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GROWTH REGULATING SUBSTANCES IN EXTRACTS OF MAPLE AND PEAR FLOWER BUDS

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In recent years several investigators have suggested that growth inhibitors may be responsible for dormancy in plants. Evanari(4) has called attention to the wide distribution of growth inhibitors in various plant parts, and Stewart and Caplin(13) have reported inhibiting substances in maple buds, potato tubers, and onion bulbs. Hemberg(7,8) proposed that growth inhibitors in potato tubers and Fraxinus buds are directly involved with dormancy, since these substances disappeared when dormancy was broken either naturally or artificially. Hendershott and Bailey(9) extracted a substance from peach flower buds which inhibited the elongation of pea epicotyl sections. However, the amount of inhibitor did not decrease as the end of the dormant period approached; in fact, the level of inhibition did not decrease when the buds had opened and flowers were evident. A later report identified this substance as a cyanide compound(10). In the present study, dormant maple and pear flower buds have been shown to contain a substance which inhibits the elongation of etiolated pea epicotyl sections and does not give a test for cyanide. Paper chromatography has been used to separate naturally-occurring growth regulators in bud extracts.

Dormant buds of Acer saccharum Marsh, and Pyrus communis L. var. E. J. Taylor were extracted at 1°C. for twenty hours with three changes of anhydrous ethyl ether. The combined ether extracts were evaporated to dryness and the residue stored at 5°C. in a dessicator over calcium chloride. The pea straight growth test was used to detect growth effects of substances obtained from buds. Pea seedlings were grown in white sand in a darkroom maintained at 25°C. and 80% relative humidity. A section was cut just below the plumule of an epicotyl when the third internode was two to five cm. long. Cutting was done in weak red light, using a tool consisting of two razor blades spaced 4.4 ± 0.2 mm. apart. Sections were randomly distributed in

experimental solutions.

The standard solution used in growth tests contained 1.0 p.p.m. 3-indoleacetic acid (IAA), 2.0% sucrose, and 0.05 M. phosphate buffer at pH 5.7. The experimental solutions contained, in addition, a measured quantity of bud extract or a zone cut from a paper chromatogram of a bud extract. Ten pea sections were used with each test solution and after twenty hours in a darkroom growth was measured by using a photographic enlarger which magnified ten times.

Growth Tests. Growth tests were made in Petri dishes containing a filter paper disc and five ml. of test solution. Bud extracts were suspended in five ml. of distilled water and filtered before addition to the standard solution at rates of 0.5, 1.0, and 2.0 ml. The results of typical tests using maple buds collected February 6, 1957 and pear buds collected February 2, 1957 are summarized in Table I. These tests demonstrate the amount of growth inhibition of pea sections obtained with maple and pear bud extracts.

Chromatography and Growth Tests. Bud extracts were fractionated by means of ascending paper chromatography using 16 x 2½-inch strips of unwashed Whatman #1 filter paper. Good separation was obtained with 80% isopropanol as the developing solvent. A mixture of water, ethanol, butanol, and isopentanol (4:2½:1:1) also gave satisfactory results. Ether extracts were redissolved in ether with one ml. of water added. The ether was evaporated and the water extract was streaked across one end of a paper strip and allowed to dry. As a standard practice the development was carried out in the darkroom and strips were equilibrated for at least four hours prior to lowering into the solvent.

Growth tests were conducted in small covered dishes containing zones cut from a chromatogram, two ml. of the standard solution, and ten pea epicotyl sections. Results of a typical test using an extract of dormant maple flower buds chromatographed in 80% isopropanol are presented in Figure 1.

The inhibition zones cover a wide range of R_fs, but those statistically significant are found only between R_fs 0.50 and 0.80. An improved separation

TABLE I

EFFECT OF MAPLE AND PEAR FLOWER BUD EXTRACTS ON GROWTH OF PEA SECTIONS, IN MILLIMETERS AND PERCENT

Plant	Ml. of extract added			
	0.0	0.5	1.0	2.0
Maple (400 buds/ml.)	6.92 ± 0.13* 100.0%	5.80 ± 0.03 56.6%	5.72 ± 0.04 52.5%	5.29 ± 0.04 35.3%
Pear (160 buds/ml.)	8.31 ± 0.01 100.0%	7.33 ± 0.09 75.0%	7.18 ± 0.09 71.1%	6.93 ± 0.09 64.8%

*Standard error of the mean.

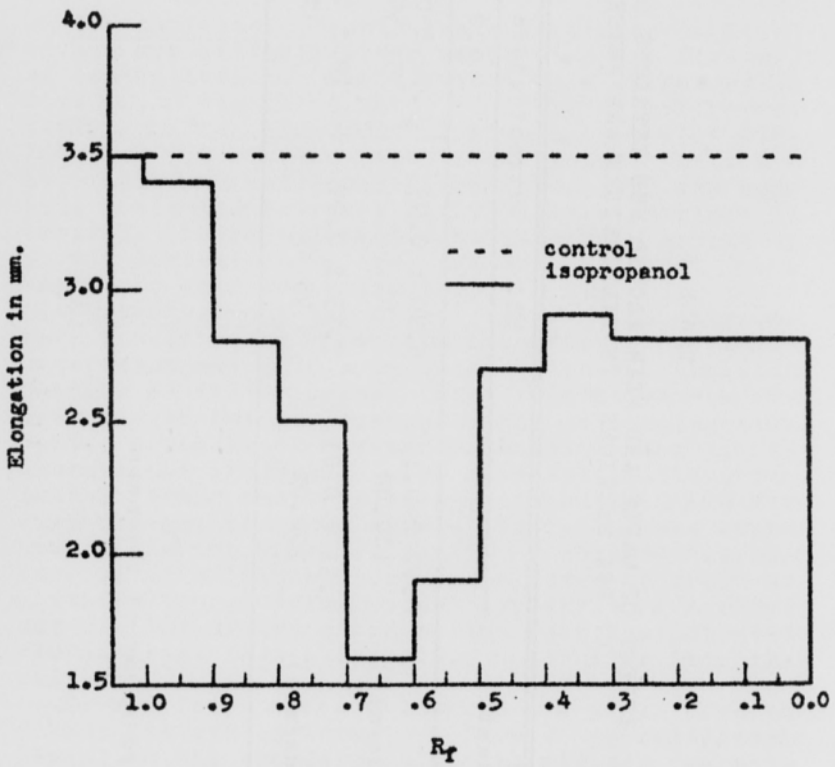

Figure 1. Results of a growth test using a chromatogram of an extract of dormant maple flower buds developed in 80% isopropanol.

was obtained by eluting these zones overnight with water at 5°C., evaporating the eluate to dryness over steam, and rechromatographing in the alcohol mixture. Growth tests with zones cut from these chromatograms demonstrated that inhibiting substances had completely masked the presence of a growth-promoting substance, as shown in Figure 2. Compounds fluorescing in ultraviolet light were located at the higher R_fs and these zones were used for separate growth tests.

Results of growth tests using six chromatograms prepared by two-step chromatography are presented in Table II. Two features are noteworthy: A growth-promotion zone most prominent at R_f 0.97, and a growth-inhibition zone between R_fs 0.60 and 0.80. No significant activity was recorded below R_f 0.50.

CHARACTERIZATION OF THE GROWTH-ACTIVE SUBSTANCES

Growth Promotion Zone. The zone above R_f 0.97 fluoresced pink under ultra-violet light, gave an acid reaction when streaked with a universal indicator, and an ash color when sprayed with Salkowski reagent(11). The zone just beneath fluoresced light purple, gave no acid reaction, and gave a faint pink color with Salkowski reagent. Synthetic IAA chromatographed with the alcohol mixture gave a pinkish fluorescence in ultra-violet and was acidic at R_f 0.97. Apparently the growth-promotion obtained at R_f 0.97 was a response to IAA in the bud extract.

The same growth-promoting zone was evident in chromatograms of non-dormant buds, with the growth response being more pronounced. Apparently, the level of active IAA increases as buds pass from the dormant to the non-dormant state, as reported previously(6).

Growth Inhibition Zone. Salkowski tests and examination in ultra-violet light failed to give any suggestion of an auxin type of compound in the zone of inhibition. Tests for cyanide were made after hydrolysis with sodium hydroxide and the addition of ferric chloride, by adding hydrochloric acid and ferrous sulfate. The presence of cyanide in this test is indicated by the formation of prus-

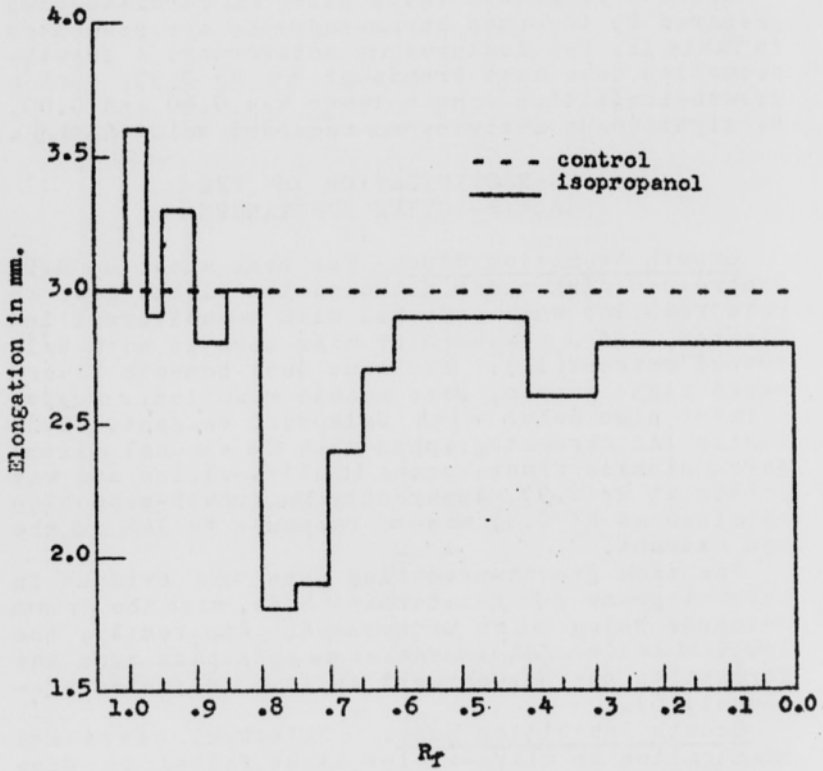

Figure 2. Growth test results with an extract of dormant maple flower buds using a two-step chromatographic process. The inhibition zone, between F_{rs} 0.50 0.80, of the 80% isopropanol chromatogram was rechromatographed in the alcohol mixture.

TABLE II

GROWTH TEST RESULTS OF SIX CHROMATOGRAMS OF 100-150 DORMANT AND NON-DORMANT MAPLE FLOWER BUDS DEVELOPED IN THE ALCOHOL MIXTURE AFTER A PRELIMINARY CHROMATOGRAPHING IN 80% ISOPROPANOL

Test	R_f .50	.60	.65	.70	.75	.80	.85	.90	.95	.97
Dormant buds										
#1		I*	I	I	I			P*	P	P
2		I	I	I	I					P
3		I	I	I	I	I	I			
4	I	I	I	I	I			P	P	P
Non-dormant buds										
#4		I		I	I	I			P	
6					I		P			P

*I -- Inhibition; P -- Promotion. Results significant at the one per cent level.

sian blue. No evidence of cyanide compounds was obtained with eluates of the inhibition zones.

Elliott and Leopold(2) proposed that dormancy in oats may be due to inhibitors that antagonize sulfhydryl-containing enzymes. An -SH compound could competitively inhibit a sulfhydryl enzyme necessary for growth. When a drop of iodine-azide solution(5) was applied to the chromatograms in the zones of inhibition, the deep brown color of the solution quickly disappeared. This is a specific reaction for thicketones (R=S) and thiols (R-SH). Zones on chromatograms that indicated the presence of thicketones and/or thiols are given in Table III, as are those that gave evidence of being acid.

With dormant buds, zones showing growth inhibition gave a positive test for divalent sulfur compounds. Also, there was a close correlation between the presence of sulfur and acidic substances, suggesting that thiols rather than thicketones were involved. This was substantiated by the failure of the zone of inhibition to catalyze the iodine-azide reaction after mild oxidation with hydrogen peroxide. Apparently, a thiol compound occurs in the zone of inhibition.

The close correlation between inhibition and the presence of thiol compounds in dormant buds was not evident with non-dormant buds (Table III). The level of inhibition in non-dormant buds dropped, but there was no corresponding loss in thiols. Similar solubility properties and R_f values indicate that the thiol compound in both dormant and non-dormant buds may be glutathione. Glutathione is soluble in water and insoluble in ether, occurred between R_fs 0.58 and 0.65 when chromatographed in the alcohol mixture, and catalyzed the iodine-azide reaction.

DISCUSSION

Considerable evidence exists which indicates that dormancy in buds results from the presence of substances specific for growth inhibition. The earlier suggestion that auxins at relatively high concentrations cause dormancy has been discredited by several investigators(12). Chan-Thom(1) showed that auxin concentrations necessary for inhibition never occurred in dormant pear buds. Hemberg (7,8)

TABLE III

CHROMATOGRAPHIC SPECTRUM OF EXTRACTS OF DORMANT AND NON-DORMANT MAPLE FLOWER BUDS FOR ACIDITY AND IODINE-AZIDE TESTS. RESULTS ARE FOR SIX CHROMATOGRAMS DEVELOPED IN THE ALCOHOL MIXTURE WITH A PREVIOUS CHROMATOGRAPHING IN 80% ISOPROPANOL

	Dormant						Non-Dormant			
Test No.	Acidity				Iodine-azide		Acidity		Iodine-azide	
	1	2	3	4	3	4	5	6	5	6
R_f										
0.97	x	x	x	x			x	x		
.95							x		x	
.90							x		x	
.85					x		x	x	x	x
.80					x		x	x	x	x
.75	x	x	x	x	x	x	x	x	x	x
.70	x	x	x	x	x	x	x	x	x	x
.65	x	x	x	x	x	x	x	x	x	x
.60	x	x	x	x	x	x	x	x	x	x
.55			x	x		x		x		x
.50						x		x		x

found a connection between inhibitors and dormancy in potato and Fraxinus buds and established that the auxin content does not change either during dormancy or immediately thereafter. According to Leopold(11) dormancy results from the occurrence of acidic inhibitors in buds. In the presence of these inhibitors endogenous auxin is ineffective. The onset of bud development is accompanied with the loss of growth inhibitors and the production of glutathione.

In this study, dormant flower buds of maple are shown to contain substances that promote and inhibit elongation of pea epicotyl sections. The growth-promoting substance apparently is 3-indoleacetic acid which increases in growth activity as dormancy is lost. The growth-inhibiting substance is not a cyanide-containing compound. On paper chromatograms growth inhibition is associated with an acidic zone of one or more compounds which contain thiol groups. As dormancy passes, inhibition in this zone disappears but the sulfhydryl reaction remains and extends over a wider zone on the chromatograms.

The persistence of the sulfhydryl reaction in chromatograms of non-dormant buds suggests that the thiol detected in dormant buds may not be related directly to the inhibitor. This thiol may be glutathione since these compounds give similar R_f values when chromatographed in the same manner and possess similar solubility properties. Glutathione increase has been reported as dormancy is lost(3) and this could account for the broad sulfhydryl zone on the chromatograms.

The possibility exists that the thiol present in dormant buds is not glutathione but a specific substance responsible for dormancy -- acting as a growth inhibitor by competitive interference with physiological activity of intracellular SH-groups. Since the two compounds chromatograph similarly, the proposed thiol inhibitor could be structurally similar to glutathione and serve as a precursor for this compound.

SUMMARY

Ether extracts of dormant flower buds of maple and pear contain one or more substances which in-

hibit the elongation of etiolated pea epicotyl sections. Paper chromatograms of bud extracts contained a growth-promoting substance, probably IAA, and a growth-inhibiting zone. The latter was acidic, contained no cyanide, and gave a reaction characteristic of thiols. As dormancy passed, inhibition disappeared but the sulfhydryl reaction remained. Glutathione may be involved in the sulfhydryl reaction, although the thiol occurring during dormancy may not be identical with that occurring after dormancy ends.

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