In typical data from such an experiment (Table 1) two salient features can be seen: (1) Maleic hydrazide at both the concentrations used (3 and 10 mg/l) inhibits growth in the presence of low concentrations of auxin (.01 and 0.1 mg/l indoleacetic acid). For example, referring to Table 1, maleic hydrazide inhibition in 0.1 mg/l auxin amounts to  $-118^\circ$  and  $-126^\circ$ , respectively, for the two inhibitor concentrations. These differences are both significant at the 1% level. (2) Maleic hydrazide at these same concentrations does *not* inhibit growth in the presence

of high concentrations of auxin (10 and 100 mg/l indoleacetic acid). Thus the differences between the auxin controls and the maleic hydrazide treatments amount to  $+9$  and  $-9^\circ$ ,  $-7^\circ$  and  $+17^\circ$ , respectively, for the two inhibitor concentrations. These differences do not approach significant levels.

TABLE 1

INHIBITION OF GROWTH BY MALEIC HYDRAZIDE AND ITS REVERSAL WITH HIGH CONCENTRATIONS OF INDOLEACETIC ACID  
(Slit pea test, 17 hr; readings made by stem-reference method.)

IAA conc (mg/l)	Curvature with-out MH (de-grees)	Curvature with 3 mg/l MH (de-grees)	Difference due to added MH (de-grees)	Curvature with 10 mg/l MH (de-grees)	Difference due to added MH (de-grees)
None	-227	-239	-12	-242	-15
.01	-135	-168	-33	-231	-96*
0.1	-13	-131	-118†	-139	-126†
1.0	162	152	-10	122	-40
10	337	346	9	330	-7
100	-125	-134	-9	-108	17

\* LSD at 5% level:  $76^\circ$ .

† LSD at 1% level:  $101^\circ$ .

The capacity of auxin to overcome maleic hydrazide inhibition completely is reproducible over a wide range of concentrations of the inhibitor, and has been confirmed using two other growth tests: the pea straight growth test and the *Avena* straight growth test. Indoleacetic acid is not the only growth regulator capable of relieving maleic hydrazide inhibition. The same effect has been observed using naphthaleneacetic acid as the growth regulator.

Maleic hydrazide inhibition is not evident in the data in Table 1 in the treatment using no auxin. In the tests we have carried out it has been generally true that where conditions did not permit much growth maleic hydrazide inhibition was greatly reduced. In cases where growth is more active, however, maleic hydrazide inhibition is much more severe. This can be seen readily from Table 1 in the treatments using 0.01–0.1 mg/l of auxin.

The ability of maleic hydrazide to act as an antiauxin will be discussed in more detail in another paper (6). It is clear, however, that maleic hydrazide inhibition can be overcome by the addition of excess auxin, and hence it may properly be called an antiauxin.

Several other compounds capable of antagonizing auxin action have been described. Triiodobenzoic acid (7), unsaturated lactones such as coumarin (8), and 2,4-dichloroanisole (9) all have been demonstrated to antagonize auxin action. However, they have not been shown to be counteracted by the addition of more auxin, and work in our laboratory has failed to demonstrate such a characteristic in the first two of these. Hence it would seem that they act in a different manner than does maleic hydrazide.

One other antiauxin has been described which is

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counteracted by auxin, namely *trans*-cinnamic acid, which was shown by van Overbeek (10) to have this interesting characteristic. Apparently its activity comes from its being an isomer of a compound showing growth-regulator activity. Maleic hydrazide, however, must not derive its activity in such a direct manner, because it has no side chain or acid group such as are required for growth regulators (4).

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## A Qualitative Analysis of Capsular Polysaccharides from *Cryptococcus neoformans* by Filter Paper Chromatography

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The technique of filter paper partition chromatography (1) has been shown to provide a convenient method for the determination of sugars present in a mixture (2) or of monosaccharide components of polysaccharides following hydrolysis (3).

This procedure has been applied to a qualitative analysis of the capsular polysaccharide which numerous investigators (4-10) have isolated from the pathogenic yeast, *Cryptococcus neoformans*. Aside from general qualitative chemical tests for carbohydrates, there have been no studies on the chemical composition of this polysaccharide.<sup>3</sup>

A representative polysaccharide was chosen from each of the three antigenic types (A, B, and C) of *C. neoformans* (5, 11, 12). The polysaccharides were isolated from neopeptone dialysate broth cultures by alcohol precipitation. Details of the method used for purification are presented elsewhere (5).

Twenty-mg samples of each polysaccharide were hydrolyzed in 0.5 ml 1 N H<sub>2</sub>SO<sub>4</sub> for 2.5 hr at 100° C.

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<sup>3</sup> Subsequent to the writing of this manuscript, personal communication with E. Drouhet and G. Segretain (4) has disclosed that they have recently submitted a paper in which it was shown that xylose, mannose, and a uronic acid were present in the polysaccharide with which they are working.

Neutralization of the hydrolysate was then effected by slowly adding solid barium carbonate. The precipitate was removed by centrifugation, and the supernatant fluid was applied to filter paper sheets for the analysis.

In performing the tests, strips of Whatman No. 1 filter paper 17.5 cm × 30-40 cm were employed for descending chromatograms. The lower end of each sheet was cut to a point to facilitate drainage of the solvent (13). Two solvents were employed with this method, *n*-butanol saturated with acetic acid (10%) and water (50%), as described by Partridge (2); the second solvent was ethyl acetate containing acetic acid and water in a 3 : 1 : 3 mixture (13).

TABLE 1

RELATIVE MIGRATION DISTANCES ( $R_F$  AND  $R_X$  VALUES) FOR HYDROLYZED POLYSACCHARIDES AND FOR REFERENCE MONOSACCHARIDES USING 4 DIFFERENT SOLVENTS

Reference monosaccharides and hydrolyzed polysaccharides	Solvents			
	Ethyl acetate ( $R_X$ )	<i>n</i> -Butanol ( $R_X$ )	Acetone ( $R_F$ )	Phenol ( $R_F$ )
D (+) xylose	1.00	1.00	0.67	0.47
L (-) fucose	1.07	1.22	0.68	.65
L (-) rhamnose	1.12	1.34	—	.61
D (-) ribose	1.11	1.16	0.72	.62
L (+) arabinose	0.95	0.90	.62	.56
D (+) galactose	.70	.71	.48	.46
D (+) glucose	.73	.77	.53	.42
D (+) mannose	.79	.86	.58	.51
D (-) fructose	.85	.88	.58	.55
D (+) glucosamine HCl	<i>T</i> *	.69	<i>T</i>	.63
D (+) galacturonic acid	.67	.47	.15	.13
D (+) glucuronic acid	0.68	0.46	0.20	0.12
Type A hydrolyzed polysaccharide	1.00 0.79 0.70	1.00 0.86 0.71 0.44	0.67 0.48 <i>T</i>	0.51 0.47 <i>T</i>
Type B hydrolyzed polysaccharide	1.00 0.79 0.70	1.00 0.86 0.71 0.44	0.67 0.49 <i>T</i>	0.51 0.47 <i>T</i>
Type C hydrolyzed polysaccharide	1.00 0.79 0.70	1.00 0.86 0.71 0.44	0.67 0.49 <i>T</i>	0.51 0.47 <i>T</i>

\* *T* = trailing spot for which no value was calculated.

On a pencil line drawn across the paper 2.5 cm from the edge of the trough, spots of the polysaccharide hydrolysates were placed with a micropipette calibrated to deliver 4  $\mu$ l. Spots of the hydrolysates and spots of known reference sugars were placed along the line at intervals of 2 cm. The concentration of the hydrolysates was increased by adding a spot, allowing it to dry, and applying another spot at the same point. The sugars employed for reference are indicated in Table 1.

The filter paper strips were irrigated with solvent