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GROWTH RETARDANTS: Effects on Gibberellin Biosynthesis and Other Metabolic Pathways

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■ **Abstract** Plant growth retardants are applied in agronomic and horticultural crops to reduce unwanted longitudinal shoot growth without lowering plant productivity. Most growth retardants act by inhibiting gibberellin (GA) biosynthesis. To date, four different types of such inhibitors are known: (*a*) Onium compounds, such as chlormequat chloride, mepiquat chloride, chlorphonium, and AMO-1618, which block the cyclases copalyl-diphosphate synthase and *ent*-kaurene synthase involved in the early steps of GA metabolism. (*b*) Compounds with an N-containing heterocycle, e.g. ancymidol, flurprimidol, tetcyclacis, paclobutrazol, uniconazole-P, and inabenfide. These retardants block cytochrome P450-dependent monooxygenases, thereby inhibiting oxidation of *ent*-kaurene into *ent*-kaurenoic acid. (*c*) Structural mimics of 2-oxoglutaric acid, which is the co-substrate of dioxygenases that catalyze late steps of GA formation. Acylcyclohexanediones, e.g. prohexadione-Ca and trinexapac-ethyl and daminozide, block particularly 3ß-hydroxylation, thereby inhibiting the formation of highly active GAs from inactive precursors, and (*d*) 16,17-Dihydro-GA_5 and related structures act most likely by mimicking the GA precursor substrate of the same dioxygenases. Enzymes, similar to the ones involved in GA biosynthesis, are also of importance in the formation of abscisic acid, ethylene, sterols, flavonoids, and other plant constituents. Changes in the levels of these compounds found after treatment with growth retardants can mostly be explained by side activities on such enzymes.

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INTRODUCTION

Plant growth retardants are synthetic compounds, which are used to reduce the shoot length of plants in a desired way without changing developmental patterns or being phytotoxic. This is achieved primarily by reducing cell elongation, but also by lowering the rate of cell division. In their effect on the morphological structure of plants, growth retardants are antagonistic to gibberellins (GAs) and auxins, the plant hormones that are primarily responsible for shoot elongation. The first growth retardants, certain nicotinium derivatives, became known in 1949 (121). Many other compounds have subsequently been detected, some of which have been introduced into agronomic or horticultural practice. Plant growth retardants represent the commercially most important group of plant bioregulators (PBRs) or plant growth regulators, although compared to herbicides, insecticides, and fungicides, they play a relatively minor role and represent only a few percent of the worldwide sales of crop-protecting chemicals, totaling approximately US $28 billion in 1999.

In addition to other agronomic tools, PBRs can be used relatively flexibly by the farmer to adjust his crop in a desired way to changes in growing conditions. Plant growth retardants have found a number of practical uses: In intensive small grain cultivation in Europe, they have become an integral part of the production system by reducing the risk of lodging due to intensive rainfall and/or wind; in cotton excessive vegetative growth may be controlled, thereby helping adjust a perennial plant species to an annual cycle of cultivation; fruit trees can be kept more compact, thereby reducing costs for pruning and obtaining a better ratio between vegetative growth and fruit production; the quality of ornamental and bedding plants is generally improved by keeping them compact, which also reduces the space in a greenhouse required for production; costs for trimming hedges and trees and for mowing turf grasses may also be reduced by applying plant growth retardants. For more details on applications of plant growth retardants the reader is referred to (43, 50, 80, 118, 129, 134, 151).

Reduction of shoot growth can also be achieved by compounds other than growth retardants. For instance, compounds with a low herbicidal activity or

herbicides applied at lower rates may cause a stunted shoot without bringing about visible symptoms of phytotoxicity. Reductions in plant productivity have to be expected, however. Examples of such plant growth suppressants are mefluidide, amidochlor, maleic hydrazide, or chlorflurenol, which might be used, for example, to reduce shoot growth of turf grasses. In principle, breeding offers an alternative way to achieve desired alterations in plant development. However, a fixed genotype is less flexible towards changing growing conditions and does not allow an active steering of growth.

We can classify the existing growth retardants into two main groups: ethylene-releasing compounds, such as ethephon, and inhibitors of GA biosynthesis. This contribution deals with the biochemical mode of action of typical representatives of the latter group. Previous reviews on this subject have been published (22, 34, 68, 55, 135). In light of the availability of new compounds and substantial progress made in the area, an update appears to be useful.

GIBBERELLIN BIOSYNTHESIS

At present, 125 different GAs are known to occur in higher plants and/or GA-producing fungi. A continuously updated list of structures and their occurrence may be found on the internet (http://www.plant-hormones.bbsrc.ac.uk/educatio.html). Only a few of the GAs possess biological activity per se, whereas the majority are precursors or catabolites. The main hormonal functions of GAs are the promotion of longitudinal growth, the induction of hydrolytic enzymes in germinating seeds, the induction of bolting in long-day plants, and the promotion of fruit setting and development. However, some of the many GAs might have functions that are still unknown.

GAs are diterpenoids and consist of 19 or 20 carbon atoms. Their biosynthesis is relatively well understood. The sequential steps involved in GA metabolism have been studied by using cell-free enzymatic systems prepared, for example, from immature seeds of pumpkin or pea. Radiolabeled substrates were converted into their respective products under distinct conditions in the presence of suitable co-factors. These in vitro biosynthesis systems have, in conjunction with analyzing the GAs present in intact plants, also been used to identify the point of interaction of inhibitors in the biosynthetic sequence leading to GAs. More recently, further details of GA biosynthesis were elucidated by employing distinct plant mutants and by the cloning and characterization of genes coding for the GA-biosynthetic enzymes.

The biosynthesis of GAs can be separated into three stages according to the nature of the enzymes involved and the corresponding localization in the cell: Terpene cyclases acting in proplastids, monooxygenases associated with the endoplasmatic reticulum, and dioxygenases located in the cytosol. Only a brief outline is given here for orientation; for more-detailed information on different aspects of GA metabolism the reader is referred to recent review articles (e.g. 51, 70, 71, 74, 75, 101, 112, 146, 157).

Formation of *ent*-Kaurene

It has been assumed until recently that GAs as well as all other isoprenoids are exclusively formed from the C_5 compound isopentenyl diphosphate (IPP) synthesized from mevalonic acid (MVA) (24). In the cytosol MVA is phosphorylated via two steps into MVA-5-diphosphate, which, after decarboxylation, yields IPP. However, new results indicate that IPP can also be formed via a non-mevalonate pathway in plastids (106, 144). In this pathway, D-glyceraldehyde 3-phosphate plus pyruvate yields 1-deoxy-D-xylulose 5-phosphate, which is converted into IPP. The mevalonate pathway gives rise to sterols, sesquiterpenes, and triterpenoids, whereas the pathway involving 1-deoxy-D-xylulose 5-phosphate yields carotenoids, phytol, plastoquinone-9, mono-, and diterpenoids. Some interchange between the pathways seems to exist. In general, it appears likely that GA precursors also are formed primarily via the 1-deoxy-D-xylulose 5-phosphate pathway in plastids of green tissues although this has not yet been conclusively demonstrated.

IPP is transformed via an isomerase-catalyzed reaction into dimethylallyl-PP. In head-to-tail condensations, three molecules of IPP are sequentially added to this compound to form geranyl diphosphate (GPP), farnesyl diphosphate (FPP), and finally, the C_{20} compound geranylgeranyl diphosphate (GGPP). GGPP is cyclized via copalyl diphosphate (CPP) to *ent*-kaurene. The latter steps are catalyzed by two distinct enzymes, namely CPP synthase (formerly known as *ent*-kaurene synthase A) and *ent*-kaurene synthase (formerly known as *ent*-kaurene synthase B). High activities of CPP synthase and *ent*-kaurene synthase were detected in the stroma of proplastids from pea and wheat shoots and in leucoplasts from pumpkin endosperm (2). In contrast, mature chloroplasts are low in such activities and, therefore, it is proposed that *ent*-kaurene is primarily produced in rapidly dividing cells (1).

Oxidation of *ent*-Kaurene to GA_{12}-Aldehyde

The reactions of stage 2 are catalyzed by monooxygenases located on the endoplasmatic reticulum, which require O_2 and NADPH for activity and involve cytochrome P450. The highly lipophilic *ent*-kaurene is oxidized stepwise at C-19 via *ent*-kaurenol and *ent*-kaurenal to *ent*-kaurenoic acid. *ent*-Kaurenoic acid is then hydroxylated to *ent*-7α-hydroxykaurenoic acid. After an oxidative ring contraction with extrusion of C-7, GA_{12}-aldehyde is formed. GA_{12}-aldehyde can be deemed the first intermediate specific for GAs.

Further Oxidation of GA_{12}-Aldehyde to the Different GAs

The conversions of stage 3 take place primarily in the cytosol. Most reactions are catalyzed by soluble dioxygenases, which require 2-oxoglutarate as a co-substrate and Fe^{II} and ascorbate as co-factors for activity. However, depending on the plant species and the tissue, some initial steps may still be catalyzed by monooxygenases. Hydroxylations at positions 13 and 12α may involve both types of enzyme. GA_{12}-aldehyde is oxidized by either a monooxygenase or a dioxygenase at position 7,

thereby converting the aldehyde function into a carboxylic acid group and leading to GA_{12}. In the early 13-hydroxylation pathway, which is common to higher plants, GA_{53} would be the next intermediate after GA_{12}. Thereafter, a stepwise oxidation of C-20 and lactone formation (involving C-19 between C-4 and C-10) with the loss of C-20 as CO_2 is catalyzed by the multifunctional GA 20-oxidase. These reactions lead via GA_{44} and GA_{19} to GA_{20}, a C_{19}-GA. Superimposed on these and further steps, species and organ specific hydroxylation patterns occur, which may lead to typical "GA families." Considerable biological activity can be found only among C_{19}-GAs, which are further hydroxylated at position 3ß (e.g. GA_1 as a product of GA_{20}, or GA_3, GA_4, GA_7 and several other GAs). In contrast, hydroxylation at position 2ß (e.g. conversion of GA_1 to GA_8) drastically reduces biological activity. This step, further oxidative reactions, and conjugation with, for example, glucose obviously have the function of terminating the mission of a GA. Evidence is available that several related or isoenzymes of GA 20-oxidase, 3ß-hydroxylase, and 2ß-hydroxylase exist that are relatively low in substrate specificity and that may have overlapping activities.

Figure 1 represents a highly simplified scheme of GA metabolism concentrating on those reactions that are involved in the formation of GA_1, since this GA seems to be of paramount importance for stem elongation in many plant species (133). The structures of some important intermediates are presented on the left side. Numbering of carbon atoms is exemplified in this figure referring to *ent*-gibberellane.

INHIBITORS OF GA BIOSYNTHESIS

Four groups of GA biosynthesis inhibitors are known: "onium" compounds, compounds with an N-containing heterocycle, structural mimics of 2-oxoglutaric acid, and 16,17-dihydro-GAs. Each of these groups inhibits GA metabolism at distinct steps. An overview on the points of interaction with GA biosynthesis is shown in the right part of Figure 1.

Onium-Type Compounds

Several compounds that possess a positively charged ammonium, phosphonium or sulphonium group block the biosynthesis of GAs directly before *ent*-kaurene. The most prominent representatives of this group are chlormequat chloride (163, 164) and mepiquat chloride (178). These compounds, which have a quaternary ammonium group, are primarily used as anti-lodging agents in cereal production and to reduce excessive vegetative growth in cotton. Piproctanyl bromide, which is used to some extent in the production of ornamental plants and AMO-1618 are further growth retardants with a quaternary ammonium function. Chlorphonium and BTS 44584 (49) should be mentioned here as possessing a phosphonium and sulphonium moiety, respectively. Further examples of onium-type compounds may

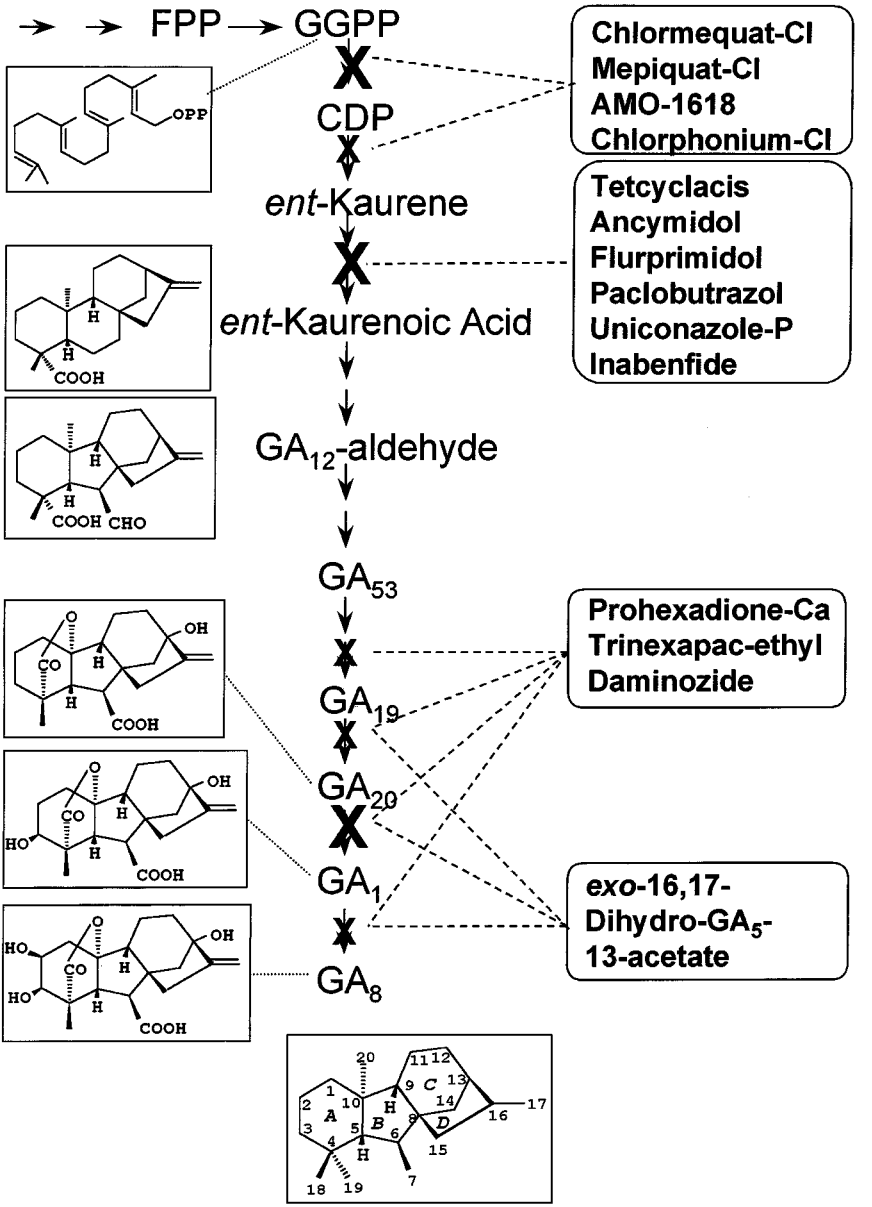

Figure 1 Simplified scheme of biosynthetic steps involved in GA biosynthesis and points of inhibition by plant growth retardants (**X**, **x** = major and minor activity, respectively). (See text for abbreviations.)

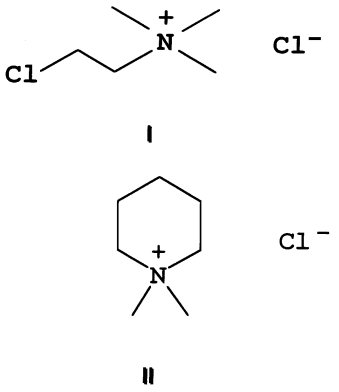

Figure 2 Onium-type plant growth retardants. I. Chlormequat chloride = (2-Chloroethyl)-trimethylammonium chloride {Chlorocholine chloride, ***CCC***}; II. Mepiquat chloride = 1,1-Dimethylpiperidinium chloride {***DPC***}.

be found in the literature (25, 34, 87, 107, 150). The structures of chlormequat chloride and mepiquat chloride are presented in Figure 2. Their official common, systematic chemical and other frequently used names and often used or suggested abbreviations (in bold and italics) are also given in this and the following figures.

Chlormequat chloride, AMO-1618, and chlorphonium inhibit CPP-synthase in both the GA-producing fungus *Gibberella fujikuroi* and cell-free preparations of this fungus and of higher plants. *ent*-Kaurene synthase is also inhibited by these compounds, but mostly at a lower degree of activity (153). The cyclization of GGPP into CPP, catalyzed by CPP-synthase, is analogous to the reaction leading from 2,3-oxidosqualene to lanosterol in mammals and fungi or cycloartenol in higher plants in the respective courses of sterol biosynthesis. Tertiary amine analogs of squalene are efficient inhibitors of oxidosqualene cyclase. It is suggested that such compounds, which are positively charged at physiological pH, mimic carbocationic high-energy intermediates in the cyclization reaction. Such intermediates are expected to bind very tightly to the enzyme, thereby blocking the reaction (8). By analogy, it appears likely that inhibitors of CPP-synthase mimic cationic intermediates in the conversion of GGPP into CPP (68). A similar mechanism would also apply for the succeeding cyclization of CPP into *ent*-kaurene.

To obtain any significant effects in cell-free preparations, relatively high concentrations of chlormequat chloride have to be used and, in some cases, the compound is even inactive (4, 46, 68, 170). The same is true of mepiquat chloride: In an enzyme system derived from pumpkin (*Cucurbita maxima*) endosperm, concentrations as high as 10^{-3} M of this compound, as well as of chlormequat chloride, did not affect the spectrum of GAs and GA precursors (77; L Schwenen & JE Graebe, unpublished data). A possible explanation of this difficulty might be the fact that these compounds are almost inactive in intact pumpkin plants. The same might also be expected for cell-free preparations from pumpkin tissues. Consequently,

chlormequat chloride has been tested with enzymes derived from germinating wheat seedlings, where it gave more pronounced effects (53).

More definite results with some of the onium-type growth retardants have also been obtained by studying their effects on GA levels in intact higher plants. Several older investigations exist, in which levels of endogenous GAs had been determined by bioassays. In general, GA levels were found to be decreased by the growth retardants, more or less parallel to reductions in shoot length (cf. 134). With regard to chlormequat chloride, these results could more recently be confirmed employing modern techniques such as combined gas chromatography-mass spectrometry: Chlormequat chloride lowered the levels of GA_1 in both the shoots and grains of *Triticum aestivum* (104). Likewise, it led to a dose-responsive reduction of all GAs (GA_{12}, GA_{53}, GA_{44}, GA_{19}, GA_{20}, GA_1, GA_8) present in two cultivars of *Sorghum bicolor* (103). In *Eucalyptus nitens*, chlormequat chloride caused a reduction of GA_{20} and GA_1 (171).

Compounds with a Nitrogen-Containing Heterocycle

Several growth retardants are known that comprise a nitrogen-containing heterocycle. The pyrimidines ancymidol (165) and flurprimidol are of some commercial relevance, especially in ornamentals. Tetcyclacis, a norbornanodiazetin (88), has been used as a dwarfing agent in the production of rice seedlings for transplanting. Certain triazole-type compounds have attained a relatively high degree of interest. Paclobutrazol (105) and the closely related uniconazole-P (84, 85) are highly active members of this group and have found practical uses in rice, fruit trees and ornamentals. Triapenthenol (110) and BAS 111..W (89) represent further triazole-type growth retardants. A compound being used to lower the risk of lodging in rice is inabenfide, a 4-substituted pyridine (154). Also distinct imidazoles, such as 1-*n*-decylimidazole and 1-geranylimidazole (167) and HOE 074784 (19) possess plant growth-retarding properties. In some instances, plant growth retardation can also be found as a side activity of some triazole-type fungicides such as triadimenol, triadimefon (14), or ipconazole (147). Particularly in oilseed rape, the growth-regulating effect of the fungicides tebuconazole and metconazole is of practical relevance. The structures of typical growth retardants possessing an N-containing heterocycle are shown in Figure 3.

These growth retardants act as inhibitors of monooxygenases catalyzing the oxidative steps from *ent*-kaurene to *ent*-kaurenoic acid (51 and references cited therein, 119). Steps lying after *ent*-kaurenoic acid, which may still involve monooxygenases, do not seem to be affected (51). The structural feature common to all these inhibitors of *ent*-kaurene oxidation is a lone electron pair on the sp^2-hybridized nitrogen of their heterocyclic ring. In each case, this electron pair is located at the periphery of the molecule (140). Most probably, the target monooxygenases contain cytochrome P450 (65, 66) and it appears likely that the lone pairs of electrons of the growth retardants displace oxygen from its binding site at the protoheme iron (30). Evidence for such a type of interaction has been presented

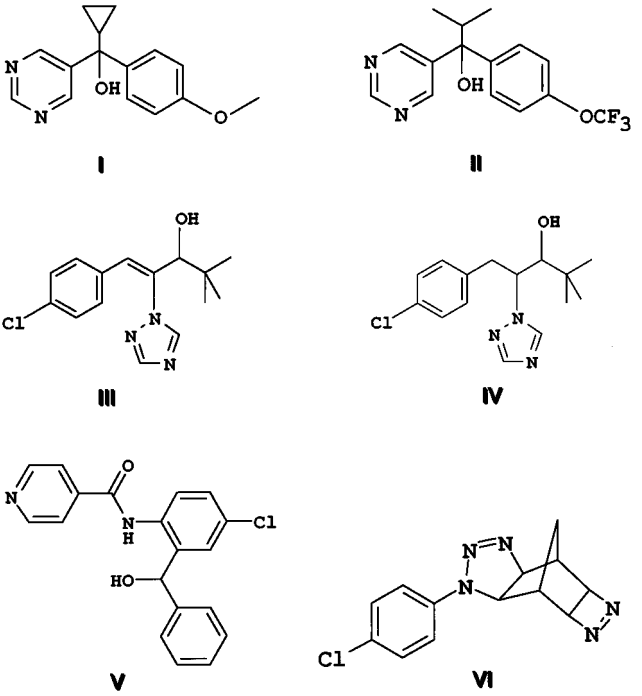

Figure 3 Plant growth retardants with an N-containing heterocycle. I. Ancymidol = α-Cyclopropyl-(p-methoxyphenyl)-5-pyrimidinemethanol {EL-531, ***Anc***}; II. Flurprimidol = α-(1-Methylethyl)-[p-4-(trifluoromethoxy)phenyl]-5-pyrimidinemethanol {EL-500, ***Flp***}; III. Uniconazole-P = (E)-(*RS*)-1-(4-Chlorophenyl)-4,4-dimethyl-2-(1H-1,2,4-triazol-1-yl) pent-1-en-3-ol {S-3307D, XE-1019, ***UCZ***}; IV. Paclobutrazol = (*2RS, 3RS*)-1-(4-Chlorophenyl)-4,4-dimethyl-2-(1H-1,2,4-triazol-1-yl)pentan-3-ol {PP333, ***PBZ***}; V. Inabenfide = [4-Chloro-2-(a-hydroxybenzyl)]-isonicotinanilide {CGR-811, ***IBF***}; VI. Tetcyclacis = 5-(4-Chlorophenyl)-3,4,5,9,10-pentaazatetracyclo-[5.4.$10^{2,6}$.$0^{8,11}$]-dodeca-3,9-diene {LAB 102 883, BAS 106 W, ***TCY***}.

for ancymidol in microsome preparations of *Marah macrocarpus* (28, 30) and for BAS 111..W, using microsomal membranes isolated from immature pumpkin endosperm (111).

Depending on the presence or absence of a double bond, uniconazole-P and paclobutrazol possess one or two asymmetric carbon atoms, respectively. Since commercial paclobutrazol consists mainly of the (*2RS,3RS*) diastereoisomer (160), this structure allows virtually only two enantiomers, as does uniconazole-P. Detailed experiments carried out with the optical enantiomers of paclobutrazol have shown that the (*2S,3S*)-form exhibits more pronounced plant growth-regulatory activity and blocks GA biosynthesis more specifically, whereas the (*2R,3R*)-enantiomer is more active in inhibiting sterol biosynthesis (15, 73, 160). Fungicidal

side activities of paclobutrazol are attributed to its effect on sterol formation (160). It has been demonstrated that the (*2S,3S*)-enantiomer is structurally similar to *ent*-kaurene whereas *the* (*2R,3R*)-form is closely related to lanosterol, the respective intermediates of GA and sterol biosynthesis (160). Similar chiralic specificities have been found for uniconazole-P (84), triapenthenol (109) and inabenfide (119): In all cases, the (*S*)-enantiomer was more inhibitory to *ent*-kaurene oxidation than the respective (*R*)-counterpart. Using computer assisted molecular modeling methods, clear structural similarities could be demonstrated between tetcyclacis and the growth-retarding forms of paclobutrazol and uniconazole-P with *ent*-kaurene and *ent*-kaurenol (94, 123, 160). This indicates that, within certain limits, distinct structural features are required to bind to and thereby block the active site of the enzyme. One may assume that the structures of the other growth retardants possessing an N-containing heterocycle would also fit into this scheme.

Clear evidence is available that reduction of shoot growth by pyridines, 4-pyrimidines, triazoles, imidazoles, and diazetines is caused by a lowered content of biologically active GAs. Reduced levels of GAs have, for instance, been analyzed by modern techniques under the influence of ancymidol in beans (155), tetcyclacis in corn cockle (*Agrostemma githago*) (179), paclobutrazol in barley and wheat (104) and in *Eucalyptus nitens* (171), uniconazole-P in rice (85) and *Sorghum bicolor* (103), BAS 111..W in oilseed rape (72), and inabenfide in rice (119).

Structural Mimics of 2-Oxoglutaric Acid

One part of this group is represented by acylcyclohexanediones such as prohexadione-calcium (prohexadione-Ca) (125), trinexapac-ethyl (3) and the experimental compound LAB 198 999 (134). Virtually all higher plants react with a reduced shoot growth after treatment (cf. 126). Stem stabilization in cereal crops, rice, and oilseed rape, growth control in turf grasses and reduction of vegetative growth in fruit trees are the main applications.

Acylcyclohexanediones interfere with the late steps of GA biosynthesis. Structural similarities between the acylcyclohexanediones and 2-oxoglutaric acid, which is the co-substrate of the involved dioxygenases, are assumed to be responsible for the blocking of GA metabolism (54). Studies with cell-free preparations have revealed that most steps after GA_{12}-aldehyde are inhibited by acylcyclohexanediones (52–54, 69, 93, 124, 126, 143). Enzyme kinetic data indicate that the retardants act largely competitively with respect to 2-oxoglutarate (54, 69). The hydroxylations at position 3ß (e.g. the formation of GA_1 from GA_{20}) and also at position 2ß (e.g. the conversion of GA_1 into GA_8) appear to be the primary targets of acylcyclohexanediones (54, 126). These findings are supported by analytical data, generally showing that growth reduction is accompanied by lowered levels of biologically active GA_1 and its metabolite GA_8 but increased concentrations of GA_{20} and earlier precursors of GA_1 (3, 11, 91, 93, 103, 126, 143, 149, 182).

Growth retardation caused by acylcyclohexanediones can be reversed only by GAs that are active per se and need not be metabolically activated, also indicating that late stages of GA formation are blocked (90, 127, 182). In selected cases, compounds like prohexadione-Ca and trinexapac-ethyl may, paradoxically, even lead to increases in shoot growth, most likely by protecting endogenous active GAs from being metabolically inactivated (78). Likewise, the inactivation of exogenously applied GA_1 by 2ß-hydroxylation can be inhibited by simultaneous treatment with an acylcyclohexanedione, resulting in increased GA-like activity (125, 158).

A number of different acylcyclohexadiones and structurally related compounds have been evaluated for their ability to inhibit GA 2ß- (54) and 3ß-hydroxylases (11, 12, 93) in cell-free systems. When the cyclohexane ring was replaced by benzene, an almost complete loss of activity resulted. In contrast, certain pyridine structures displayed a relatively high degree of activity. In structures related to prohexadione, a free carboxylic acid function resulted in higher activity as compared to the corresponding methyl or ethyl esters, most likely due to a higher degree of similarity to 2-oxoglutaric acid. Longer acyl side-chains lead to increased inhibitory activity as compared to shorter ones. However, when applied to intact plants, too long chains caused phytotoxicity. Therefore, substituents such as ethyl or cyclopropyl appear to be optimal for practical uses. In addition to these findings, one has also to consider that esters are often more easily taken up by leaves after spray application than ionized forms. In the plant cell, the acid might be formed again by saponification. Furthermore, esters may be easier to handle for preparing formulated products. Under practical conditions trinexapac-ethyl (an ester) and prohexadione-Ca (a salt of an acid) display similar degrees of activity when applied in appropriate formulation to graminaceous species, such as small grains or turf grasses. However, in dicots prohexadione-Ca generally outperforms trinexapac-ethyl (W Rademacher, unpublished results). This may indicate that trinexapac-ethyl is easily saponified into its active acidic form in grasses, whereas this process is not as pronounced in dicots.

The growth retardant daminozide has been used for many years to reduce excessive shoot growth. Its growth-retarding activity is, however, restricted to relatively few plant species, such as apple, groundnuts and chrysanthemums. Due to toxicological concerns, the importance of daminozide has declined markedly in recent years, particularly for edible crops. Until a few years ago, the mode of action of daminozide had been unclear. In light of structural similarities between daminozide and 2-oxoglutaric acid and re-evaluating older results from the literature, it has been proposed that daminozide, like acylcyclohexanediones, would block GA formation as an inhibitor of 2-oxoglutarate-dependent dioxygenases (137). This hypothesis has later been proven by working with an enzyme preparation derived from cotyledons of *Phaseolus coccineus* and by analyzing the GAs of treated peanut plants (11).

Figure 4 shows the structures of several compounds mentioned and of 2-oxoglutaric acid for comparison.

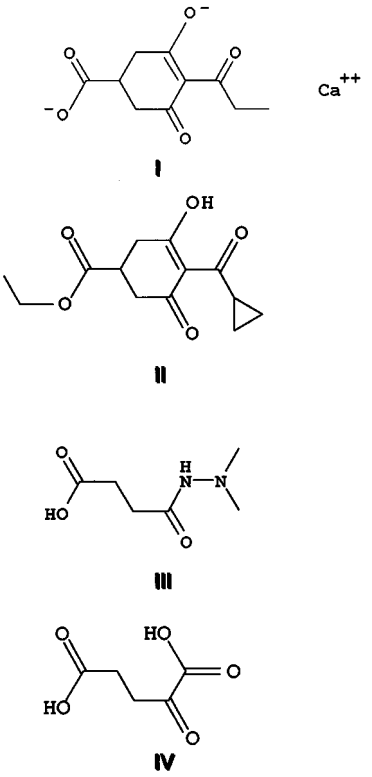

Figure 4 Structural mimics of 2-oxoglutaric acid. I. Prohexadione-calcium = Calcium 3-oxido-4-propionyl-5-oxo-3-cyclohexenecarboxylate {KIM-112, BAS 125..W, ***ProCa***/free acid: KUH 833, BX-112, ***ProH***}; II. Trinexapacethyl (=cimectacarb) = Ethyl-(3-oxido-4-cyclopropionyl-5-oxo) oxo-3-cyclohexenecarboxylate {CGA-163′935, ***TrixE***}; III. Daminozide = Succinic acid 2,2-dimethyl hydrazide {B-995, ***SADH***}; IV. 2-Oxoglutaric acid.

16,17-Dihydro-GAs

16,17-Dihydro-GAs represent the most recent group of growth retardant. A number of different structures of this type, mostly GA_5 derivatives, have been described to reduce shoot elongation in *Lolium temulentum* (41, 114–116) and other grasses (96). Evidence is available that their growth-retarding activity is due to an inhibition of dioxygenases, which catalyze the late stages of GA metabolism, particularly 3ß-hydroxylation (45, 91, 161). Similar to acylcyclohexanediones, such GA derivatives also increase the biological activity of GA_1, when applied simultaneously to seedlings of wheat and barley (W Rademacher, unpublished). Hence, most likely GA_1 2ß-hydroxylation is inhibited as well, although this may be less pronounced in species such as *Lolium temulentum* (91). Treating plants with 16,17-dihydro-GA_5 results in changes of GA levels similar to the ones caused by acylcyclohexanediones: In *Lolium temulentum* (91) and *in Sorghum bicolor* (45) the levels of GA_1 declined, whereas GA_{20} accumulated significantly.

With a view to finding new anti-lodging compounds for small grains, several 16,17-dihydro-GA_5 derivatives have recently been retested. Applying the compounds in conjunction with suitable adjuvants has, in general, significantly raised

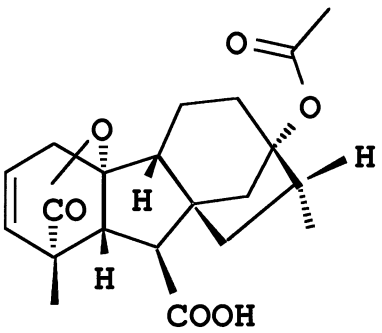

Figure 5 16,17-Dihydro-GAs exo-16,17-Dihydro-GA_5-13-acetate.

their biological activity. Any comparison with older data is almost impossible, however. As a result of these investigations, *exo*-16,17-dihydro-GA_5-13-acetate (Figure 5) represents the most active growth retardant ever known for graminaceous plants. Under greenhouse conditions effects of as little as 500 mg per hectare can be detected in wheat and barley (141). However, in order to reduce the risk of lodging under practical conditions, rates in the range of 20 g per hectare have to be used (141). In contrast to graminaceous plants, *exo*-16,17-dihydro-GA_5-13-acetate and related structures are virtually inactive in reducing shoot growth in any other plant species tested (141). Likewise, 16,17-dihydro derivatives of GA_{19}, GA_{20}, and GA_1 did not cause growth retardation in willow (*Salix pentandra*). As compared to their naturally occurring counterparts, GA-like activity of these compounds was significantly reduced (132). These results demonstrate that 16,17-dihydro derivatives, particularly of GA_5, interact very specifically with GA formation only in graminaceous species. This could be due to distinct peculiarities of GA metabolism or uptake, translocation, and degradation in these species.

It appears logical that *exo*-16,17-dihydro-GA_5-13-acetate and related structures are highly specific in competing in grasses with the natural GA substrates, e.g. GA_{20}, for the respective enzymatic sites (161). The *endo* form of 16,17-dihydro-GA_5-13-acetate is somewhat less active than its *exo* counterpart (141). Similar observations have also been made with slightly different and, in general, less active structures, such as 16,17-dihydro-GA_5 (141) and 17-alkyl derivatives (114). A number of substituents of 16,17-dihydro-GA_5 at C-13, in particular esters and ethers of different chain length, have been assayed in wheat and barley. The 13-acetate function was clearly the most active one. However, a fairly high degree of activity is still observed with groups such as *n*-propionate or O-ethylether (141). According to our current knowledge, it appears that a double bond between C-2 and C-3 is of importance for high growth-retarding activity. Also, the absence of hydroxy groups on these carbon atoms seems to be an essential element for pronounced growth-retarding activity: In sharp contrast to its GA_5 analog, *exo*-16,17-dihydro-GA_1-13-acetate, which displays a single bond between C-2 and C-3 and is 3ß-hydroxylated, is virtually inactive in wheat and barley seedlings

(LG Mander & W Rademacher, unpublished results). Several naturally occurring GAs, in particular GA_5, also reduce the activity of 3ß-hydroxylases obtained from immature *Phaseolus vulgaris* seeds. Unlike several other GAs tested, the inhibiting GAs did not possess any carbonyl functions in the A-ring of the molecule except for the lactone group (100, 156). GA_5 also reduced the conversion of GA_{20} into GA_1, although at a clearly lower degree of activity than 16,17-dihydro-GA_5 (91). Inhibition of GA metabolism has also been reported for other structural GA variants. For instance, deoxygibberellin C, which is an isomer of GA_{20} and displays a keto function at C-16 and a methyl group at C-13, inhibits shoot growth in normal rice and maize (64, 148). Earlier suggestions that deoxygibberellin C would act by inhibiting 3ß-hydroxylation (63) were proven in an enzyme system derived from embryos of immature *Phaseolus vulgaris* seeds (92). In contrast, 3ß-hydroxylase was unaffected in a cell-free system derived from pumpkin endosperm (92), which indicates that species-specific differences may exist. Note also that deoxygibberellin C inhibits shoot growth in rice and maize, but not in cucumber (148) and pea (Y Kamiya, personal communication). This would indicate that, similar to 16,17-dihydro-GA_5 derivatives, graminaceous species, but not dicots, respond with retarded growth. Thus it appears that a double bond between C-2 and C-3 and the absence of hydroxy groups on these carbon atoms, combined with the 16,17-dihydro function, are the main important structural elements of this new class of growth retardant. Derivatization of the hydroxy function at C-13 seems to be of secondary relevance only.

Several 16,17-dihydro GAs occur naturally in higher plants or in GA-producing fungi (GA_2, GA_{10}, GA_{41}, GA_{42}, GA_{82}, GA_{83}). Likewise, some synthetically produced 16,17-dihydro-GAs were dealt with a number of years ago (10, 79). None of these compounds has ever been described as possessing growth-retarding activity. At that time, testing was rather performed to determine GA-like activity and, except for some dwarfing genotypes, graminaceous species were not employed in the assays. As a consequence, the growth-retarding properties of compounds such as 16,17-dihydro-GA_5 (10) did not show up.

EFFECTS OF GROWTH RETARDANTS ON GA METABOLISM IN GA-PRODUCING FUNGI

Many investigations on growth retardants have been conducted with GA-producing fungi, since the analysis of GAs, for instance, from cultures of *Gibberella fujikuroi* and *Sphaceloma manihoticola*, is relatively easy owing to the presence of much higher amounts than in higher plants. In general, the steps of fungal GA metabolism are deemed to be closely related to those in higher plants, although distinct differences must not be overlooked (138).

Both in *G. fujikuroi* and in *S. manihoticola*, onium compounds such as chlormequat chloride, AMO-1618 and mepiquat chloride cause a clear inhibition of GA formation. Only chlorphonium is relatively inactive in these fungi, which is most

likely due to rapid disintegration throughout fermentation (61, 95, 130, 136). Fungal GA production is also blocked by a number of growth retardants with a nitrogen-containing heterocycle (29, 136).

Contrasted with the situation in higher plants, GA formation in *G. fujikuroi and S. manihoticola* is not affected by acylcyclohexanediones such as LAB 198 999 (136) or prohexadione-Ca (W Rademacher, unpublished results). This could be explained by a relatively rapid disintegration in fungal cultures, since both compounds are known to be relatively short-lived in biological systems (40). However, daminozide, which has a similar mode of action, did not affect GA synthesis of *G. fujikuroi* even though it remained intact during fermentation (130). *exo*-16,17-dihydro-GA_5-13-acetate does not interfere with GA production in *G. fujikuroi*, nor at the same time is it metabolized (W Rademacher, unpublished results). Altogether, one should not rule out the possibility that the late steps of GA metabolism in fungi are catalyzed by enzymes, which are different from the ones in higher plants. This suggestion is supported by the fact that to date only cyclases and monooxygenases, but not dioxygenases, could be detected in *G. fujikuroi* (166).

SIDE EFFECTS OF GA BIOSYNTHESIS INHIBITORS

The commercially available plant growth retardants have undergone intensive testing in the processes of selection and registration. Therefore, any side effects can be expected to be either neutral or even positive to the growth of treated plants. Early precursors of GA formation, such as IPP, are shared with other terpenoids, and thus there are links, for example, to the biosynthesis of sterols, carotenoids, abscisic acid (ABA), and cytokinins. In addition, related enzymatic reactions may be found in other pathways. Cytochrome P450-dependent monooxygenases would appear to be of particular relevance in this context, since many isoforms exist capable of modifying a variety of substrates (152). Furthermore, the possibility that indirect effects will also influence certain metabolic reactions cannot be ruled out. From the wealth of information available, the following parts of this contribution will concentrate on side effects that are relevant for plant development and plant defense reactions.

Effects on the Levels of Other Phytohormones

Plant growth retardants have often been reported to interfere with the endogenous levels not only of GAs but also of other plant hormones. Here, reference is made only to reports in which reliable methods, as seen from today, have been employed. Thus, many older contributions, most of which involve the long-known onium compounds, have not been considered.

Many investigations have dealt with the effect of growth retardants with a nitrogen-containing heterocycle on levels of hormones other than GAs (see surveys by 43, 55, 134). Typically, these compounds induce increased contents of

cytokinins, whereas ethylene levels are lowered. ABA concentrations may be significantly increased under distinct conditions whereas the auxin status is not significantly affected. Resulting primarily from these effects, a delay in senescence and increases in resistance to environmental stresses are often found (43, 55). At present, the observed effects on cytokinin and ethylene levels cannot be explained satisfactorily, since no metabolic links are obvious. It rather appears that nonspecific effects are responsible for the hormonal changes observed: Under the influence of growth retardants, assimilates are often shifted into the roots, which are known to be a major site of cytokinin formation. The resulting stimulation of root growth may lead to an increased formation of cytokinins, which are then exported into the shoot (42). Work with the triazole-type retardants BAS 111 W (56) and uniconazole-P (98) and paclobutrazol (120) indicated that ethylene formation might have been reduced by blocking aminocyclopropanecarboxylic acid (ACC) oxidase. Again, this must be an indirect effect since ACC oxidase is a dioxygenase-type enzyme (33, 117) and not a cytochrome P450-dependent monooxygenase, as suggested earlier (98). Inhibited conversion of ACC is also proposed as a reason for increased levels of polyamines (58, 81). The situation is clearer with regard to the mechanisms leading to increased ABA levels: By using detached leaves *of Xanthium strumarium*, it could be shown that tetcyclacis is capable of inhibiting the oxidative metabolism of ABA into phaseic acid (181), which is biologically inactive. As a result, ABA accumulates. Similar observations have been made in embryos of maize (6), primary leaves of barley (168), and in the moss *Riccia fluitans* (76). Since this inactivation involves 8′-hydroxylase, a monooxygenase that is cytochrome P450-dependent (32, 99, 180), the enzyme is likely blocked in a manner similar to *ent*-kaurene oxidase. Blocking ABA 8′-hydroxylase with tetcyclacis may lead to an accumulation of this hormone in a relatively short time (86). Other growth retardants of the group of monooxygenase inhibitors affect ABA metabolism in a similar fashion in other plant species. However, this effect is clearly not achieved by all retardants of this type in all plant species (20, 134, 139). It rather appears that the right compound has to match the right species. This would be in line with other reports, which indicate that many different cytochrome P450-dependent monooxygenases may occur in different species, the substrate specificity of which is not very pronounced (152). Knowing about the existence of monooxygenases that may affect, at the same time, key enzymes of GA and ABA metabolism, it is tempting to suggest that such enzymes could be part of the plant's rapid response mechanism to cope with stressful situations (139). Under favorable growing conditions, these monooxygenases might be "switched on," resulting in low ABA but high GA levels, thereby allowing intensive assimilation and shoot growth. Conversely, under situations such as drought stress, these enzymes would be "switched off," leading to low GA but high ABA levels. As a consequence, shoot growth, photoassimilation, and transpiration would be diminished.

Acylcyclohexanediones, although affecting different types of enzymes in GA metabolism, seem to have similar side effects on other hormones as N-heterocyclic compounds. Prohexadione-Ca, trinexapac-ethyl and LAB 198999 reduce ethylene

levels in sunflower cell suspensions and in leaf disks of wheat (55). In shoots of wheat and oilseed rape, prohexadione-Ca leads to increased concentrations of cytokinins and ABA, while no major changes of indole-3-acetic acid contents occur (57). Since no immediate effect of acylcyclohexadiones on the metabolism of cytokinins and ABA are conceivable, indirect effects seem to play a role. In contrast, effects on ethylene levels may, at least partly, be explained by a more direct interaction: Ethylene is generated from aminocyclopropanecarboxylic acid (ACC) in a reaction catalyzed by ACC oxidase. This is a dioxygenase that requires ascorbic acid as a co-substrate. 2-Oxoglutaric acid and similar compounds inhibit its activity (83). It seemed, therefore, appropriate to investigate the effect of prohexadione-Ca, due to its structural relationship to 2-oxoglutaric and, also, ascorbic acid, on this reaction. Employing an enzyme system prepared from ripe pear, it was demonstrated that prohexadione-Ca was inhibitory to ACC oxidase at an I_{50} of approximately 10^{-5} M (142). Daminozide is known to delay the onset of ethylene formation in apple (108). Obviously, this is due to prevention of ACC formation (60) and, unlike the structurally related prohexadione-Ca, daminozide does not affect ACC oxidase.

Effects on Sterol Metabolism

The formation of sterols in fungi and in higher plants involves enzymatic reactions that are similar to certain steps in the biosynthesis of GAs (8, 17, 24, 62, 128). Therefore, it is not surprising that several growth retardants show some side effects on sterol metabolism. In the group of onium compounds, chlormequat chloride, AMO-1618, and chlorphonium, applied at high rates, restricted the biosynthesis of sterols and other terpenoids in tobacco and some further plant species. Growth retardation induced by these compounds could be reversed not only by GAs, but also by emulsions of different phytosterols (35, 36). Most likely these growth retardants inhibit 2,3-oxidosqualene cyclase in the course of plant sterol formation, just as a number of other quaternary ammonium compounds do (8, 18, 68). AMO-1618 applied to tobacco seedlings caused an accumulation of 2,3-oxidosqualene and inhibited the incorporation of radiolabeled mevalonic acid into sterols (37, 38). However, this effect could not be repeated in pea microsomes (39) which may indicate the existence of species-specific differences or the necessity of AMO-1618 to be metabolically activated in vivo. Furthermore, concentrations of the different onium compounds well in excess of 10^{-4} M have been used in most cases, indicating that the growth retardants inhibit sterol formation only at a relatively low degree of specificity.

Fungicides of the pyrimidine-, imidazole- and triazole-type often show a growth-regulatory side activity. These compounds act by blocking the oxidative 14α-demethylation in the course of fungal ergosterol biosynthesis (8, 17). Similarly, such fungicides, as well as tetcyclacis, paclobutrazol, triapenthenol and other triazole-type growth retardants, reduce the formation of 14α-demethylated sterols in higher plants by blocking obtusifoliol 14α-demethylase (13, 14, 16, 162).

In general, relatively high rates, which induce extreme growth reduction, are required to obtain such changes (140). However, species-specific reactions must be expected: For instance, the triazole fungicide epoxiconazole induces significant growth retardation selectively in cleavers (*Galium aparine*), which is paralleled by a significant accumulation of 14α-methyl sterols (7). Tetcyclacis, applied at moderate rates, totally changes the sterol spectrum of oat, with cholesterol becoming the dominant sterol (19). A similar reaction was induced in roots of fenugreek (*Trigonella foenum-graecum*) (23). This phenomenon cannot be attributed solely to an inhibition of 14α-demethylase, because different sterols had to be expected then. As a possible explanation, the authors suggested that tetcyclacis would inhibit cholesterol 26-hydroxylase, a cytochrome P450-dependent monooxygenase. 26-Hydroxycholesterol, in turn, leads to the synthesis of saponins, which are of major relevance particularly in oats and fenugreek (see 23). Under similar conditions (reduction of shoot length to 50 to 30% of the respective control), tetcyclacis did not induce such changes in the sterol spectrum of maize, pea, bean, and sunflower (140) and of wheat and barley (RS Burden, personal communication).

Using the pure optical enantiomers of paclobutrazol it could be shown that the (*2R,3R*)-enantiomer is more specifically blocking fungal ergosterol biosynthesis while the (*2S,3S*)-form is the more specific inhibitor of GA biosynthesis (160). Likewise, (*2R,3R*)-paclobutrazol reduced plant sterol formation much more intensely than its (*2S,3S*)-analog (16). The (*2R,3S*)-enantiomer, mentioned in this contribution as being even more active as an inhibitor of phytosterol formation, is practically absent in commercial paclobutrazol (see 160). It could be shown that the (*2R,3R*)-form relates closely to lanosterol, while the (*2S,3S*)-enantiomer is structurally similar to *ent*-kaurene (160). Thus it is likely for higher plants also that the different enantiomers compete in distinct cytochrome P45O species with the substrates of sterol or GA biosynthesis, respectively. Similar chiralic specificities have been found for uniconazole-P (48) and triapenthenol (109).

Tetcyclacis, applied at relatively high doses, reduces cell division in cell suspension cultures of maize and simultaneously leads to qualitative and quantitative changes in the sterols present. Adding cholesterol or other plant sterols to the cell cultures can restore normal growth. GA_3, in contrast, has no effect (59). Equivalent results were later reported with cell cultures of celery (*Apium graveoIens dulce*) treated with paclobutrazol (67). Conversely, intense growth retardation caused by tetcyclacis and several triazoles in intact wheat and sunflower plants could not even partly be overcome by external application of emulsions of different phytosterols (W Rademacher, unpublished results). This finding may indicate that sterols play only a minor role in longitudinal growth. However, one has to keep in mind the high degree of lipophilicity of such sterols, which inhibits uptake.

In general, one may conclude that influences of plant growth retardants on phytosterol formation will affect longitudinal shoot growth only to a small extent. Cell elongation, primarily occurring in the growth zones outside the meristems, is the more sensitive process as compared to cell division in the meristems itself. The regulation of cell elongation appears to be closely linked to the availability

of GAs, which can be affected by relatively low retardant concentrations. Higher rates of retardants will additionally inhibit cell proliferation. Most likely, this is still primarily a result of increased GA deficiency. However, one should not rule out that altered sterol levels and, thus, a change in membrane properties may also be of relevance under such conditions (55, 131).

Effects on Brassinosteroid Formation

Brassinosteroids represent a group of plant steroids known to cause remarkable effects in higher plants (26, 113, 176). Their hormonal status is widely accepted due to results obtained from molecular genetic investigations combined with studies of the biosynthetic pathway. The regular plant sterols, campesterol in particular, function as precursors of brassinosteroids (47, 176). In addition to obtusifoliol 14α-demethylation, which is part of the metabolism of regular plant sterols, many of the reactions committed to brassinosteroid formation are catalyzed by cytochrome P450-type monooxygenases (47). It is therefore not surprising that growth retardants such as uniconazole-P (177) and antimycotic imidazoles such as clotrimazole and ketoconazole (174) inhibit brassinosteroid biosynthesis. However, convincing evidence is not yet available that such interactions are specific for brassinosteroid metabolism. Reducing the formation of sterol precursors by inhibiting obtusifoliol 14α-demethylase would also result in a reduction of brassinosteroid levels. Likewise, it remains to be clearly proven that inhibition of shoot growth in brassinosteroid-deficient mutants is caused by a lack of brassinosteroids or of phytosterols in general. The availability of specific inhibitors of brassinosteroid biosynthesis (5) should certainly help to clarify such questions.

Effects on Flavonoid Metabolism

The biosynthesis of flavonoids and other phenylpropanoids comprises steps that are catalyzed by cytochrome P450-dependent monooxygenases and by dioxygenases requiring 2-oxoglutaric acid as a co-substrate (44, 169). Cinnamate 4-hydroxylase, a cytochrome P450-type monooxygenase, is inhibited by relatively high dosages of tetcyclacis in a cell-free system prepared from soybean cell cultures. A higher degree of activity could be observed employing enzymes prepared from pea apices. Several triazole-type retardants also tested were inactive in the soybean system and gave only weak effects in the pea preparation (97, 140). Results varying between different inhibitors and different plant species were also obtained when ancymidol, tetcyclacis and ketoconazole were tested on flavonoid 3′-hydroxylase and flavone synthase II (159) and chalcone 3-hydroxylase (172). Likewise, anthocyanin formation was inhibited in buckwheat hypocotyls and sorghum coleoptiles by low dosages of tetcyclacis whereas the triazole-type inhibitor BAS 111. . .W was almost inactive (143). These observations provide further evidence that a great number of isoenzymes exist among cytochrome P450-dependent monooxygenases. Most likely, according to their sterical fit, typical inhibitors of GA metabolism and related compounds may or may not affect such enzymes involved in the metabolism of flavonoids as of sterols or other plant components.

High dosages of prohexadione-Ca and other acylcyclohexanediones inhibit the formation of anthocyanins in flowers and other parts of intact higher plants (143). Inhibition of anthocyanin production could also be observed in carrot cell-suspension cultures to which prohexadione had been added (82). It has been suggested that 2-oxoglutarate-dependent dioxygenases, in particular flavanon 3-hydroxylase (FHT), involved in the biosynthesis of anthocyanidins would represent targets for these growth retardants (143). This hypothesis has meanwhile been supported by the finding that treated young shoots of apple are unable to convert eriodictyol by 3-hydroxylation into flavonoids such as catechin. Instead, eriodictyol accumulates and large amounts of luteoliflavan, which does not normally occur in apple tissue, can be found (142, 145). Apple and pear trees treated with prohexadione-Ca are significantly less affected by fire blight, caused by the bacterium *Erwinia amylovora* (31, 173, 175). Likewise, the incidence of fungal diseases, e.g. scab on apple shoots and gray mold on grape shoots and berries, can also be reduced when plants are pretreated with prohexadione-Ca or trinexapac ethyl (E Ammermann, J Speakman, G Stammler & W Rademacher, unpublished). Morphological and anatomical effects caused by the growth retardants should not be ruled out as being of some relevance for these observations. However, there are several indications of an induction of physiological resistance and it is hypothesized that changes in flavonoids or other phenylpropanoids play a major role (142, 145). Daminozide, recently also identified as an inhibitor of 2-oxoglutarate-dependent dioxygenases involved in GA biosynthesis (11), obviously possesses more parallels to acylcyclohexanediones: It may also block anthocyanin formation, for instance in chrysanthemums (118), and it may also cause a slight induction of resistance against fire blight in apple (KS Yoder, personal communication).

Effects on Other Metabolic Reactions

Further side effects of plant growth retardants have been reported for the inhibitors of cytochrome P450-dependent monooxygenases. Under certain circumstances, compounds of this type may have a relatively high efficiency in blocking the oxidative metabolism of certain herbicides and other xenobiotics (e.g. 21, 27, 102, 122). Tetcyclacis is able to inhibit the formation of jasmonic acid in osmotically challenged barley leaves. It is suggested that allene oxide synthase, which is involved in its biosynthesis, is the target enzyme (168). In a cell-free system derived from the blue-green alga *Aphanocapsa sp.*, tetcyclacis and an experimental triazole-type growth retardant inhibited hydroxylating reactions in the course of xanthophyll formation (9).

Further Important Features of the Different Growth Retardants

Under practical conditions, the growth-retarding effect of a given compound is not necessarily determined by its type of interaction with GA metabolism. Rather, factors such as plant responsiveness, uptake, translocation, persistency, and side

effects are of relevance (cf. 43, 134, 151). It is known, for instance, that cotton is relatively sensitive towards mepiquat chloride. A typical dosage would be around 50 g of active ingredient per hectare and season. In contrast, a dosage of approximately 1000 g/ha is required for proper growth regulation in wheat. Even other plant species are virtually insensitive to this retardant. Most growth retardants are applied as a spray, after which they are absorbed via the leaves and translocated to the growing shoot tissues. However, compounds such as paclobutrazol, uniconazole-P, or tetcyclacis are translocated almost entirely acropetally and are absorbed relatively poorly by shoot parts. Hence, in order to obtain appropriate results, they are often applied as a soil drench. Tetcyclacis should even be fed via the roots in hydroponics or similar systems, since it is almost immobile in soil. Extreme differences among different growth retardants are found with regard to their longevity. Whereas the half-life period of paclobutrazol or uniconazole-P in a plant or in a soil is in the range of several months, compounds such as trinexapac-ethyl, prohexadione-Ca, or *exo*-16,17-dihydro-GA_5-13-acetate are much more rapidly degraded. For example, the half-life period of prohexadione-Ca in the soil is in the range of hours rather than days. A long-lasting effect may be desirable, for instance, in order to regulate the growth of perennial ornamentals. In contrast, shorter-lived compounds give more flexibility to the grower, who may have better means for applying a retardant as needed.

CONCLUDING REMARKS

The majority of plant growth retardants are inhibitors of distinct steps of gibberellin biosynthesis. They have been selected from a vast number of other candidate compounds making any negative effects on their target plants highly unlikely. Against this background, it may appear surprising that a number of other metabolic reactions can still be affected by such compounds, albeit only at very high dosages usually. When used in agriculture and horticulture, such side effects often add to the benefit of mere growth retardation. To plant scientists, growth retardants, with their typical and with their additional biochemical effects, are valuable tools to gain better insight into the objects of their work.

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