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Tannins as Gibberellin Antagonists[1]

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MARY RITZEL CORCORAN, T. A. GEISSMAN, AND BERNARD O. PHINNEY
Department of Biology, San Fernando Valley State College, Northridge, California 91324 and Departments of Chemistry and Botany, University of California, Los Angeles, California 90024

ABSTRACT

Fourteen chemically defined hydrolyzable tannins and six impure mixtures of either condensed or hydrolyzable tannins were found to inhibit the gibberellin-induced growth of light-grown dwarf pea seedlings. The highest ratio of tannins to gibberellic acid tested (1000:1 by weight) inhibited from 80 to 95% of the induced growth for all tannins tested except for two monogalloyl glucose tannins which inhibited only 50% of the induced growth. The lowest ratio tested (10:1) inhibited the induced growth by less than 25% except for the case of terchebin where 50% inhibition was found. The inhibition of gibberellin-induced growth was found to be completely reversed by increasing the amount of gibberellin in three cases tested. Tannins alone did not inhibit endogenous growth of either dwarf or nondwarf pea seedlings. Eight compounds related to tannins, including coumarin, *trans*-cinnamic acid, and a number of phenolic compounds were also tested as gibberellin antagonists. Most of these compounds showed some inhibition of gibberellin-induced growth, but less than that of the tannins. At the highest ratio (1000:1) the greatest inhibition was 55%; at the lowest ratio (10:1) no more than 17% was observed. These compounds did not inhibit endogenous growth, and the inhibition of gibberellin-induced growth could be reversed by increasing the amount of gibberellin in two cases tested.

Six chemically defined tannins were found to inhibit hypocotyl growth induced by gibberellic acid in cucumber seedlings. Growth induced by indoleacetic acid in the same test was not inhibited. The highest ratio of tannin to promotor tested gave strong inhibition of gibberellic acid-induced growth, but actually enhanced the growth induced by indoleacetic acid. This difference in action suggests a specificity between the tannins and gibberellic acid.

Tannins are well known constituents of many plants. They are of widespread occurrence taxonomically and have been reported from all parts of the plant body (2, 14). Although tannins have been known in plants for a long period of time, their function is obscure. It has been suggested that they act to prevent infection in the plant. As early as 1911 and 1912 tannins were shown to retard the growth of many plant parasites (4, 5). Correlation between specific disease resistance and tannin content has been noted (23). Since these early reports, however, the direct effect of tannins in inhibiting disease has been questioned (10, 33, 35).

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Several authors have noted inhibitory or stimulatory effects of tannins on plant growth and development. Treatment with tannins has been reported to inhibit cambium tissue culture (17), root growth (13, 21), germination (12), and to cause growth disturbances (31, 32). In contrast, there are reports of stimulation of root growth (20, 29), seedling growth (29), and germination (12). More recently Zinsmeister (36, 37) reported that Chinese tannins, gallic acid, and *d*-catechin reduced the IAA-induced growth of oat coleoptile sections. The three substances influenced growth in a similar manner. The endogenous growth was either unaffected or reduced by no more than 8.3%. The IAA-induced growth was much more strongly inhibited, and the inhibition could not be overcome by increasing the amount of IAA.

The present paper describes the effect of tannins on gibberellin-induced growth in two test systems and presents evidence indicating that tannins are effective gibberellin antagonists.

MATERIALS AND METHODS

Pea Seedling Test. Pea seedlings (*Pisum sativum* L., cvs. Little Marvel and Alderman) were used. Little Marvel is a dwarf strain, Alderman, a tall strain. The assay was conducted as described earlier (8) with modifications already discussed (7).

Cucumber Hypocotyl Test. Seedlings of cucumber, *Cucumis sativus* L. cv. National Pickling were used. The test was adopted from Katsumi *et al.* (19) with a few changes previously described (6).

Tannins. Twelve crystalline tannins used in this study were obtained from O. T. Schmidt, University of Heidelburg. Two crystalline tannins, β-1-O-galloyl-D-glucose and β-D-1,6-di-O-galloylglucose, were obtained from M. A. Joslyn, University of California, Berkeley as were also the three purified extracts from carob. The walnut pelicle tannin was provided by L. Jurd of the United States Department of Agriculture, Albany, California. The tannic acid and tara tannin were obtained from Mallinckrodt.

The tannins were added to distilled water and, if necessary, the mixture heated to dissolve the tannins.

RESULTS

Effects of Tannins and Some Related Compounds on GA_3-induced Growth of Dwarf Peas. Fourteen tannins, coumarin, *trans*-cinnamic acid, and seven related phenolics were tested for their abilities to inhibit gibberellin-induced growth in the dwarf pea assay. Three concentrations of each compound were mixed separately with aqueous GA_3 containing 0.05% Tween-20 (sorbitan polyoxyethylene monolaurate). Each seedling received 50 ng GA_3. The weight ratios of tannins or related compounds to GA_3 were 10:1, 100:1, and 1000:1. These mixtures were applied to the seedlings, and measurements

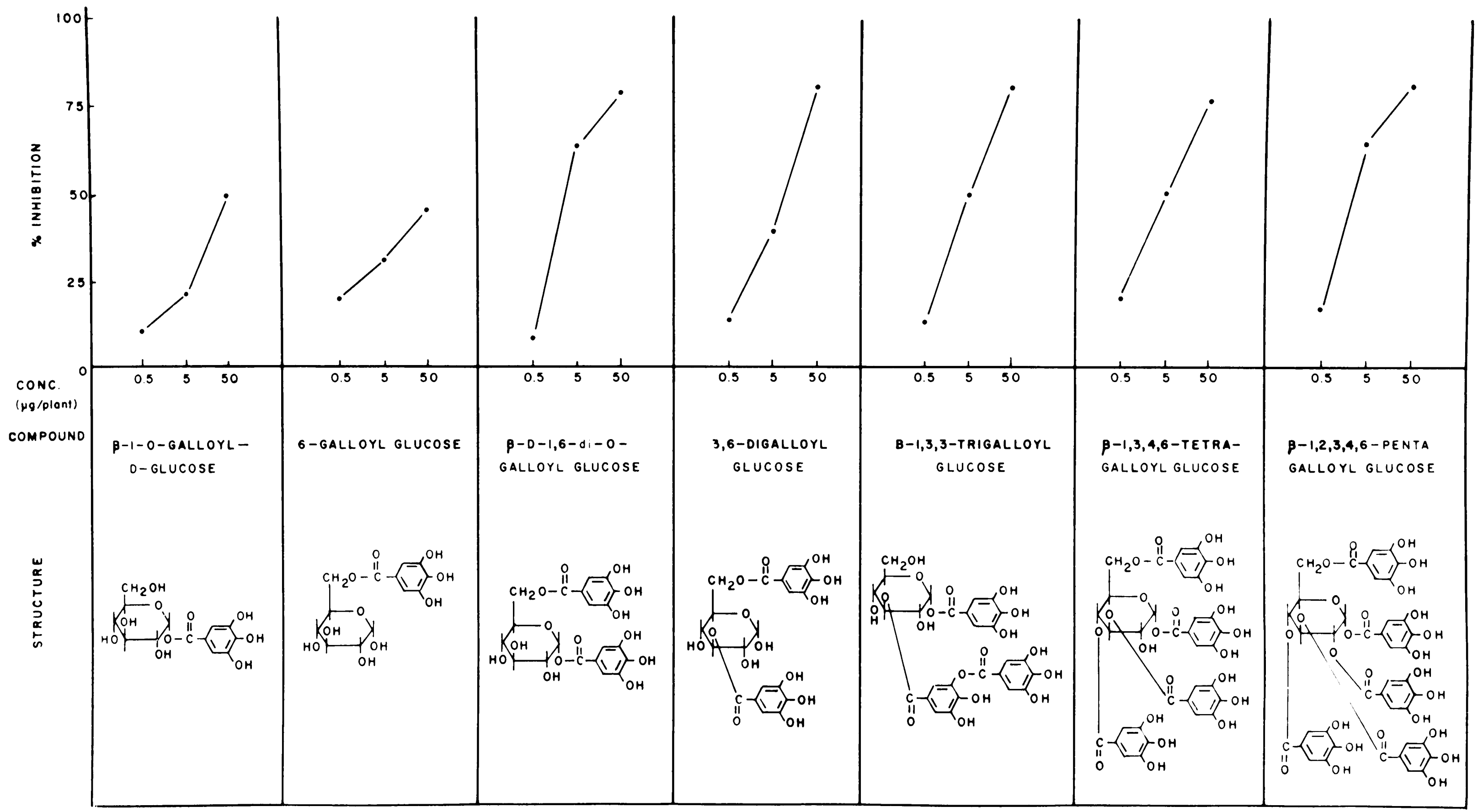
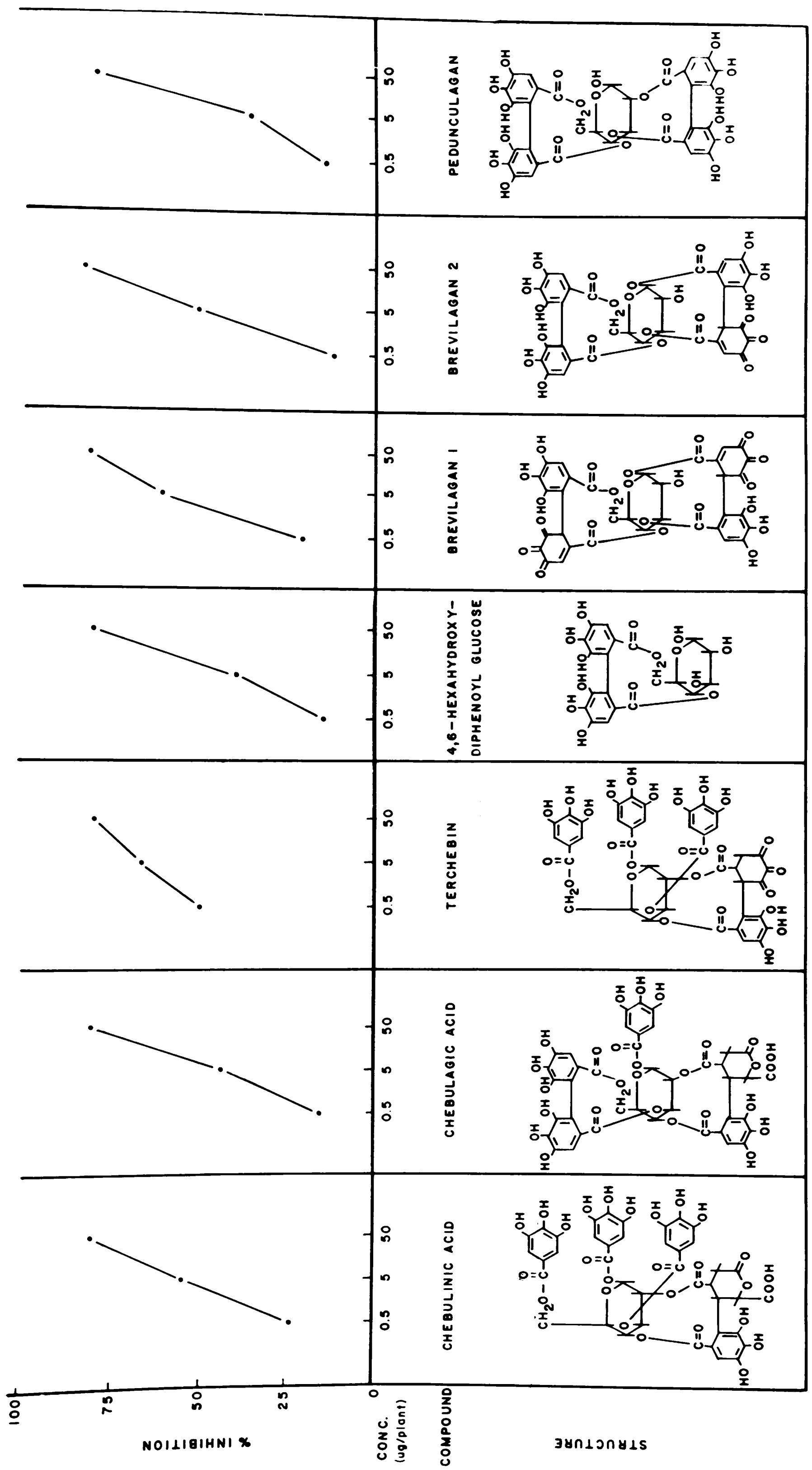


FIG. 1. Effect of tannins and related compounds on growth induced by GA_3. Seedlings received 50 ng GA_3 and three concentrations of tannin or related compounds. Inhibition is given as percentage reduction of the gibberellin-induced growth. The data represent the average values from two to six replica runs. Each run consisted of the three inhibitor concentrations mixed with GA_3 and each mixture was assayed on 10 seedlings.



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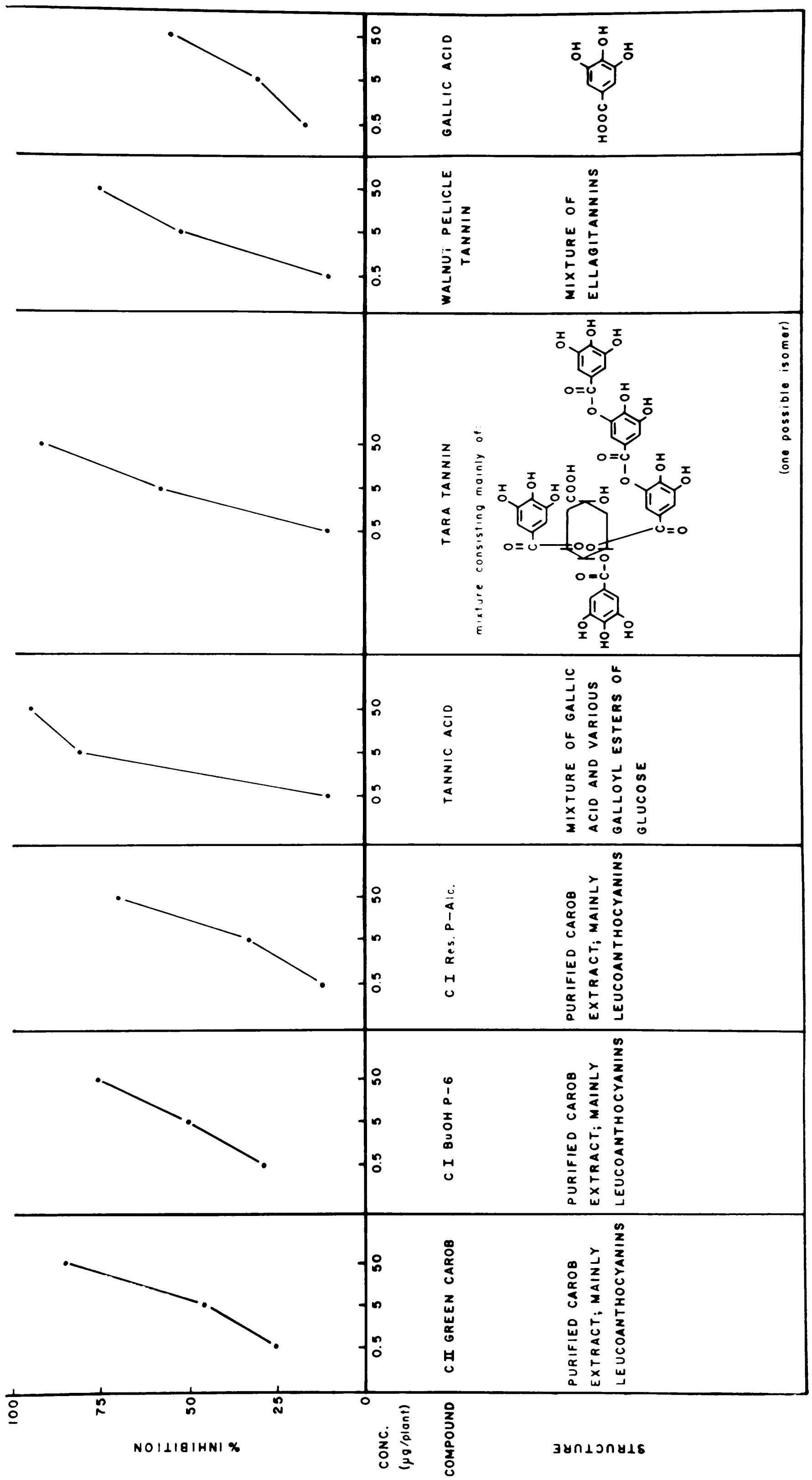

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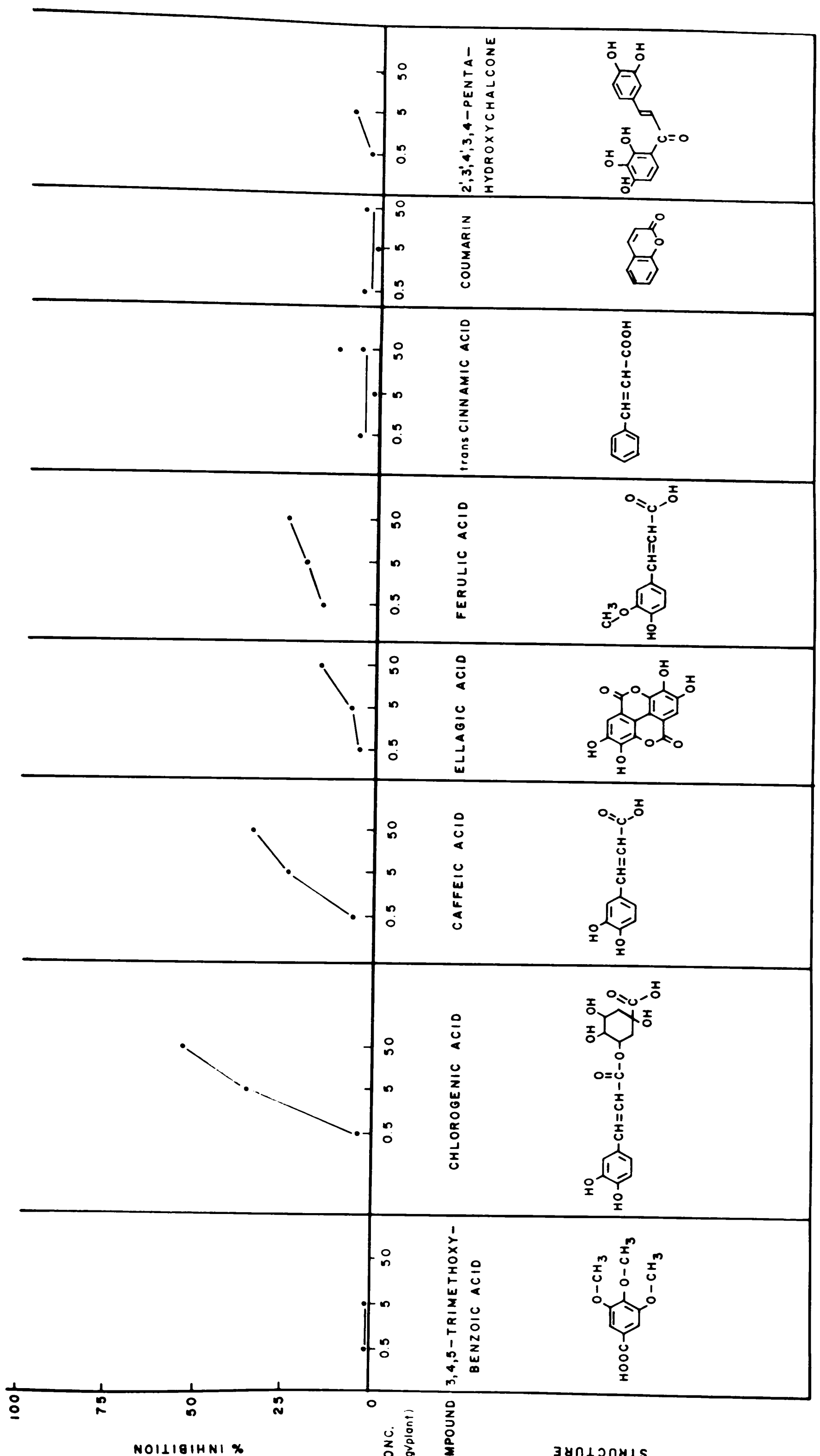

FIG. 1.—*Continued*

Table I. *Effect of Tannins on Endogenous Growth of Alderman Pea Seedlings*

Treatment[1]		Shoot Length[2]
Tannin	Concn	
	μg/plant	*mm*
	0	187.1 ± 7.2
β-1,2,3,4,6-Pentagalloyl glucose	50	178.7 ± 11.4
Chebulinic acid	50	186.3 ± 4.8
4,6-Hexahydroxydiphenoyl glucose	50	181.3 ± 6.0
Brevilagin 2	50	183.2 ± 6.8
Pedunculagin	50	190.9 ± 5.4
CII Green carob Res. P-Alc	50	193.2 ± 6.1
Walnut pelicle tannins	50	185.3 ± 8.0

[1] Each seedling received a single application of 0.1 ml.

[2] Averages and standard errors of 10 plants of Alderman peas.

were taken 5 to 7 days later. In the absence of GA_3, the tannins or related compounds had no effect on the endogenous growth of the seedlings. All of the tannins reduced the growth caused by GA_3 (Fig. 1). Terchebin, in particular, was especially inhibitory. A ratio of 10:1 (terchebin-GA_3) caused a 50% reduction of the induced growth. The two monogalloyl glucose compounds were less inhibitory than those with more than one gallic acid group and were about as inhibitory as gallic acid alone. The other compounds were markedly less inhibitory than the tannins, and the two concentrations of 3,4,5-trimethoxy benzoic acid tested showed no inhibition at all. Gallic acid occupies an interesting position in the middle range of activity between most of the phenolics and the tannins. It is a phenolic and is one of the constituents of tannins.

Effect of Tannins on the Endogenous Growth of Nondwarf Peas. Each of seven tannins was mixed with Tween-20 and tested on Alderman pea seedlings. Nondwarf peas were used because an inhibitor might show a greater effect where more endogenous growth is involved. The concentration of tannins was equal to the highest concentration previously used with dwarf peas. None of the tannins affected the endogenous growth of the seedlings (Table I).

Reversibility of Inhibition. Overcoming the action of an inhibitor by increasing the amount of promotor is consistent with the interpretation that the inhibitor and promotor are involved in the same system. The reversibility of the inhibitor effect was tested by adding increasing amounts of GA_3 to constant amounts of tannins or related compounds. The gallotannin, β-1, 3,3-trigalloyl glucose, and the ellagitannins brevilagan 1 and pedunculagan were tested in this way. A constant amount of 5 μg per plant was used. The phenolics, gallic acid and caffeic acid, were also tested using 50 μg per plant. The amount of GA_3 used ranged from 0.005 to 50 μg per plant. It was added alone or in solution with the inhibitors. The inhibitory activity of all five compounds was reversed by GA_3. The data for trigalloyl glucose are graphed in Figure 2. This tannin inhibited most of the growth induced by the two lowest GA_3 concentrations. The inhibition was completely reversed by the highest concentration of GA_3.

Effect of Tannins on IAA- and GA_3-induced Growth of Cucumber. The growth of cucumber hypocotyls is promoted similarly by GA_3 and IAA. The cucumber hypocotyl test then becomes a useful one to detect any differences in the effect of inhibitors on growth induced by IAA or GA_3. Six tannins were tested for their ability to inhibit the growth induced by these promotors on cucumber hypocotyls. Seedlings received 1 μg of IAA or GA_3 either alone or in solution with each of three concentrations of the tannins. The same amounts of tannin (0.5, 5, and 50 μg/plant) were tested alone. Chebulagic acid, chebulinic acid, pedunculagin, brevilagan, trigalloyl glucose, and penta-galloyl glucose were all tested with both IAA and GA_3 in this test. Data for pentagalloyl glucose are shown in Figure 3. At the highest concentration, the tannin inhibited over 50% of the GA_3-induced growth and enhanced the IAA-induced growth by almost doubling it. Similar results were found for the other tannins used.

DISCUSSION

Evidence presented in this paper shows that several tannins of established structure are potent inhibitors of gibberellin-induced growth. The inhibition can be overcome with additional GA_3, and there is no effect on endogenous growth. Thus, tannins qualify as gibberellin antagonists. Several groups of tannins are represented in this study. Among the hydrolyzable tannins, a number of examples of both gallotannins and ellagitannins were used. From the condensed tannins, mixtures obtained from carob extracts (25) and consisting mainly of leucoanthocyanin were used.

Both hydrolyzable and condensed tannins are widely distributed in the plant kingdom. Extensive surveys of the occurrence of tannins have been carried out by Bate-Smith (2), who analyzed extracts of crushed leaf material by paper chromatography. He found a characteristic distribution of different tannins among various groups. For example, ellagitannins are found only in dicots of certain orders, whereas leucoanthocyanins are found in ferns, gymnosperms, and angiosperms although not in nonvascular plants. The amount of tannins in

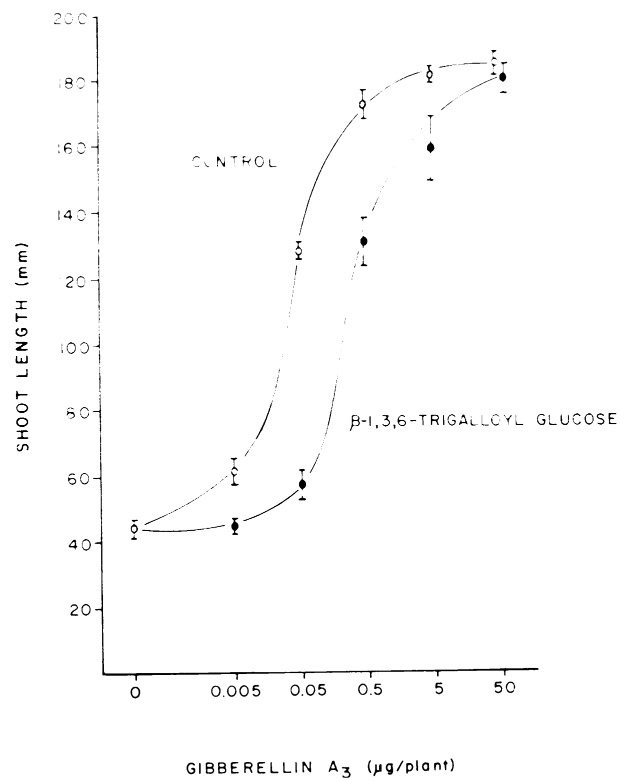

FIG. 2. Effect of gibberellic acid on the growth of pea seedlings in the presence or absence of 5 μg β-1,3,6-trigalloyl glucose per plant. Each seedling received a total volume of 10 μl of a solution containing 0.05% Tween-20. Each point represents the mean and standard error of 10 seedlings.

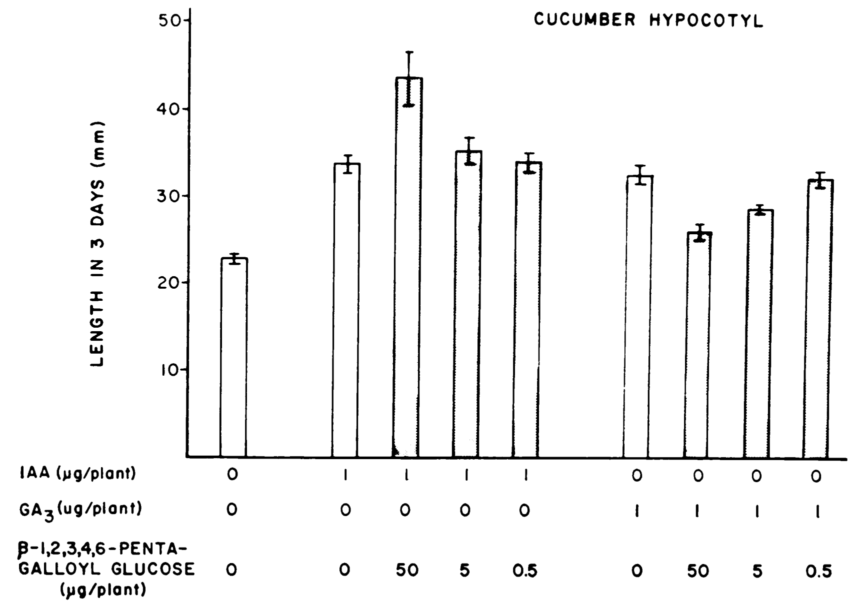

FIG. 3. Interaction of a gallotannin with GA_3 or IAA in hypocotyl growth of cucumber. The growing tip of each seedling received 10 μl of ethanol alone or combined with IAA or GA_3 or mixtures of IAA or GA_3 with different concentrations of the tannin. The mean and standard error from 10 plants are shown for the total final length of the marked segment.

plants varies greatly. Only a relatively few contain large enough quantities to make them useful as commercial sources. Tannins may differ in kind and amount in different parts of the plant and in the same organ in different ages (16).

The widespread occurrence of substances which are strong inhibitors of gibberellin-induced growth causes a re-evaluation of the possible role of tannins in the economy of the plant. Gibberellins appear to play a dominant role in plant growth, particularly that of shoots. It is possible that tannins play a growth-regulatory role by inhibiting growth caused by gibberellin in the plant. A report by Hillis and Swain (16) presents information suggestive of a regulatory role by tannins. They followed changes in total phenols, leucoanthocyanins, and flavanols in plum leaves during a growing season. They found that the proportions of leucoanthocyanin and flavanols to total phenols were less in younger leaves. The amounts increased rapidly until the leaves reached maximum size and then tended to decrease. This correlation of growth stoppage with maximum amounts of tannins might be expected if tannins were acting to inhibit growth.

Tannins are well known to be inhibitors of enzymes and proteins in general (33, 35). Several kinds of reactions are involved and they result in the formation of cross-links between the protein and the tannins (33). The action of tannin in gibberellin-induced growth might be dismissed as the result of nonspecific bonding to GA_3 or some intermediate of GA_3-stimulated growth. However, the difference in effect of the tannins on auxin-induced growth *versus* that of gibberellin-induced growth argues against such nonspecificity. Rather, it would suggest that GA_3 or the system upon which GA_3 acts is particularly sensitive to tannins or readily available to tannin action.

While the tannins used in this study failed to inhibit IAA-induced growth in cucumber hypocotyls, there is evidence that tannins can inhibit such growth in oat coleoptile sections (36, 37). In this test "Chinese Tannin" inhibited IAA-induced growth by about 40%. Gallic acid and *d*-catechin, components of tannins, caused a 20% reduction of IAA-induced growth in the same test. In each report the maximum inhibition was considerably less than the 80 to 95% inhibition of GA_3-induced growth shown by most of the tannins in the present study.

Most of the phenolics and related compounds used here have previously been shown to be inhibitory in growth processes such as seed germination (11), elongation of *Avena* coleoptile, or curvature of slit pea stems (26–28, 34). Usually these reports also mentioned an enhancement of auxin phenomena at low concentrations of the inhibitor. The enhancement is similar to the enhancement of IAA-induced hypocotyl growth of cucumber reported in this paper.

There have been several reports of plant extracts which inhibit gibberellin-induced growth. Some of these extracts may contain tannins which might account for much if not all of the growth-inhibiting property. Radley (30) reported an inhibitor from *Fucus vesiculosus* which reduced the growth induced by GA_3 in dwarf peas, but did not reduce the endogenous growth. Similarly, Bünsow (3) found extracts from *Aesculus hippocastanum, Lapsana communis,* and *Mycelis muralis* which showed the same properties in the same assay. Jennings (18) reports that an extract from *Ecklonia radiata* will also reduce GA_3-induced growth but not endogenous growth in the dwarf maize assay.

Another case of similarity between the properties of a plant extract and those of tannins involves extracts from carob. Both these extracts and tannins are GA_3 antagonists in that they inhibit GA_3-induced growth but not endogenous growth. The inhibition can be overcome with additional GA_3 (8, 9). The dry weight ratio of carob fraction C to GA_3 for 50% inhibition is 17:1 (8). Several tannins (β-D-1, 6-di-O-galloyl glucose, pentagalloyl glucose, terchebin, brevilagan 1, and tannic acid) have a ratio close to that. The difference in activity between GA_3 and IAA on cucumber seedlings reported here for tannins has also been shown for inhibitory fractions B_1 and C from carob in the cucumber test and also in two other auxin tests (6). Two tannins (β-1-O-galloyl-D-glucose and β-D-1, 6-di-O-galloyl glucose) included in this study have been isolated from carob fruit (25), and one of them was among the more inhibitory tannins. It has been suggested that these carob inhibitors consist mainly of abscisic acid (1, 22, 24). However, chromatographic, biological, and solubility differences exist between abscisic acid and the carob inhibitors (7).

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