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Physiological Effects of Gibberellic Acid. VIII. Growth Retardants on Barley Endosperm [1, 2]

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Introduction

Several types of growth-retarding chemicals have been described in recent years. The first was 2-isopropyl-4-dimethylamino-5-methylphenyl-1-piperidinecarboxylate methyl chloride (Amo-1618) and some related compounds (7, 21). It was followed by (2-chloroethyl) trimethylammonium chloride (CCC or Cycocel) (19), tributyl-2, 4-dichlorobenzylphosphonium chloride (Phosfon D) (13) and by N-dimethylamino maleamic acid (C-011) and N-dimethylamino succinamic acid (B-995) (14).

These compounds differ somewhat in their effectiveness and the range of species which they affect. When they are active, however, they usually produce dwarfed plants with shortened, in extreme cases almost rosette-like stems, and dark-green, thickened leaves. These plants are essentially normal in other respects. The substances have therefore been called dwarfing agents or growth retardants. Their overall effect is the opposite of the growth effects produced by the gibberellins, and when a retardant and a gibberellin are applied together, the retardant effects may be overcome, resulting, at least in some cases, in normal growth (6, 16, 20). Some authors have therefore called the retardants antigibberellins, although

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they made it clear that this term was used only in a general, descriptive sense and not to imply any definite type of interaction (6, 20). Using the principles of reaction kinetics in his analyses, Lockhart (6) concluded that there is indeed a specific interaction between the 2 types of growth-regulating compounds, gibberellins and growth retardants. However, more direct evidence as to their mode of interaction is lacking.

This paper reports the effects of a number of synthetic growth-retarding chemicals on the gibberellic acid (GA_3)-induced reducing sugar release by excised barley endosperm. The barley endosperm response (10, 11) was chosen for our studies since the tissue responds to applied gibberellin in an apparently direct manner. The system, it was hoped, would permit the distinction between effects on GA_3 synthesis and GA_3 action.

In addition to the 5 retardants named above, maleic hydrazide (MH) was included in the tests since it bears a structural similarity to C-011 and B-995 and also inhibits the growth of plants (2, 17). The effects of MH, however, may be more complex than those of the other retardants, particularly Amo-1618, CCC and Phosfon D, which are more selective in nature.

Materials and Methods

Barley seed (variety Naked Blanco Mariout) was used in all experiments. The techniques employed resembled those reported in detail elsewhere (8, 10). Briefly, the intact seed was sterilized for 2 to 3 hours in calcium hypochlorite, cut in half transversely (discarding the embryo half), and weighed. Endosperm halves were incubated, 4 at a time, in 3 or 4 ml of solution containing the compounds to be tested, at 30° for about 24 hours. Samples of the solutions surrounding the endosperm were analyzed for reducing sugar content with the Somogyi reagent (18). The gibberellin used was always GA_3, and all of the growth retardants were tested in the way described. In addition, CCC and Phosfon D were also compared in a somewhat modified test. Seeds were sterilized for 6 hours, rinsed, and cut so that the endosperm halves measured 4 mm in length. The endosperm were soaked in 400 ml water at 2° for 16 hours. They were then distributed at random into 25 × 50 mm stoppered vials (2 endosperm/vial) and incubated with 1 ml solution at 30° for 24 hours.

At the end of this time, 9 ml water and about 1 g Amberlite IR-120 (H^+) resin (10) was added to each vial. The contents were shaken and filtered and samples of the solutions were analyzed for reducing sugar content. The results are expressed as mg reducing sugar (glucose equivalents) per vial.

All compounds were tested in the sugar assay, and where necessary, corrections for interference were made.

Results

As demonstrated by figure 1, presence of CCC in the incubation medium neither significantly depressed nor significantly enhanced the GA_3-induced reducing sugar release from excised barley endosperm. Identical results were obtained with the other retardants. Phosfon D was tested at 2.6×10^{-8} to 2.6×10^{-4} M; Amo-1618 at 0.7×10^{-8} to 0.7×10^{-4} M; C-011 at 4.8×10^{-5} to 4.8×10^{-4} M; B-995 at 1.7×10^{-8} to 1.7×10^{-4} M; MH at 10^{-6} to 10^{-4} M. All concentrations of growth retardants were examined at at least 3 levels of GA_3 (usually 0, 10^{-8}, 10^{-6} M), and frequently 4.

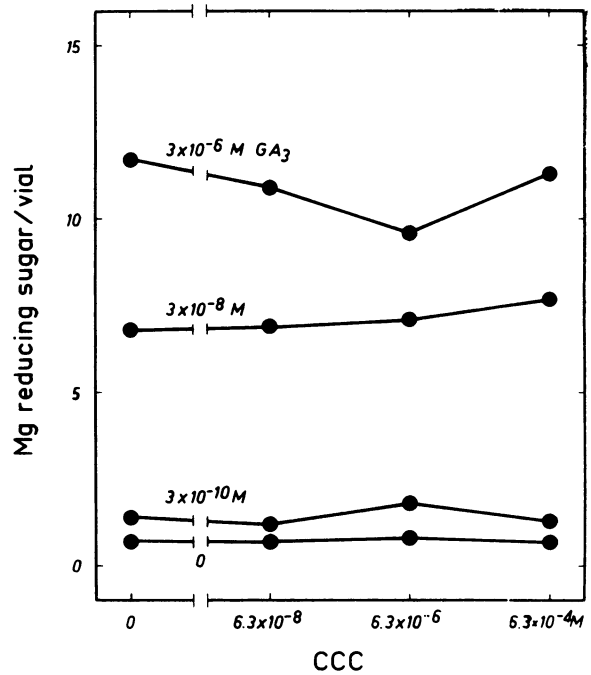


FIG. 1. Lack of effect of CCC on the GA_3-induced reducing sugar release from barley endosperm.

In addition, endosperm were preincubated in water, Amo-1618 (0.7×10^{-4} M), CCC (2×10^{-4} M) and Phosfon D (0.6×10^{-4} M) for 18 hours at 30° before rinsing and transferring them to a 0.75×10^{-7} M GA_3 solution. The reducing sugar levels determined after a subsequent 24 hour incubation at 30° were: water, 106.6; Amo-1618, 112.0; CCC, 103.5; Phosfon D, 103.4 mg per g endosperm (fig 2). The differences were not significant. We can conclude that differential rates of entry are not a factor in the ineffectiveness of the retardants in this system.

Discussion

In order to integrate the results obtained in this work, i.e., lack of any effect of the retardants, with published data, it is necessary to consider the basic features of the test system. The response measured, reducing sugar release, is initiated by treating detached endosperm with gibberellin. The question to be considered first is whether this system bears any relationship to other situations in which gibberellin exerts a demonstrable effect on growth.

While no direct experimental information is yet available bearing on this point, some circumstantial

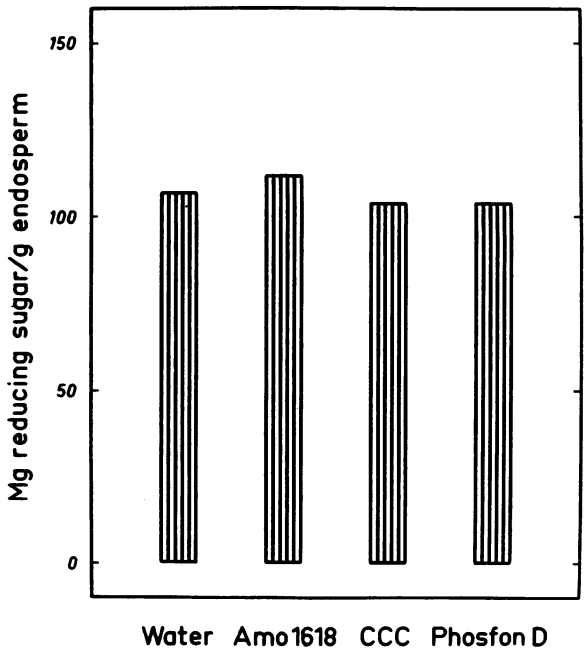


FIG. 2. Effect of growth-retardant pretreatment on subsequent GA_3-induced sugar release.

evidence can be adduced. It can be suggested that there is at present no reason to assume more than 1 action for gibberellin. On this basis all reactions initiated by gibberellin will eventually be traced back to 1 hormonal mechanism. A more weighty point is that the test system resembles, to a remarkable degree, the normal processes which lead to germination and growth in the intact barley seed. Furthermore, the indirect action of GA_3 on the endosperm can now account for the original observation of increased growth following GA_3 treatment of barley seeds (4). Further evidence, in which growth is more directly involved, derives from recent work by Flemion (1), in which she demonstrated that physiologically dwarfed peach seedlings are unable to normally metabolize starch stored in the apex unless a cold treatment is given or GA_3 is applied. Presumably, endogenous gibberellin initiates the hydrolysis of starch in the apex leading to a growth reaction in the elongating cells of the stem, in the same way that it initiates the hydrolysis of starch in the endosperm leading to a growth reaction in the embryo.

From these considerations it seems justifiable to conclude, at least in a preliminary way, that the hormonal effect of GA_3 on barley endosperm is similar to its hormonal effect on other tissues.

A second question that must be answered is, even though the endosperm response is similar to other GA_3-induced responses, is there any reason for believing that the growth retardants interact in the response, i.e., perhaps the retardants act on processes completely unrelated to those controlled by GA_3?

There is a considerable amount of pertinent evidence relating to this point. With intact bean plants, Lockhart (6) showed that the effects of CCC and Phosfon D on growth rate can be overcome by sufficiently high doses of applied GA_3. On the organ or tissue level, Sachs, et al. (14) have demonstrated that the suppression of mitotic activity in the subapical shoot meristem of chrysanthemum by Amo-1618 can be overcome by application of GA_3. The mutual reversibility of effects of Amo-1618 and GA_3 on catalase and peroxidase activity, as reported by Halevy (3), is further strong evidence on an intracellular level.

On the basis, therefore, that the action of GA_3 on barley endosperm is fundamentally comparable to its action in other gibberellin-dependent responses, the lack of a retardant interaction must be due to the intrinsic nature of the endosperm system.

There are 5 general ways in which a growth retardant may inhibit gibberellin-induced responses. An inhibition of the biosynthesis of endogenous gibberellin may be considered as the first type. The second is a decrease in the level of the compound, or class of compounds, on, or with which gibberellin acts or reacts. This may be due to inhibition of biosynthesis or to outright destruction. Destruction or inactivation of gibberellin is the third, and an action which prevents gibberellin from fulfilling its primary, or hormonal role, the fourth avenue of inhibition. Lastly, the compounds may directly or indirectly prevent one of the numerous series of gibberellin-induced reactions from taking place in treated tissue, thus blocking the physiological response in question. These 5 possibilities are illustrated in figure 3.

Because of the nature of the endosperm response, we can consider possible routes 1 and 2 separately from 3, 4 and 5. The tissue does not synthesize gibberellin since controls do not react similarly, and, indeed, exogenous gibberellin is supplied. Biosynthesis of the gibberellin substrate is not involved since the tissue responds immediately to a wide range of concentrations and different gibberellins (12). In other words, inhibition pathways 1 and 2 are not operative with this system. Consequently, if any inhibition of the gibberellin-induced response was

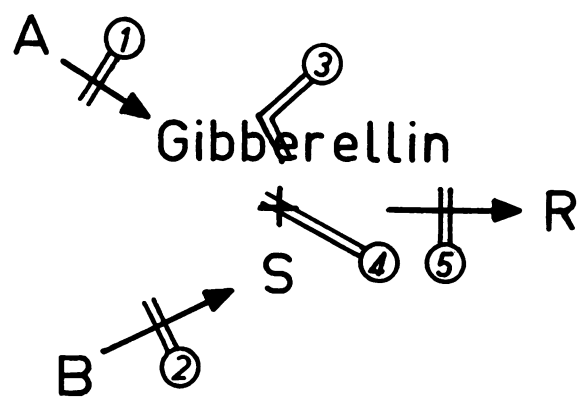
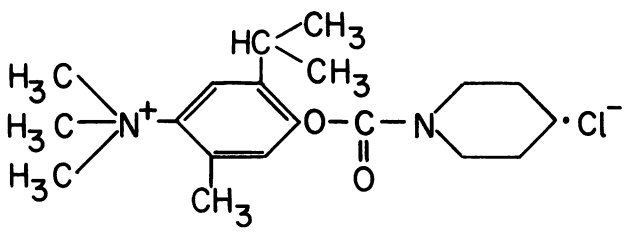


FIG. 3. Where A = gibberellin precursor; B = gibberellin substrate precursor; S = gibberellin substrate (compound or class of compounds with or on which gibberellin reacts or acts); R = physiological response.

noted, it would have to be at possibility 3, 4 or 5. No such effects were observed, and the conclusion can be drawn that the growth retardants do not act through routes 3, 4 or 5 in the gibberellin-induced endosperm response.

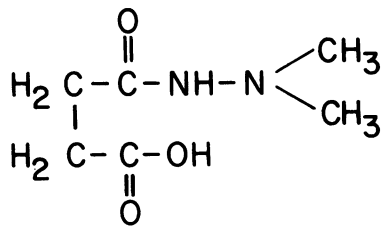
It must, then, be concluded that the growth retardants act at pathway 1 or 2, that is, that they inhibit the biosynthesis of either gibberellin or the compound with, or on which gibberellin acts. One feature of growth retardant action is the complete reversibility of inhibition by gibberellin which has been mentioned earlier. Since pathway 2 would not be reversed by increased gibberellin concentrations, the growth retardants must be acting as inhibitors of endogenous gibberellin biosynthesis.

Lockhart (6) also concluded that inhibition of gibberellin synthesis might be the action mechanism of the retardants. More recently, it has been shown (5, 9), that Amo-1618 and CCC suppress gibberellin production by *Fusarium moniliforme* (*Gibberella fujikuroi*) without affecting the growth of the fungus. A priori it appears unlikely that the pathways of gibberellin synthesis in *Fusarium* and in higher plants are fundamentally different, and the results of Kende



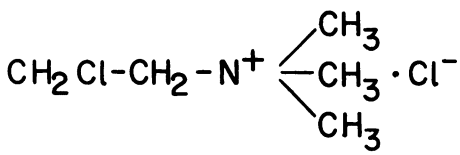
AMO-1618

2- Isopropyl-4-dimethylamino-5-methylphenyl-1-piperidine-carboxylate methyl chloride



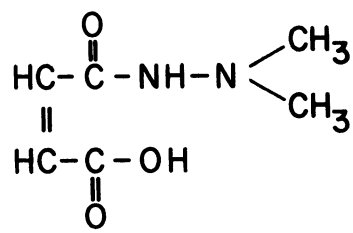
B Nine (B-995)

N-dimethylaminosuccinamic acid



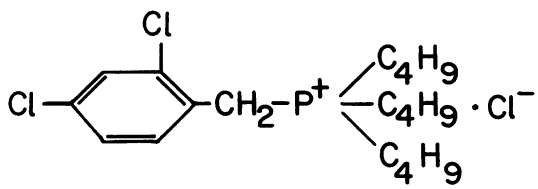
CCC (Cycocel)

(2-Chloroethyl) trimethylammonium chloride



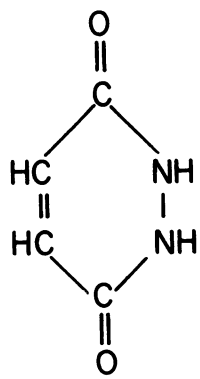
C-011

N-dimethylaminomaleamic acid



Phosfon-D

Tributyl-2,4-dichlorobenzylphosphonium chloride



MH

Maleic hydrazide

(1,2-dihydropyridazine-3,6-dione)

FIG. 4. Structural relationships between the growth retardants tested.

et al. lend strong, though circumstantial support to the conclusions reached in the present work.

As evident from figure 4, the growth retardants are chemically a very heterogenous group although there are structural similarities between some of them (C-011, B-995 and MH). The reactive groups of the retardants (keto, quaternary ammonium and phosphonium) are different enough to assume that these compounds may have more than 1 in vivo action. Although this question clearly requires further investigation, our results, when considered in conjunction with other experimental information, allow certain conclusions. They indicate that at least some of the growth retardants are acting by inhibiting gibberellin biosynthesis and not by interfering with the action of gibberellin in the cell. Because of this, the application of the term antigibbberellin to these compounds is inaccurate and should be abandoned since the concept of antimetabolites is properly reserved for compounds which interfere with the action of the corresponding metabolite on the basis of competitive inhibition. Growth retardant is a more acceptable term, and if, as suggested by our results, these compounds do inhibit the biosynthesis of endogenous gibberellins, we have in them an extremely powerful tool for investigating the formation of the gibberellins, and in this way, their physiological role in the plant.

Summary

2-Isopropyl-4-dimethylamino-5-methylphenyl-1-piperidinecarboxylate methyl chloride; (2-chloroethyl) trimethylammonium chloride; tributyl-2,4-dichlorobenzylphosphonium chloride; N-dimethylamino maleamic acid; N-dimethylamino succinamic acid; and maleic hydrazide were all tested for their ability to retard the gibberellic acid-induced reducing sugar release from barley endosperm. They were all found to be inactive.

It is suggested, on the basis of the above results, that the compounds be termed growth retardants and not antigibberellins since they probably exert their effect as inhibitors of gibberellin biosynthesis.

Acknowledgments

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