Inhibitory Action of Five Tannins on Growth Induced by Several Gibberellins

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ABSTRACT

The following tannins, Chinese gallotannin, 1,2,3,4,6-pentagalloyl glucose, chebulinic acid, procyanidin dimers, and procyanidin trimers were tested and found to be antagonists of seven gibberellins (GAs). Each tannin inhibited the growth induced by any of the gibberellins GA1, GA3, GA4, GA7, GA8, GA12, and GA14 in the dwarf pea assay. Endogenous growth was not affected. The highest ratio of tannin to gibberellin tested (1000:1 by weight) inhibited from 60 to 95% of the induced growth for all tannins and all gibberellins tested. The tannins were particularly inhibitory against GA1 and GA4, where a ratio of 10:1 (tannins: GA by weight) resulted in up to 85% growth reduction. Inhibition could be completely reversed by increasing the amount of gibberellin in all combinations studied. The procyanidin dimers and trimers were the first purified components of condensed tannins to be tested in this system and were potent inhibitors particularly against growth induced by GA4 and GA14. Inhibition by these compounds along with similar inhibition by previously tested hydrolyzable tannins demonstrates that the effect is general to tannins of all classes.

Research reported by Corcoran, et al. (3) established specific, chemically defined tannins as GA3 antagonists in that they will inhibit GA3-induced growth, have no effect on endogenous growth and the inhibition can be completely reversed with additional GA3. There was also evidence of specificity because growth induced by IAA was not reduced, although growth induced by GA3 in the same system was blocked. Harada and Nakayama (8) have since reported that tannic acid inhibits GA3-induced growth in rice seedlings and has no effect on their endogenous growth. All work was restricted to interaction with GA3 only; no other gibberellins were used. The crystalline tannins used were all hydrolyzable tannins and only crude mixtures of condensed tannins were employed.

The purpose of this study is to examine the interaction of five tannins with several of seven gibberellins. Three of the tannins had been tested previously with GA3 and are now used with gibberellins other than GA3. Two others which are chemically defined components of condensed tannins had not been previously investigated and are studied with GA4 as well as other GAs.

MATERIALS AND METHODS

Pea Seedling Test. Pea seedlings of a dwarf strain (Pisum sativum L., cv. Little Marvel) were used. Seeds were planted without soaking in greenhouse flats which were placed in an incubator at 20°C with alternating 12-hr periods of light and dark. Seedlings were treated 7 days after planting by applying a 0.01-ml droplet of test solution to the apical region. Measurements of the shoot length were taken 5 to 7 days after treatment. More complete information on the assay is given in the original description (4) and later modification (2).

Tannins. The five tannins for this study were supplied by Dr. E. Haslam, University of Sheffield. Procyanidin dimers and trimers are purified components of condensed tannins and were obtained from Aesculus hippocastanum. Their extraction, isolation, and chemical analysis have been published (10, 17). The dimers consist of a mixture of fractions B-1 and B-5 (17); their structures are shown in Figure 1. The procyanidin trimers consist of a mixture of fractions D-1 and D-2; their structures as reported previously (10) are shown in Figure 3. Tannic acid which is also known as Chinese gallotannin is an extract of galls produced by Aphis chinensis on leaves of Rhus semialata. It consists of a mixture of a gallotannin with smaller amounts of gallic acid, m-digallic acid, and trigallic acid. The gallotannin portion is based on a β-penta-o-galloylglucose core to which three to five additional galloyl groups are attached as depside linkages (9). The arrangement of these galloyl groups is not known with certainty. One possible isomer of a gallotannin containing eight galloyl groups is shown in Figure 5. The tannic acid used in this study is of a lighter color and hence perhaps greater purity than the tannic acid tested previously (3). In tests with GA3 its inhibitory strength was similar to that of the tannic acid in the earlier report (3) so this paper includes only investigations with gibberellins other than GA3. The final two tannins β-1, 2, 3, 4, 6-pentagalloyl glucose and chebulinic acid were tested previously with GA3. In all cases the tannins were added to distilled H2O and, if necessary, the mixture was heated to dissolve the tannins.

Gibberellins. Gibberellins A1, A4, A9, A12, A13, and A14 were obtained from Dr. J. MacMillan, University of Bristol. The final experiments attempting to reverse inhibition of GA3-induced growth were performed with a second sample of GA1 also sent by Dr. J. MacMillan. It consisted of a mixture of 85% GA1 and 15% GA2. The GA2 was purchased as the sodium salt from Calbiochem, Los Angeles.

RESULTS

Tannins and Gibberellin-induced Growth. Five tannins were tested for their ability to inhibit growth induced by several of seven gibberellins in the dwarf pea assay. Three concentrations of each tannin were mixed separately with aqueous GA containing 0.05% Tween 20. Each seedling received 0.5 or 0.05 ng of GA. These mixtures were applied to seedlings and measurements of shoot length were taken 7 days later. In the absence of GA the tannins had no effect on the endogenous growth of the seedlings. The effect of procyanidin dimers combined with each of four GAs is shown in Figure 1, and the chemical structure of the
The dimers with dimers in natural (position of structure at different concentrations of GAs. Each solution of values received 0.05% Tween 20. Inhibition is given as percentage reduction of the gibberellin-induced growth, and points represent the average values of means from two replicate runs. Each run tested the three inhibitor concentrations mixed with GA along with 0 and GA controls. Each solution was assayed on 10 seedlings. The individual means used to determine the per cent inhibition were compared with the mean of the GA control. All means giving inhibition above 26% were significantly different from the mean of the GA control at the 5% level. All means giving less than 15% inhibition were not significantly different.

**Fig. 1.** Effect of procyanidin dimers on growth induced by each of four GAs. Seedlings received 0.05 or 0.5 ng of GA and one of three concentrations of tannin in a total volume of 0.01 ml. The solutions contained 0.05% Tween 20. Inhibition is given as percentage reduction of the gibberellin-induced growth, and points represent the average values of means from two replicate runs. Each run tested the three inhibitor concentrations mixed with GA along with 0 and GA controls. Each solution was assayed on 10 seedlings. The individual means used to determine the per cent inhibition were compared with the mean of the GA control. All means giving inhibition above 26% were significantly different from the mean of the GA control at the 5% level. All means giving less than 15% inhibition were not significantly different.

**Fig. 2.** Structures of procyanidin dimers as previously reported (16). The dimers used were a mixture of fractions B-1 (shown) and B-5. Fraction B-5 is an isomer of B-2 (shown). The isomerism may be structural (position of linkage to "lower" flavan-3-ol unit) or stereochemical (at position 4 of "upper" flavan-3-ol).

dimers in Figure 2. The effect of procyanidin trimers combined with each of four GAs is shown in Figure 3, and the chemical structure of the trimers in Figure 4. The effect of Chinese galloyl tannin combined with each of three GAs is shown in Figure 5 and the structure of this tannin in Figure 6. The effect of pentagalloyl glucose with each of six GAs is presented in Figure 7, and that of chebulinic acid with each of four GAs in Figure 8. The structures of these last two tannins were given earlier (3).

In all cases tested, the tannins inhibited the growth induced by each GA. The highest amount of tannin used caused a reduction
of 60 to 100%. The GAs were used in either of two concentration series depending on how active each GA was in producing growth.

The amounts of tannins were the same in each case so that the ratio of tannin to GA was either 1:1, 10:1, and 100:1 or it was 10:1, 100:1, and 1000:1. Despite the differences in ratios the curves in most cases are similar. An exception to this generalization seems to be with GA$_1$ and GA$_4$, where large inhibition is achieved with small amounts of tannin. Growth induced by these GAs is strongly inhibited by tannin:GA-ratios of 10:1. Procyanidin trimers, pentagalloyl glucose, and chebulinic acid all cause over 70% inhibition of GA$_4$-induced growth at a ratio of 10:1, while procyanidin dimers and trimers and chebulinic acid all caused close to or over 50% reduction of GA$_4$-induced growth at the same ratio.

**Reversibility of Inhibition.** If the inhibitor and the promoter are acting on the same physiological system, the growth reduction should be reversible with additional gibberellins. Reversibility was tested by adding increasing amounts of gibberellin to a constant amount of tannin.

**DISCUSSION**

Tannins are native compounds of widespread occurrence in the plant kingdom, and they are present in most tissues of the plant (1). Despite this, reviews on tannins seldom discuss the function of these compounds in the plant. Brief reference may be made to a role in disease prevention but even in this regard modern pathology relegates their role to a supportive one in which tannins aid the factors primarily responsible for reducing infection (5, 18).

There are some reports of tannins affecting growth and development in plants; however, these reports are often inconsistent. Root growth may be inhibited (7) or enhanced (11, 14) and germination also may be inhibited or enhanced (6). While the role of tannins in plants is unclear, it has recently been suggested that they may play a growth-regulatory role by inhibiting growth caused by gibberellin in the plant (3). Two pieces of evidence in this paper support this suggestion. First, tannins are antagonists of a variety of chemically different gibberellins. These GAs are naturally occurring in higher plants and represent both early and later steps in the GA biosynthetic pathway (16). Second, purified tannins representing three of the four major groups of tannins have now been tested and found to be gibberelin antagonists. The antagonistic action of several naturally occurring tannins against several native gibberellins demonstrates the generality of this phenomenon among all tested tannins and gibberellins and lends support to the idea that tannins may be involved in the normal control of plant growth.

Antagonism of GA by tannin is demonstrated by the inhibition of induced growth, lack of effect on endogenous growth, and most important by the reversibility of the inhibition with additional GA. Reversibility is shown by dose-response curves in which the shoot length after treatment with the highest amount of GA and tannin is the same as shoot length after treatment with the same concentration of GA alone. Such interaction in simpler systems (i.e. enzymes) is frequently illustrated by the double reciprocal plot technique of Lineweaver and Burk (12). In order for this method to be used no endogenous substrate can be present; if it is present straight lines will not result (13). Stem growth of intact plants cannot be freed of GA and hence a Lineweaver-Burk plot is not appropriate. When it can be used the converging lines at
Fig. 7. Effect of β-1,2,3,4,6-pentagalloylglucose on growth induced by each of six GAs. Seedlings received 0.05 or 0.5 ng of GA and one of three concentrations of tannin in a total volume of 0.01 ml. The solution contained 0.05% Tween 20. Inhibition is given as percentage reduction of the gibberellin-induced growth, and points represent the average values of means from two replicate runs. Each run tested the three inhibitor concentrations mixed with GA along with 0 and GA controls. Each solution was assayed on 10 seedlings. The individual means used to determine the percent inhibition were compared with the mean of the GA control. All means giving inhibition above 44% were significantly different from the mean of the GA control at the 5% level. All means giving less than 29% inhibition were not significantly different.
infinite substrate and maximum velocity in the double reciprocal plot compare precisely to the converging curves in a dose-response plot. Tannins give experimental results similar to those of competitive inhibitors in enzyme studies. Tannins could not be competitive inhibitors of gibberellins in the usual sense because the chemical structures of the two groups of substances are so dissimilar. Lockhart (13) has pointed out that in a whole plant growth system, it is usually experimentally impossible to differentiate between inhibitors that compete with the promoter for an active site, and inhibitors which directly react with, and thus tie up, the promoter or its precursor.

Tannins are substances that convert animal skins from a form vulnerable to bacterial decomposition into the stable product leather; by definition, they are protein reactants. Tannins could function as GA inhibitors by acting as protein inhibitors. There are some differences between their activity as GA antagonists and as protein reactants in tanning. The primary difference is one of specificity. The tanning reaction is a general one against many different proteins. The growth inhibition, at least in the cucumber hypocotyl test (3) is more specifically against GA-induced growth; IAA-induced growth in the same system is not blocked. Inhibition is completely reversible by treatment with additional GA. Such reversibility would not be expected if general protein inhibition were responsible. Another indication of difference in activity is the action of the procyandin dimers. They are just as inhibitory to GA as the trimers and most of the other tannins tested. However, they do not possess significant tanning properties (15), and in a strict sense dimers would not classify as tannins since they cannot tan leather. There are two possible mechanisms of tannin action which would most easily agree with these findings. Either the tannins could act as inhibitors of a protein that specifically recognizes gibberellins or they could act directly on the gibberellin molecule to render it incapable of promoting growth.

The way in which tannins interact in GA-induced growth might be clarified by considering some of the chemical characteristics of all the purified tannins already tested with GA3 in the pea seedling test. Table I gives 16 tannins and 2 related compounds that have significant inhibitory activity. These compounds are listed according to the molar ratio of tannin to GA3 at which 50% of the GA-induced growth is inhibited. In the case of the most inhibitory compound, terchebin, less than four molecules of the tannin will inhibit growth induced by one molecule of GA3 at the 50% level. There is, in general, an increase in mol wt., potential quinones, and phenolic OH groups per molecule with increasing activity. None of these correlates exactly, however. The three least active compounds certainly have smaller mol wt., and fewer potential quinones and phenolic OH groups than the other materials. The 14 compounds from tannic acid to hexahydroxydiphenoyl glucose all have the same activity on a weight basis and have molar ratios.

Fig. 8. Effect of chebulinic acid on growth induced by each of three GAs. Seedlings received 0.005, 0.05 or 0.5 ng of GA and one of three concentrations of tannin in a total volume of 0.01 ml. The solution contained 0.05% Tween 20. Inhibition is given as percentage reduction of the gibberellin-induced growth, and points represent the average values of means from two replicate runs. Each run tested the three inhibitor concentrations mixed with GA along with 0 and GA controls. Each solution was assayed on 10 seedlings. The individual means used to determine the per cent inhibition above 23% were significantly different from the mean of the GA control at the 5% level. All means giving 23% inhibition or less were not significantly different.

Fig. 9. Effect of gibberellin A3 on the growth of pea seedlings in the presence or absence of 5 μg of β-1, 2, 3, 4, 6-penta galloylglucose per plant. Each seedling received a total volume of 0.01 ml of a solution containing 0.05% Tween 20. Each point represents the mean and standard error of 10 seedlings.
which are within the same order of magnitude. Within this group the potential quinones and phenolic OH groups are more numerous in the more active than in the less active examples. The major exception to the general trend is terchebin which is not higher in any of the characteristics than the half dozen compounds nearest to it, but is about ten times more inhibitory on a molar ratio basis. It may well be that mol wt and numbers of particular groups are too crude a measure, and that activity is also dependent on a particular configuration at some part of the molecule.

LITERATURE CITED


