PLANT GROWTH SUBSTANCES

In Plant Growth Substances; Mandava, N.;
ACS Symposium Series; American Chemical Society: Washington, DC, 1979.

Plant Growth Substances

N. Bhushan Mandava, EDITOR
Beltsville Agricultural Research Center

Based on a symposium
presented at the
13th Middle Atlantic
Regional Meeting of the
American Chemical Society
at West Long Branch, New Jersey,
March 19–23, 1979.

ACS SYMPOSIUM SERIES 111

AMERICAN CHEMICAL SOCIETY
WASHINGTON, D. C. 1979

Library of Congress CIP Data

Plant growth substances.
(ACS symposium series; 111 ISSN 0097–6156)

Based on a symposium presented at the 13th Middle Atlantic Regional Meeting of the American Chemical Society at West Long Branch, New Jersey, March 19–23, 1979.
Includes bibliographies and index.

1. Plant regulators—Congresses.
I. Mandava, N. Bhushan, 1934– . II. American Chemical Society. III. Series: American Chemical Society. ACS symposium series; 111.

SB128.P57 581.3'1 79-18933
ISBN 0-8412-0518-3 ACSMC8 111 1–310 1979

ACS Symposium Series

M. Joan Comstock, *Series Editor*

Advisory Board

In Plant Growth Substances; Mandava, N.;
ACS Symposium Series; American Chemical Society: Washington, DC, 1979.

FOREWORD

The ACS SYMPOSIUM SERIES was founded in 1974 to provide a medium for publishing symposia quickly in book form. The format of the Series parallels that of the continuing ADVANCES IN CHEMISTRY SERIES except that in order to save time the papers are not typeset but are reproduced as they are submitted by the authors in camera-ready form. Papers are reviewed under the supervision of the Editors with the assistance of the Series Advisory Board and are selected to maintain the integrity of the symposia; however, verbatim reproductions of previously published papers are not accepted. Both reviews and reports of research are acceptable since symposia may embrace both types of presentation.

In Plant Growth Substances; Mandava, N.;
ACS Symposium Series; American Chemical Society: Washington, DC, 1979.

PREFACE

Plant growth substances occupy an important place in the growth and developmental processes of all plant species. Although Charles Darwin, Boycen–Jensen, and many others were credited for their pioneering work in recognizing that the plant growth phenomenon was under the control of some chemical substances produced by the plants, an actual beginning for the hormone concept was made in 1928 when F. W. Went successfully demonstrated the existence of growth-regulating substances in plants. In fact, two landmark events took place simultaneously in this field. Went and his co-workers in the West discovered auxins from oat seedlings and Kurosawa in the East isolated gibberellins from the fungus *Gibberella fujikuroi*, the causal agent of Bakanae disease in rice. Auxins later were identified as a group belonging to indoles derived from tryptophan; all gibberellins share a common basic *ent*-gibberellane (gibbane) skeleton. Recent additions to these two groups of natural plant growth substances are cytokinins (1964) with a common 6-aminopurine ring system, abscisic acid (1967), a growth inhibitor, and a fruit-ripening agent, ethylene (1962). Thus a correlation was made between the chemical structures and the physiological responses of these compounds. It now is believed that they are present in all plant species, particularly in higher plants, and that plant growth is regulated by these compounds, commonly known as plant hormones.

Several secondary plant products such as phenolics, lipids, steroids, and terpenoids also were shown to be responsible for growth and development and some of them elicit growth responses in conjunction with these endogenous growth hormones. Several synthetic compounds, although different from the natural growth substances, also induce similar biological responses. There was considerable interest in synthetic growth substance research mainly in view of practical applications and some have found limited agricultural uses. Because of the presence of natural plant growth substances in very minute amounts, the progress in this field, particularly in the characterization, was really slow until the recent introduction of new analytical methods.

Food production in the last few decades has been improved greatly by applying chemical fertilizers, irrigation methods, rotation of crops, and plant-breeding practices. Despite these new technologies for increased food production, the crop losses caused by insect pests, pathogens,

In Plant Growth Substances; Mandava, N.;
ACS Symposium Series; American Chemical Society: Washington, DC, 1979.

and weeds are so great that in the United States alone the resulting damage amounts to several billion dollars. It appears likely that future research (e.g., allelopathic compounds) will provide a solution to this problem, and proper pest management by natural methods might further increase agricultural production. In view of the increasing worldwide demand for food, feed, energy, and safe environment, several new approaches are being sought now for increasing agricultural productivity, particularly the crop and biomass production, by modifying such plant control mechanisms as photosynthetic efficiency, light- and stress-related phenomena, and nitrogen fixation of the plants. Undoubtedly, plant growth substances play a key role in understanding these physiological processes.

It was considered timely for the American Chemical Society to take an inventory by reviewing the progress relating to the chemistry, biochemistry, and physiology of plant growth substances at a symposium at the Middle Atlantic Regional Meeting (MARM) in March, 1979. The titles and subjects for this symposium were selected to provide broad coverage in both synthetic and natural growth substances including the analytical methods for their detection and characterization. The participants, experts in their respective fields, were chosen to provide a well-balanced program covering auxins, gibberellins, cytokinins, abscisic acid, ethylene and other natural products, and synthetic growth substances. Nine chapters on natural plant growth substances are followed by two chapters on synthetic growth regulators. It is hoped that this volume will initiate and stimulate work by chemists, biochemists, plant physiologists, and other related scientists. This multidisciplinary approach provides a better understanding of plant internal control mechanisms via growth substances and results in finding practical applications of these compounds for increasing agricultural productivity.

U.S. Department of Agriculture
Beltsville, MD
June 19, 1979

N. BHUSHAN MANDAVA

In Plant Growth Substances; Mandava, N.;
ACS Symposium Series; American Chemical Society: Washington, DC, 1979.

Chemistry and Physiology of Conjugates of Indole-3-Acetic Acid

ROBERT S. BANDURSKI

Department of Botany and Plant Pathology, Michigan State University, East Lansing, MI 48824

Auxins are hormones that promote plant growth. They occur naturally and, in structure, generally possess a planar aromatic ring, an unsubstituted, or electronegatively substituted position ortho to a side chain of, at least, two carbons, and with a carboxyl group on the side chain (1, 2). Examples of auxins are indole-3-acetic acid (3), phenylacetic acid (4), and 4-chloroindole-3-acetic acid (5, 6). Knowledge of the structure of indole-3-acetic acid--IAA--(7) led to the chemical plant growth regulator industry with annual agricultural savings in Michigan alone equal to the cost of all U.S. financed plant hormone research.

Utilization of auxins date to antiquity, as for example, the use of germinating seeds to promote rooting of cuttings (8). The discovery of hormones in roots by the Polish horticulturalist, Teofil Ciesielski (9) and in shoots by the British naturalist, Charles Darwin (10) was made one century ago. They observed that the tip of the root or shoot controlled growth of the tissue some distance from the tip. Thus, an "influence" must have diffused from tip to growing region. Sixty years later, F.W. Went developed the "*Avena* curvature test" for the "influence" (3), and Bonner developed the "straight growth assay" (11). These techniques plus knowledge that tryptophan could be converted to auxin in fungal cultures (12) and that "precursors" in seeds could be converted to auxins by alkaline hydrolysis (13, 14, 15, 16) led to knowledge of the structure of IAA (7).

We stress these assays because, while leading to the discovery of IAA, they imposed structure-activity requirements precluding study of the IAA conjugates--and possibly the discovery of other auxins. For a substance to be active in the assays required that they: 1) permeate membranes in a cut tissue surface, 2) be transported to the growing zone, and 3) promote growth in that zone. Hopefully these three requirements may someday be studied independently.

The biological effects of the auxins are diverse and range from rapid effects, usually growth promotion and occurring within

0-8412-0518-3/79/47-111-001$05.00/0

In Plant Growth Substances; Mandava, N.;
ACS Symposium Series; American Chemical Society: Washington, DC, 1979.

minutes, to growth differentiation, obvious only in days (17). How IAA can elicit profound changes in the size and form of a plant is totally unknown. At the cellular level it is known that the plant cell walls must be "softened" for growth promotion to occur (cf. 18). Such effects, however, may be concomitants of growth and not the "primary" effects of auxin (19).

We suggest that research directed towards correlating and developing biological and physiochemical assays of the auxins, structural characterization of auxins and auxin conjugates and studies of what permits auxins to move to different parts of the plant and, perhaps selectively into different organelles, will be productive approaches. Thus, I shall confine my remarks to, 1) the chemistry of IAA conjugates, 2) the quantitative assay of IAA and its conjugates, 3) the "turnover" of the indolylic compounds of the plant, 4) an indication of how knowledge of pool size and turnover permitted identification of the seed auxin precursor, 5) a demonstration of the equilibrium between IAA and its conjugates, 6) a demonstration of the perturbability of the equilibrium and, lastly, 7) a working hypothesis concerning how a hormonal homeostatic system can be attuned to the environment.

The Structure and Concentrations of Indoles of *Zea mays*

Figure 1 summarizes the structures and concentrations of the IAA conjugates of the kernels of corn (*Zea mays*), the only plant to have been studied in detail. This work was done by my colleagues Drs. Labarca, Nicholls, Ueda, Piskornik and Ehmann (20-25). We have not detected appreciable amide linked IAA in *Zea* but there are three major classes of esters: the IAA-*myo*-inositols, constituting about 15%; the IAA-*myo*-inositol glycosides, about 25%; and the high molecular weight IAA β1→4 glucan, about 50% of the total IAA. Free IAA, the 2-O, 4-O and 6-O IAA glucose esters and the $(IAA)_n$ inositols comprise the remainder. The vegetative tissue of corn contains 300 μg/kg fresh weight of ester IAA and 30 μg/kg of free IAA (26). A major portion of the esters of the shoot is IAA-*myo*-inositol (cf. 27 and Nowacki, unpublished).

The seeds of oats (*Avena sativa*) have been studied by Dr. Percival and shown to have 85% of their IAA esterified to a glucoprotein (28). The glucan is of the lichenan type having both β1→3 and β1→4 linkages. Recently, Ms. P. Hall in our laboratory (personal communication) has isolated IAA-*myo*-inositol from rice (*Oryza sativa*) thus showing the compound originated early in cereal evolution.

This completes our knowledge of the chemistry of the naturally occurring IAA conjugates. IAA-aspartate is known to be formed following exogenous application of IAA to plants of *Pisum sativum* and was the first IAA conjugate to be structurally characterized (29). There are some data indicating that IAA-aspartate occurs naturally (30). In addition, 1-O glucosyl IAA has been reported to be formed following application of IAA to plants (31).

In Plant Growth Substances; Mandava, N.;
ACS Symposium Series; American Chemical Society: Washington, DC, 1979.

Definitive characterization of the biosynthesized 1-O glucose ester has not been published although the compound has been synthesized chemically (32).

Quantitative data on the amounts of free IAA, ester IAA and amide linked IAA have been supplied by Ms. A. Schulze (33). Her data, shown in Table I, permit several conclusions: first, all plants examined contain greater amounts of IAA conjugates than free IAA; secondly, the cereals contain mainly ester IAA; and, thirdly, legumes contain mainly amide linked IAA.

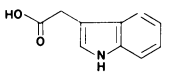
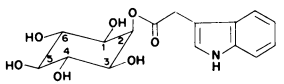
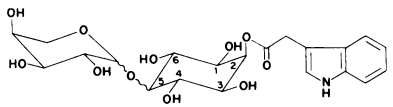
The discovery by Ms. Schulze of the ubiquity of IAA-conjugates convinced us that covalently bonded hormone conjugates were of metabolic importance. Especially significant was the occurrence of IAA conjugates in seedlings where growth rate is a function of IAA concentration. We concluded that, if an equilibrium exists between IAA and its conjugates, then anything that shifts that equilibrium would affect growth rate. To further study this system for hormonal homeostasis two additional things were required, one, a more convenient and sensitive assay for IAA and, two, knowledge of pool size and turnover rates of the indolylic components of the plant.

Methods of Assay

Bio-assays, in the hands of careful workers (cf. 3), have provided almost all of our knowledge of plant hormonal metabolism. Such assays, however, measure activities of extracts and are not equatable to amounts of a chemical entity. Thus, one must rely upon both chemical and bioassays. With regard to chemical assays, it is our contention that the planar indole structure and the number of π bonding electrons renders IAA so unstable that recoveries will be both low and variable thus making internal standards obligatory (cf. 33, 34). We first used ^{14}C-IAA-isotope dilution assays in 1961 (35) and refined the assays in 1974 (33). The original assays required kilogram amounts of tissue and a week to obtain a single value. More recently we developed two new assay procedures which permit one assay in a day or two of work and require only 10 to 50 grams of tissue (36, 37). Our assays are laborious but, in this early stage of chemical assays of IAA, such labor may be desirable.

One method involves the use of 4,5,6,7 tetradeutero-IAA, recently synthesized by Dr. V. Magnus (36). There are two advantages to use of this as an internal standard: first, the deuterium in these positions is stable to the alkaline hydrolysis we use to assay the IAA in the conjugates, and secondly, the presence of four deuterium in the standard moves the ions of the standard away from the isotope cluster normally observed in mass spectrometry owing to the naturally occurring heavy isotopes. We add d_4-IAA, plus a trace of ^{14}C-IAA, to the acetone in which we homogenize the plant material. Then, with or without alkaline hydrolysis, depending upon whether we wish to measure free or

In Plant Growth Substances; Mandava, N.;
ACS Symposium Series; American Chemical Society: Washington, DC, 1979.

COMPOUND	STRUCTURE	AMOUNT IN DRY SEED MG/KG		PERCENT OF TOTAL
Indole-3-acetic acid			0.5	0.8%
Indoleacetylinositols			10.1	15.2%
2-*O*-(indole-3-acetyl)-*myo*-inositol		7.0	10.5%	
1-DL-(indole-3-acetyl)-*myo*-inositol		3.1	4.7%	
Indoleacetylinositol-arabinosides			15.4	23.2%
5-*O*-β-L-arabinopyranosyl-2-*O*-(indole-3-acetyl)-*myo*-inositol		11.7	17.6%	
5-*O*-β-L-arabinopyranosyl-1-DL-(indole-3-acetyl)-*myo*-inositol		3.7	5.6%	

In Plant Growth Substances; Mandava, N.;
ACS Symposium Series; American Chemical Society: Washington, DC, 1979.

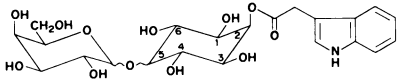
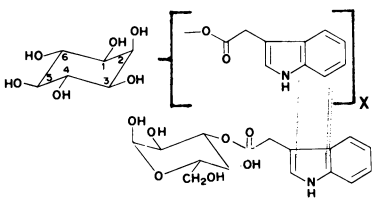
5-*O*-β-L-galactopyranosyl-2-*O*-(indole-3-acetyl)-*myo*inositol		5.4	8.1%
Trace compounds		0.2	0.3%
Di-*O*-(indole-3-acetyl)-*myo*-inositol		0.08	-
Tri-*O*-(indole-3-acetyl)-*myo*-inositol		0.03	-
2-*O*-(indole-3-acetyl)-D-glucopyranose		0.02	-
4-*O*-(indole-3-acetyl)-D-glucopyranose		0.02	-
6-*O*-(indole-3-acetyl)-D-glucopyranose		0.05	-
LOW M.W. COMPOUNDS -- TOTAL		31.2	47.6%
(indole-3-acetyl)-glucan	β 1 4 cellulosic glucan with 7 to 50 glucose units per IAA	35.0	52.5%

Figure 1. The structure and concentration of indolylic compounds in kernels of Zea mays

In Plant Growth Substances; Mandava, N.;
ACS Symposium Series; American Chemical Society: Washington, DC, 1979.

TABLE I

Concentrations of Free and Bound IAA in Various Plant Tissues

Species	Tissue	IAA content		
		Free IAA[1]	Ester IAA[2]	Peptidyl IAA[3]
		µg/kg		
CEREALS				
Avena sativa	vegetative tissue	16	5	69
Avena sativa	seed	440	7620	n.d.
Hordeum vulgare	seed (milled)	40[5]	329	-
Oryza sativa	seed	1703	2739	-
Panicum miliaceum	seed	366	3198	-
Triticum aestivum	seed	123	511	-
Zea mays	vegetative tissue	24	328	60
Zea mays	seed	500 to 1000	71600 to 78500	-
LEGUMES				
Glycine max	seed	4	50[5]	524
Phaseolus vulgaris	seed	20[5]	30[5]	136
Pisum sativum	vegetative tissue	35	5	43
Pisum sativum	seed	93	n.d..	202
OTHERS				
Cocas nucifera	liquid endosperm	0	905	-
Fagopyrum esculentum	seed	40	127	25
Helianthus annus	seed	30[5]	110[5]	-
Lycopersicum esculentum	fruit	trace	trace	-
Saccharomyces cerevisea	packed cells	290	n.d.	-

[1]No alkaline hydrolysis.
[2]IAA after hydrolysis with 1 N alkali minus the free IAA.
[3]IAA after hydrolysis with 7 N alkali minus the free and ester IAA.
[4]Seedlings and fruits are fresh weight, seeds are air dry and yeast cells contain 30% dry matter.
[5]A visual estimate of IAA on a TLC plate as colorimetry was precluded by contaminants.

Reprinted by permission of Plant Physiology (33).

In Plant Growth Substances; Mandava, N.;
ACS Symposium Series; American Chemical Society: Washington, DC, 1979.

total IAA, we re-isolate the IAA by partitioning, DEAE-sephadex, and high pressure liquid chromatography. The resultant mixture of d_4-IAA and the plant derived IAA is methylated and then the indole nitrogen is acylated with heptafluorobutyric anhydride. The methylheptafluorobutyryl IAA derivative is used for gas chromatography-selected ion mass spectrometry (gc-sim-ms). As shown in Figure 2, we monitor four masses, 385 and 389, the molecular ion for methylheptafluorobutyryl IAA (and its d_4 analog), and 326 and 330, the base peaks for IAA and the d_4 standard. The agreement between the ratios of d_4-IAA to IAA at the molecular ion and at base peak give assurance of the validity of the assay. Is this finally an absolute assay, giving data such that error is impossible? We think it is close in that for an error to occur a compound would have to cofractionate with IAA on a DEAE and HPLC column and coemerge from the gc column and then yield the same percentages of ions at m‡ and at base peak as IAA. Still, we do occasionally observe anomalous results and can only warn other workers that dealing with nanogram amounts of indoles is difficult.

A second method of assay of IAA has been developed by Mr. J. Cohen and involves a "double internal standard" usually ^{14}C-IAA and ^{14}C-indole-3-butyric acid (37). I will not discuss this method of assay except to indicate that it is possible to develop assays not involving mass spectrometry but with comparable sensitivity and good selectivity.

Metabolic "Turnover" of Plant Indoles

Ms. Pat Hall and Drs. J. Nowacki and E. Epstein have provided our knowledge of the amounts and rate of metabolic turnover of the indolylic components of the kernels of *Zea mays* (cf. 27, 28; Nowacki, unpublished; Epstein, unpublished). This knowledge has enabled us, 1) to identify the "seed auxin precursor"--that is the compound which is transported from the seed to the growing shoot (39), and 2) has provided a portion of the proof that IAA-*myo*-inositol and IAA are in reversible equilibrium in the shoot tissue. Proving that IAA and IAA esters are in reversible equilibrium in the tissue is essential if we wish to postulate hormonal homeostasis.

These experiments required labeled IAA and tryptophan, which are available commercially, and ^{14}C-labeled IAA-*myo*-inositol. This compound was synthesized by Dr. Nowacki by reacting ^{14}C-IAA-imidazole with *myo*-inositol (40). Application of these labeled compounds to corn kernels, followed immediately by homogenization of the tissue in acetone permitted us to determine the amounts of each constiuent in the kernel by the isotope dilution method of Rittenberg and Foster (41). An extension of this method, whereby the kernels are incubated for varying periods of time after application of the isotopically labeled compound permits determination of the "turnover" of the pool. Such data are shown in

In Plant Growth Substances; Mandava, N.;
ACS Symposium Series; American Chemical Society: Washington, DC, 1979.

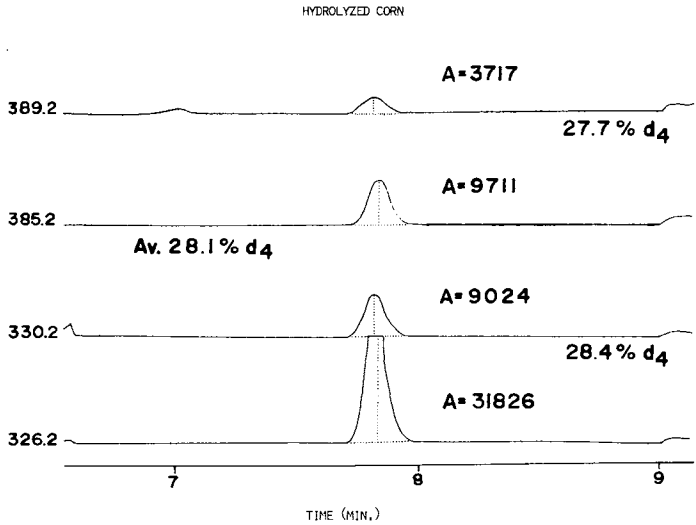


Figure 2. Selected ion chromatogram of a mixture of the methyl esters of tetrafluorobutyryl IAA and d_4-IAA. The IAA was from an extract of corn seedlings and the d_4-IAA added during homogenization. Retention time is in minutes and the masses monitored are 326.2 and 385.2 for IAA and 330.2 and 309.2 for d_4-IAA. The percent d_4-IAA has been computed by the area of the peaks at 330.2/326.2 + 330.2 (base peak) and 389.2/385.2 + 389.2 (molecular ion).

In Plant Growth Substances; Mandava, N.;
ACS Symposium Series; American Chemical Society: Washington, DC, 1979.

Table II. They showed that tryptophan, IAA and IAA-*myo*-inositol are turning over--that is made and then destroyed, or used, at rates such that $t_{1/2}$ was 5, 3.2 and 12 hrs, respectively. Such data permitted several important conclusions concerning the metabolism of these compounds, for example, that it is the IAA esters, and not tryptophan, which serve as a source of IAA for the germinating seed and secondly that the IAA-inositols are turning over at such a rapid rate that they must be in equilibrium with the IAA-*myo*-inositol glycoside pool perhaps acting as glycosylation reagents (Epstein, unpublished).

TABLE II

Concentration and Metabolic Turnover of Some Indolylic Compounds in *Zea* Kernels

Compound	Incubation Time	Recovered Sp. Act.	k	$t_{1/2}$
	hrs	dpm/μg	hrs^{-1}	hrs
	0	31,000		
IAA	4	8,900	0.22	3.2
	8	5,400		
Tryptophan	0	15,200		
	8	5,000	0.14	5.0
IAA-*myo*-inositol	0	935		
	8	590	0.06	12.0

The "Seed Auxin Precursor"

Of great importance, for these studies, was knowledge of both pool size and turnover. This knowledge permitted calculation of the specific activity of the applied isotopically labeled compound at any desired time. Thus, radioactivity, from a labeled compound applied to the endosperm and appearing in the shoot could be translated in amounts of compound moved from seed to shoot.

For these experiments, minute amounts of labeled IAA, tryptophan, or IAA-*myo*-inositol were applied to an incision in the semi-liquid endosperm of 4 day germinated *Zea* seedlings. After 8 hrs of incubation, the shoots were harvested and the IAA, tryptophan or IAA-*myo*-inositol isolated using rigorous purification techniques. Now, knowing the specific activity of the applied compound at the mid-point of the experiment we could calculate the

In Plant Growth Substances; Mandava, N.;
ACS Symposium Series; American Chemical Society: Washington, DC, 1979.

average rate of transport of that compound from endosperm to shoot. A summary of a portion of these data (cf. 27; Nowacki, unpublished; Epstein, unpublished) is shown in Table III. As can be seen IAA-*myo*-inositol moves from endosperm to shoot at a rate of 6 pmols·shoot^{-1}·hr^{-1}. We had previously estimated that about 9 pmols·shoot^{-1}·hr^{-1} of IAA compound must be moving from endosperm to shoot to sustain indole concentrations in the shoot (38) and Gillespie and Thimann (42)measured 5 pmols·shoot^{-1}·hr^{-1} of IAA diffusing down from excised *Zea* tips. On the assumption that what goes down must come up, at least 5 pmols·shoot^{-1}·hr^{-1} must be going up. By contrast free IAA moves from endosperm to shoot at a rate of 0.015 pmol·shoot^{-1}·hr^{-1} and tryptophan in the endosperm appears as IAA in the shoot at less than 0.2 pmol·shoot^{-1}·hr^{-1}. This last figure is high and could, in fact, be zero since non-enzymatic conversion of tryptophan to IAA occurs so readily. Thus these data establish that IAA-*myo*-inositol is the "seed auxin precursor" for *Zea mays* (cf. 27 and Nowacki, unpublished).

TABLE III

Rate of Transport of Indolylic Components From Endosperm to Shoot

Compound applied to endosperm	Compound isolated from shoot	Rate pmols·shoot^{-1}·hr^{-1}
^{3}H-IAA	IAA + ester IAA	0.015
^{3}H-tryptophan	IAA + ester IAA	0.15
^{14}C-IAA-*myo*-inositol	IAA + ester IAA	6.2

The Equilibrium Between IAA and IAA-*myo*-inositol *in vivo*

Dr. Hamilton earlier observed that ether extraction of tissue induced autolysis, liberating active esterases and glycosidases, and thus leading to more free IAA than extraction of tissue by polar, and thus, enzyme-denaturing solvents (35). Thus, we knew then that there were enzymes in the tissue capable of hydrolyzing IAA esters. Much later, Kopcewicz demonstrated the presence of an enzyme system which could synthesize IAA-*myo*-inositol from IAA, ATP, Mg^{++} and CoASH (43). More recently, Mr. Lech Michalczuk (unpublished) has shown that IAA-CoA will acylate inositol only in the presence of other nucleotides. Thus, the reaction is complex, but there is no doubt that enzymes to make and hydrolyze the IAA esters are present in corn.

Now, are the enzymes active *in vivo*? The data of Ms. Schulze and Hall and Nowacki, Epstein and Cohen (27, 38; Nowacki, unpublished; Epstein, unpublished) demonstrate that they are. We

In Plant Growth Substances; Mandava, N.;
ACS Symposium Series; American Chemical Society: Washington, DC, 1979.

showed in 1972 that there is 93% esterified IAA and 7% free IAA in the shoots of *Zea* seedlings (26). The desired experiment then was to apply labeled IAA-*myo*-inositol or labeled IAA to the endosperm of *Zea* kernels and determine whether we approach the same value--93% ester, 7% free--starting either from ester or free labeled IAA. Hydrolysis or synthesis of ester in the endosperm becomes unimportant since the pools are so large that we would not see appreciable radioactivity in the shoot if hydrolysis or esterification occurred in the endosperm. Thus, we can determine whether equilibrium is attained after the labeled compound enters the shoot. Mr. Nowacki applied labeled IAA-*myo*-inositol to the endosperm and found 94% ester and 6% free IAA in the shoot (unpublished). Ms. Hall applied labeled IAA to the endosperm and found 70% ester and 30% free IAA in the shoot (38). These values approximate those found for natural *in vivo* concentrations by Ms. Schulze. The conversion of free IAA to ester, as studied by Ms. Hall, are low but these were early experiments done before we were aware of the ease of hydrolysis of the esters. The results demonstrate that one approaches the same equilibrium amounts of ester and free IAA starting from either compound. We use the word equilibrium to denote that ester IAA can be hydrolyzed to free IAA and free IAA can be converted to ester IAA. We do not imply that this is a reversible reaction catalyzed by a single enzyme (43). This constitutes the first demonstration in biology of an *in vivo* equilibrium between a hormone and its covalently linked conjugates.

Is the Equilibrium Between IAA and its Conjugates Perturable by an Environmental Input

An attempt to answer the question of whether the environment controls plant growth by perturbing the equilibrium between free and covalently conjugated hormone is the major effort of our laboratory. To date only one environmental input has been tested and that is photoinhibition of growth. It has been known for many years that a brief flash of light will inhibit the extension growth of an etiolated seedling plant (cf. 44). The question then becomes, when photoinhibition of growth occurs, will there be a concomitant decrease of free IAA and a commensurate increase in ester IAA? The results of this experiment are shown in Table IV. A 20 second light flash resulted in a 43% inhibition of growth as measured 90 minutes after the light flash. The free IAA decreased by 35% and ester IAA increased by a commensurate amount (45). Thus, our working hypothesis that growth is controlled by the relative amounts of free and conjugated hormone and that it is this ratio which reflects the environment is, in this case, confirmed.

In Plant Growth Substances; Mandava, N.;
ACS Symposium Series; American Chemical Society: Washington, DC, 1979.

TABLE IV

Photo-Induced Change in Growth and in Free and Free Plus Ester IAA

	Dark	Light	Δ	%
		mm/90 min		
Growth	3.6	2.6	-1.1	-34
		μg/kg		
Free IAA	23	13	-10	-42
Free plus ester IAA	68	77	+9	+11

An Hypothesis Concerning Hormonal Homeostasis

The "take home lesson" I wish to leave with you is that the hormones, IAA, the gibberellins, cytokinins, and abscisic acid, all occur in free and conjugated form (46, 47, 48, 49) and that anything that affects the relative amounts of free and conjugated hormone will control growth. A similar hypothesis, concerning mainly the gibberellins has been made (50). In the special case of IAA we have demonstrated that IAA is in equilibrium with its conjugates and that this equilibrium can be shifted by light. Thus, from these limited data, we propose, as a working hypothesis, that the environment affects the rate of plant growth by causing changes in the relative amounts of free and conjugated hormone. This concept is illustrated diagramatically in Figure 3.

We envisage, Figure 3, that environmental stimuli, as for example light, heat, gravity, water stress, etc., impact upon one or more sensory apparatuses. In the case of light this would be a pigment, whereas other stimuli would impact upon the plant counterpart of a "solion" (51). A "solion" senses changes in heat, sound, pressure, gravity, etc., using a reversible redox system and a minute applied potential. Since plant cells have a suitable bio-electric potential (52) and redox systems in their cytoplasm, they can be, in a very real sense, "solions". The sensor then transfers its signal, perhaps a hydride ion from a flavin, to one of the transducer enzymes. Chemically this could mean using the hydride ion to reduce a disulfide bond in an enzyme that synthesizes or hydrolyzes hormone conjugates--thus changing the activity of the enzyme. If the hydrolyzing transducer is activated then more active hormone results. If the synthesizing transducer is activated then there is less free hormone and less hormone effect occurs. We do not know what hormones do to control growth nor how many processes must occur for

In Plant Growth Substances; Mandava, N.;
ACS Symposium Series; American Chemical Society: Washington, DC, 1979.

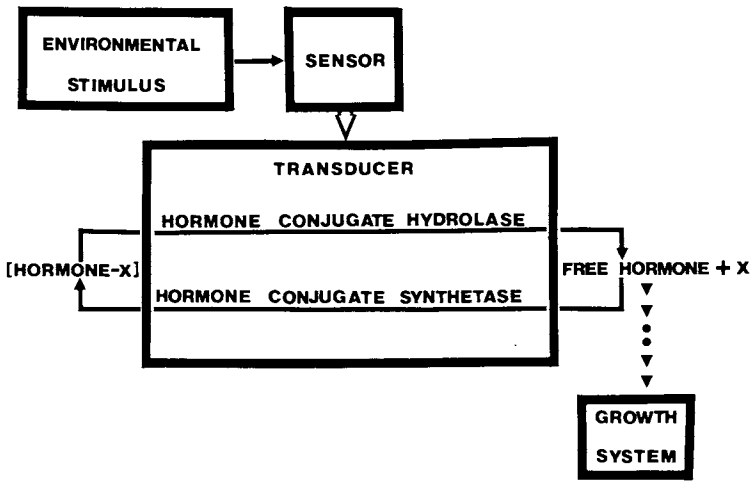


Figure 3. Diagram of a system for control of plant growth by varying the relative amounts of free and conjugated hormone

In Plant Growth Substances; Mandava, N.;
ACS Symposium Series; American Chemical Society: Washington, DC, 1979.

growth to result so we show a dotted arrow leading to growth. My personal feeling is that what the hormone does cannot be discovered until *in vitro* hormone-responsive systems are available (19). Here we encounter the Heisenberg uncertainty principle as applied to biology.

Conclusion

I wish to close now upon an optimistic but cautious note. Ultra-micro scale chemical assays of labile plant hormones will never be easy and routine. Nonetheless, use of internal standards and the detectors now available for gc and hplc, or the sensitive and selective mass spectrometer, permits assays of the hormones in 1 to 10 gm amounts of tissue thus permitting physiological experiments. We must someday return to bio-assays, using a definitive chemical assay and a physiologically sensitive bioassay simultaneously. This is true because the tissues contain both hormones and high amounts of, usually inhibitory, phenylpropanes, as will be discussed later in this symposium. Also, as Professor J. van Overbeek has indicated (personal communication), we will not understand the physiology of the organism until we simultaneously know what happens to all the hormones and their conjugates during complex developmental phenomenon. Difficult as this sounds, it will be possible, provided we confine ourselves to a few plants and seriously try to understand the hormonal system.

Lastly, I believe our working hypothesis of hormones and their conjugates as homeostatic hormone systems will lead to new and answerable questions. I emphasize, however, our data apply to one hormone, and to one environmental input and only to seedling *Zea* plants. Will there be a generality to our working hypothesis that the environment controls the ratio of free to conjugated growth hormone and thus controls growth? We feel that the answer to this question has great implications for the control of food and fiber production by applied growth regulators. I think we should work hard to answer this question.

Abstract

Most of the indole-3-acetic acid (IAA) in plants occurs as ester or amide-linked conjugates. This preponderance and apparent ubiquity led to a study of the functions of the conjugates. Evidence for four physiological roles has been found: 1) Conjugation is reversible and provides the plant with a way to regulate its IAA levels, and thus its growth rate, in accordance with the environment; 2) one of the conjugates (IAA-*myo*-inositol) is the chemical form in which IAA is transported from the seed of corn to the shoot, suggesting that conjugation provides the plant with information concerning where the hormone-precursor should be delivered; 3) IAA conjugates serve as a source of IAA for the seed

In Plant Growth Substances; Mandava, N.;
ACS Symposium Series; American Chemical Society: Washington, DC, 1979.

and seedling; and 4) conjugation of IAA protects it against peroxidative attack. To our knowledge, this is the first case in biology where hormone levels are controlled by the formation and hydrolysis of a covalent bond.

Acknowledgements

The support of the Metabolic Biology Section of the National Science Foundation PCM 76-12356 is acknowledged. Without sustained support this work could not have been accomplished. This is journal article 8963 from the Michigan Agricultural Experiment Station. I am indebted to my colleagues Drs. Cohen, Epstein, and Nowacki and to Mr. Michalczuk, Ms. Schulze, and Ms. Hall for permission to use their unpublished data; to Ms. Joanne Di Lucca Schlub for valuable help in manuscript preparation; and to Dr. R. Chapman and a grant to Professor C. C. Sweeley (NIH-RR00480) for the use of the MSU-NIH mass spectral facility.

Literature Cited

1. Wain, R.L.; Fawcett, C.H. *In* F.C. Steward, Ed. "Plant Physiology VA"; Academic Press: New York, 1966, pp. 231-296.

2. Katekar, G.F. Phytochem., 1979, 18, 223.

3. Went, F.W.; Thimann, K.V. "Phytohormones"; Macmillan: New York, 1937.

4. Haagen-Smit, A.J.; Went, F.W. Konikl. Ned. Akad. Wetenschap., Proc., 1935, 38, 852.

5. Gandar, J.C.; Nitsch, J.P. *In* J.P. Nitsch, Ed. "Regulateurs Naturels de la Croissance Végétale"; Centre Nat. de la Rec. Sci. Quai-Anatole: Paris, 1964, pp. 169-178.

6. Marumo, S.; Hattori, H.; Abe, H.; Manakata, K. Nature, 1968, 219, 959.

7. Haagen-Smit, A.J.; Leech, W.D.; Bergen, W.R. Amer. J. Bot., 1942, 29, 500.

8. Weaver, R.J. "Plant Growth Substances in Agriculture"; Freeman: San Francisco, 1972, p. 120.

9. Ciesielski, T. Beitr. Bio. Pflanzen, 1872, 1, 1.

10. Darwin, C.; Darwin, F. "The Power of Movement in Plants"; D. Appleton: London, 1880.

11. Bonner, J. J. Gen. Physiol., 1933, 17, 63.

12. Thimann, K.V. Ann. Rev. Biochem., 1935, 4, 545.

13. Avery, G.S.; Creighton, H.B.; Shalucha, B. Amer. J. Bot., 1940, 27, 289.

14. Avery, G.S.; Berger, J.; Shalucha, B. Amer. J. Bot., 1941, 28, 596.

15. Haagen-Smit, A.J.; Leech, W.D.; Bergren, W.R. Science, 1941, 93, 624.

16. van Overbeek, J. Amer. J. Bot., 1941, 27, 1.

17. Evans, M.L. Ann. Rev. Plant Physiol., 1974, 25, 195.

18. Cleland, R. Ann. Rev. Plant Physiol., 1971, 22, 197.

19. Bandurski, R.S.; Piskornik, Z. *In* F. Loewus, Ed. "Biogenesis of Plant Cell Wall Polysaccharides"; Academic Press: New York, 1973, pp. 297-314

20. Labarca, C.; Nicholls, P.B.; Bandurski, R.S. Biochem. Biophys. Res. Commun., 1966, 20, 641.

21. Ueda, M.; Bandurski, R.S. Phytochemistry, 1974, 13, 243.

22. Piskornik, Z.; Bandurski, R.S. Plant Physiol., 1972, 50, 176.

23. Ueda, M.; Bandurski, R.S. Plant Physiol., 1969, 44, 1175.

24. Ehmann, A. Carbohy. Res., 1974, 34, 99.

25. Ehmann, A.; Bandurski, R.S. Carbohy. Res., 1974, 36, 1.

26. Bandurski, R.S.; Schulze, A. Plant Physiol., 1974, 54, 257.

27. Bandurski, R.S. *In* W.W. Wells and F. Eisenberg, Eds. "Cyclitols and Phosphoinositides"; Academic Press: New York, 1978, pp. 35-54.

28. Percival, F.; Bandurski, R.S. Plant Physiol., 1970, 58, 60.

29. Andreae, W.A.; Good, N.E. Plant Physiol., 1955, 30, 380.

30. Tillberg, E. Physiol. Plant, 1974, 31, 271.

31. Zenk, M.H. Nature, 1961, 191, 493.

32. Keglevic, D. Carbohy. Res., 1971, 20, 293.

In Plant Growth Substances; Mandava, N.;
ACS Symposium Series; American Chemical Society: Washington, DC, 1979.

33. Bandurski, R.S.; Schulze, A. Plant Physiol., 1977, 60, 211.

34. Little, C.H.H.; Heald, J.K.; Browning, G. Planta, 1978, 139, 133.

35. Hamilton, R.H.; Bandurski, R.S.; Grigsby, B.H. Plant Physiol., 1961, 36, 354.

36. Magnus, V.; Bandurski, R.S. Plant Physiol., 1978, 61(S), 63.

37. Cohen, J.D.; Schulze, A.; Bandurski, R.S. Plant Physiol., 1978, 61(S), 63.

38. Hall, P.L.; Bandurski, R.S. Plant Physiol., 1978, 61, 425.

39. Skoog, F. J. Gen. Physiol., 1937, 20, 311.

40. Nowacki, J.; Cohen, J.D.; Bandurski, R.S. J. Labelled Comp., 1978, 15, 325.

41. Rittenberg, D.; Foster, G.L. J. Biol. Chem., 1940, 133, 737.

42. Gillespie, B.; Thimann, K.V. Plant Physiol., 1963, 38, 214.

43. Kopcewicz, J.; Ehmann, A.; Bandurski, R.S. Plant Physiol., 1974, 54, 346.

44. Elliott, W.M.; Shen-Miller, J. Photochem. and Photobiol., 1976, 23, 195.

45. Bandurski, R.S.; Schulze, A.; Cohen, J.D. Biochem. Biophys. Res. Commun. 1977, 79, 1219.

46. Yogota, T.; Hiraga, K.; Hisakazu, Y. Phytochem., 1975, 14, 1569.

47. Milborrow, B.V. Ann. Rev. Plant Physiol., 1974, 25, 259.

48. Peterson, J.B.; Miller, C.O. Plant Physiol., 1977, 59, 1026.

49. Morris, R.O. Plant Physiol., 1977, 59, 1029.

50. Sembdner, G. *In* K. Schreiber, H.R. Schütte, and G. Sembdner, Eds. "Biochemistry and Chemistry of Plant Growth Regulators"; Inst. of Plant Biochem.: Halle, GDR, 1974, pp. 283-302.

51. Hurd, R.M.; Lane, R.N. J. Electrochem. Soc., 1957, 104, 727.

52. Lund, E.J. "Bioelectric Fields and Growth"; The University of Texas Press: Austin, 1947.

RECEIVED June 19, 1979.

Aspects of Gibberellin Chemistry

PETER HEDDEN

Department of Biology, University of California, Los Angeles, CA 90024

Since their discovery as secondary metabolites of the phytopathogenic fungus, Gibberella fujikuroi, gibberellins (GAs) have been identified from numerous species of angiosperms and some gymnosperms. In addition there are many more reports of GA-like substances (detected by biological assays) occurring in species of both groups. It seems probable that GAs are ubiquitous in seed plants. There are also reports of GA-like substances occurring in lower organisms, including fungi other than G. fujikuroi, algae and bacteria, but none of these have been conclusively identified as GAs.

Gibberellins elicit a variety of physiological responses in seed plants and are well established as hormones controlling plant growth and development. Gibberellic acid (GA_3) is used extensively in agriculture, and is produced commercially from large scale cultures of G. fujikuroi. Other GAs have been found to have specific agricultural applications where they are more effective than GA_3. There is therefore interest in methods for producing GAs, other than GA_3, in commercially useful quantities. GAs are also required for research purposes, both for testing their biological activity and as standards for GA identification and quantitation. In most cases it is impractical to extract sufficient quantities of GAs from their plant sources and they must be prepared chemically or microbiologically from more-accessible compounds.

This review discusses aspects of GA chemistry which may be useful to plant physiologists or biochemists. It covers GA identification and quantitation, particularly difficult tasks considering the low levels of GA in plant tissues. However the problems of GA analysis have been considerably alleviated by recent technology, particularly combined gas chromatography-mass spectrometry. Also described are methods for preparing less-accessible GAs and for isotopically labeling GAs for metabolism studies. Finally some consideration is given to structure-activity relationships. Such correlations may shed light on the mechanism of action of GAs at the molecular level and

0-8412-0518-3/79/47-111-019$09.50/0

In Plant Growth Substances; Mandava, N.;
ACS Symposium Series; American Chemical Society: Washington, DC, 1979.

suggest how the GA structure might be modified to produce compounds with enhanced bio-activity or specialized application.

Structure

The gibberellins (GAs) are a class of tetracyclic diterpenoid acids of which 53 members (Figure 1) have been identified from higher plants or the fungus, Gibberella fujikuroi. For convenience, each fully-characterized, naturally-occurring GA is allocated a number (1), thus the GAs are referred to as GA_1 - GA_{53}. Structurally, the GAs can be subdivided into two groups, the C_{20} and C_{19} GAs. The C_{20} GAs contain the ent-gibberellane skeleton as is typified by the simplest member of this group, GA_{12} (Figure 2). The C_{19} GAs have an ent-20-norgibberellane skeleton in which carbon-20 has been replaced by a hydroxyl group. With one exception, the C_{19} GAs contain a 19,10 γ-lactone, as, for example, in GA_9 (Figure 2). Within these two groups the GAs differ mainly in the degree and position of oxidation of the basic skeleton. The C_{19} GAs are derived biosynthetically from the C_{20} GAs by an as yet unknown mechanism. C_{20} GAs are found naturally having carbon-20 at each possible oxidation level and C_{19} GA biosynthesis may involve successive oxidation of this carbon atom. GAs with an alcohol function at carbon-20 are isolated as the 19,20 δ-lactones in which carbon-20 is prevented from further oxidation (2). It is likely that this lactone is formed during isolation and that these GAs occur as free alcohols in vivo. Those GAs with C-20 at the aldehyde oxidation level appear to exist as the 19,20 lactols in the solid state and as an equilibrium mixture of lactol and aldehyde in solution (3). The C_{20} and C_{19} GA ring systems can be hydroxylated at a number of positions, 2β, 3β and 13 hydroxyled GAs being encountered most frequently. Hydration of the 16,17 double bond is observed in G. fujikuroi to give the saturated C-16 alcohol. Other less frequent functionalities include a double bond at either the 1,2 or 2,3 positions in C_{19} GAs; ketone and epoxide functions.

Stability

The general chemistry of the GAs has been reviewed (4). Many of the GAs contain a high concentration of functional groups rendering them susceptible to rearrangement and degradation. Therefore, as a general rule high temperatures and extremes of pH should be avoided when working with them. In mineral acid 13-hydroxy GAs undergo a Wagner-Merwein rearrangement of the C/D ring system (Figure 3) (5). When the 13-hydroxyl group is absent the 16,17 double bond may be isomerized by acid to the endocyclic 15,16 position or may be hydrated to give the saturated 16-alcohol. Four GAs with the 16-hydroxyl group (GA_2, GA_{10}, GA_{41}, GA_{42}) have been identified from G. fujikuroi (6,7,8). Since the fungus is usually grown at acidic pH, these GAs could be

In Plant Growth Substances; Mandava, N.;
ACS Symposium Series; American Chemical Society: Washington, DC, 1979.

the result of non-enzymatic hydration.

GAs such as GA_1 or GA_4 with only a 3β-hydroxyl group in the A ring are sensitive to dilute aqueous alkali, underoing epimerization at the 3 position to give a mixture of epimers. A retro-aldol mechanism has been proposed for this epimerization (Figure 4) (9), a mechanism supported by the finding that the 3α-hydrogen is retained in the rearrangement (10). The epimerization does not occur if there is also a 2-hydroxyl group or a 1,2 double bond in the A ring. In the latter case there is a shift of the 1,2 double bond to the 1,10 position and the formation of a 19,2 lactone. This isomerization is rather facile and can occur during gas chromatography of GAs, such as GA_3 or GA_7, resulting in broad double peaks.

Many GAs in aqueous solution are slowly degraded, the process being accelerated at higher temperatures as, for instance, during autoclaving. After heating aqueous solutions of GA_3 in an autoclave at 120° for 20 minutes only 1-2% GA_3 remains (11). The chemical processes involved in this degradation of GA_3 have been studied in some detail (12). The proposed pathway of decomposition is shown in Figure 5. The major products are isogibberellic acid, gibberellenic acid, allogibberic acid, 9-*epi*-allogibberic acid and 9,11-didehydroallogibberic acid. The last compound is formed from gibberellenic acid *via* a proposed triene intermediate by an oxidation which appears to involve hydroperoxide intermediates.

Qualitative and Quantitative Analysis

Extraction and Purification. Many methods have been used to extract and purify GAs from plant material, the procedure often depending on the tissues being extracted. Graebe and Ropers (13) in their review on GAs have critically discussed extraction and purification techniques. The concentration of GAs in higher plant tissues varies from about 10 μg per g fresh weight in seeds of certain species to less than 1 ng per g fresh weight in vegetative tissues. The extent of purification required will depend on the particular plant tissue under investigation. Typically the material is homogenized in a water-methanol mixture (about 75% methanol) at low temperature. Acetone has been used as the organic solvent but can cause problems due to the formation of acetonides with vicinal diols in slightly acid conditions (14,15). After the aqueous methanol extraction, the homogenate is filtered and the methanol removed from the filtrate under reduced pressure at 40° or below. At this stage it is common to buffer the aqueous residue, usually with potassium phosphate. With some tissues, for example liquid endosperm, it is convenient to extract directly with a buffer solution at about pH 8 resulting in a cleaner extract (16). The buffered aqueous extract is adjusted to pH 8 and neutral and basic compounds are extracted with an organic solvent,

In Plant Growth Substances; Mandava, N.;
ACS Symposium Series; American Chemical Society: Washington, DC, 1979.

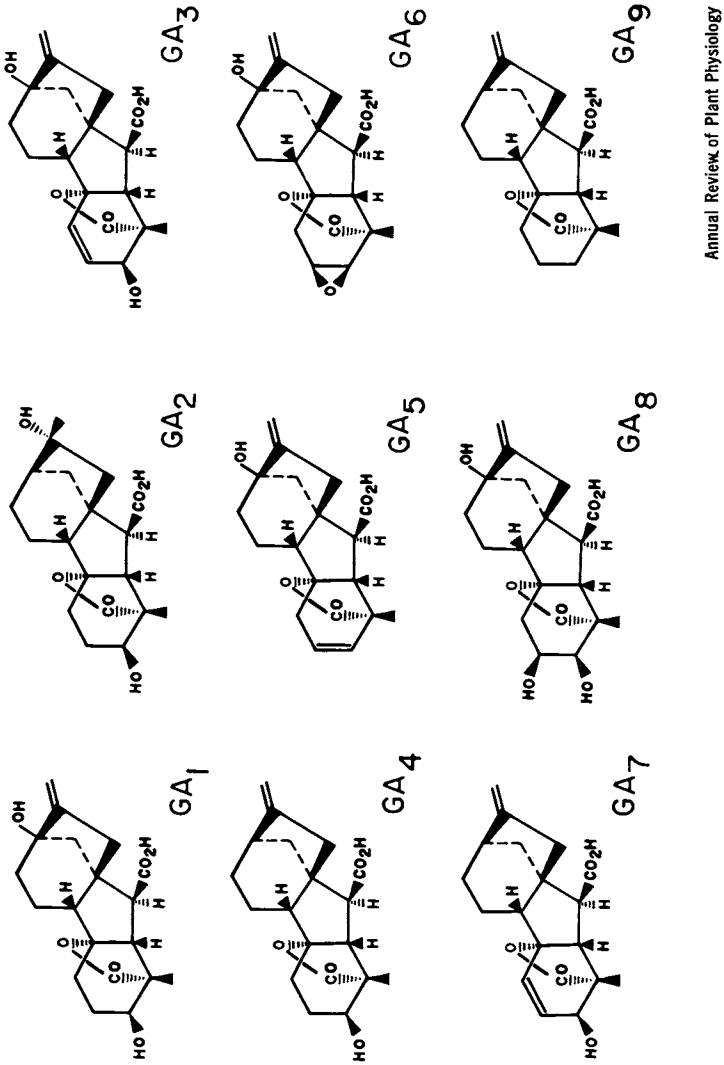


Figure 1. Structures of gibberellins (60)

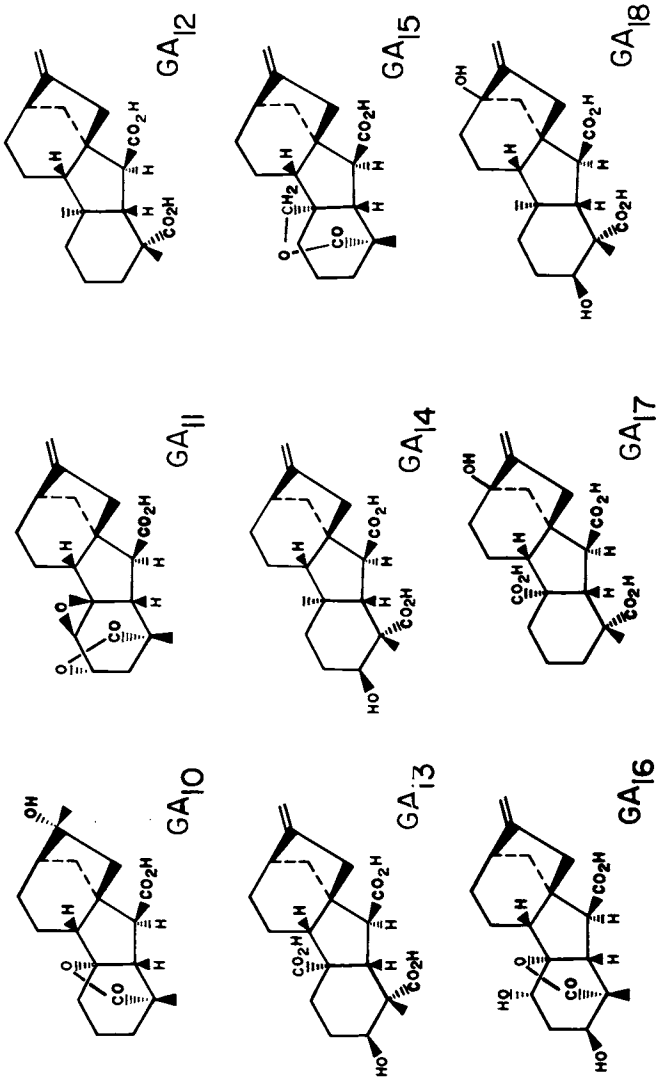


Figure 1. (Continued)

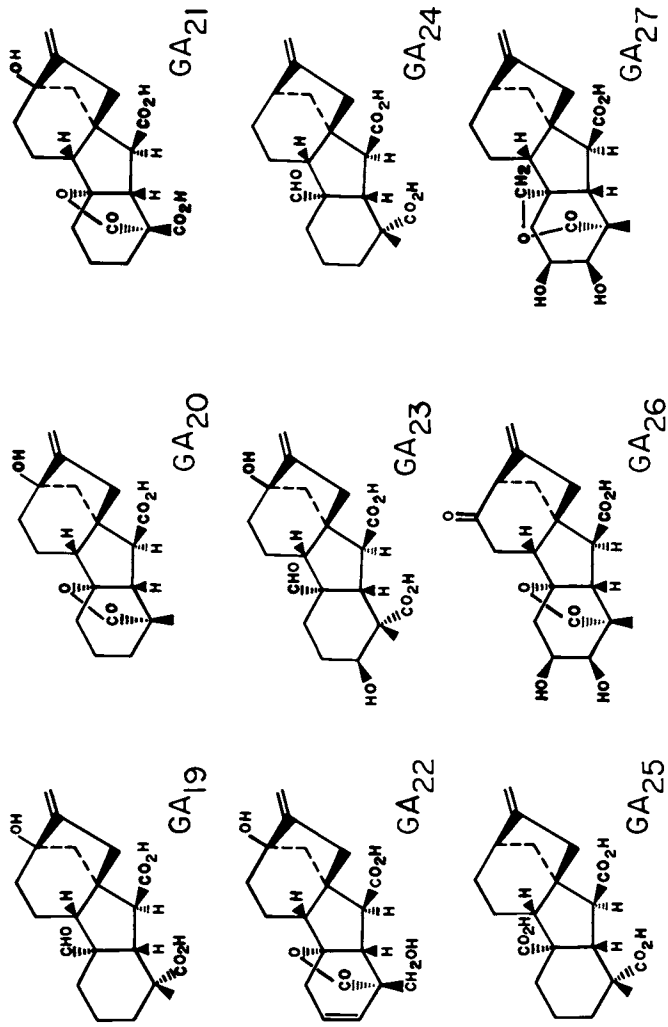


Figure 1. (Continued)

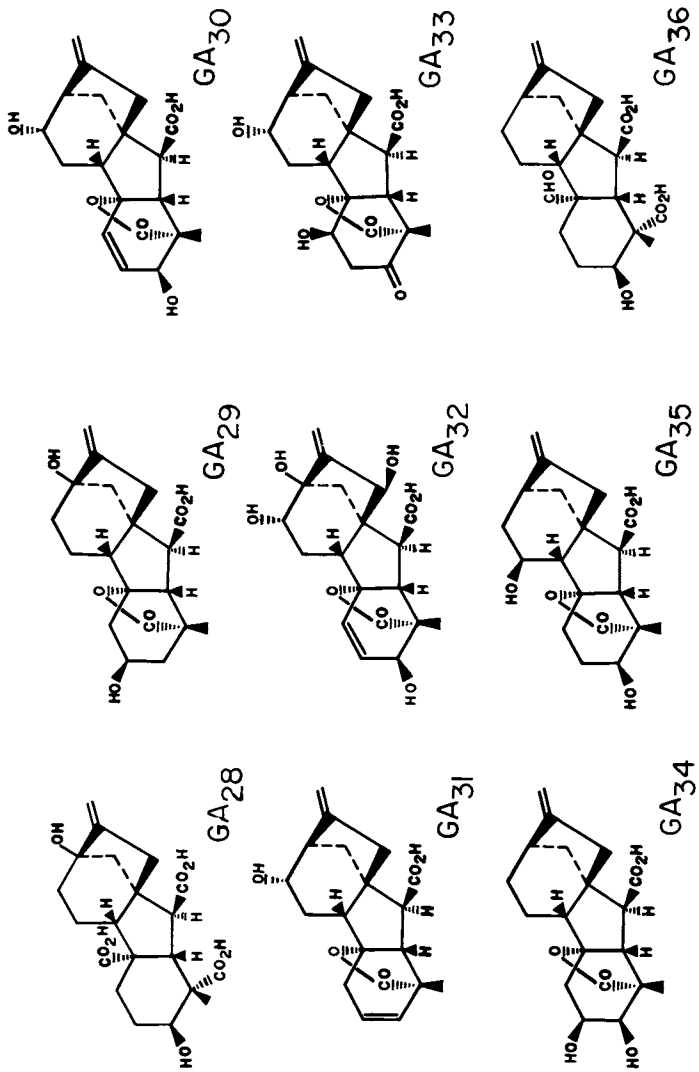


Figure 1. (Continued)

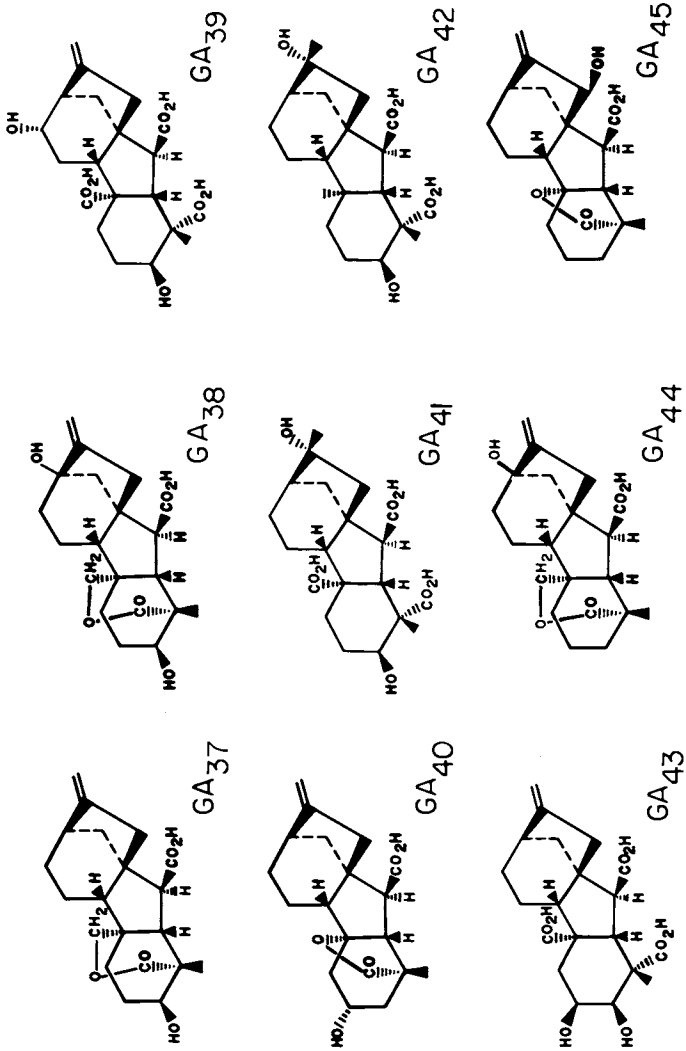


Figure 1. (Continued)

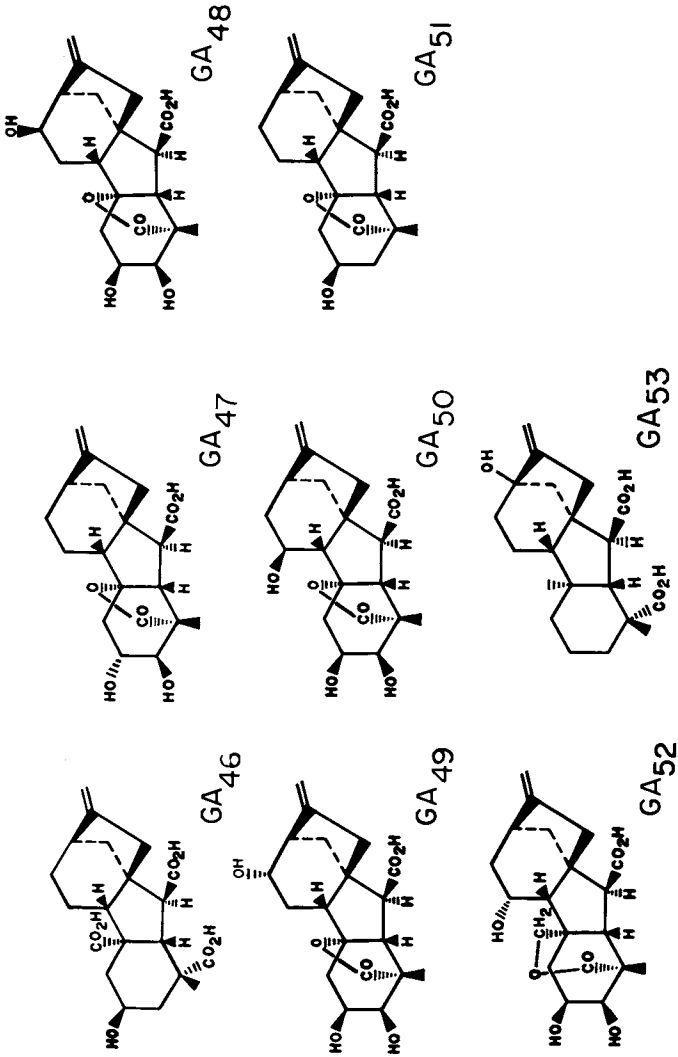


Figure 1. (Continued)

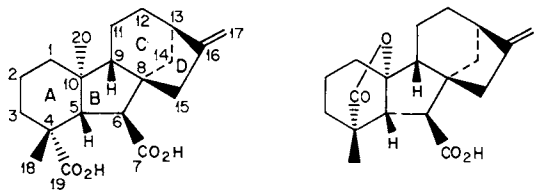


Figure 2. Structures of GA_{12} (a C_{20} GA) and GA_9 (a C_{19} GA) possessing the ent-*gibberellane and* ent-*20-norgibberellane skeletons, respectively. The numbering system and ring designations are shown also.*

In Plant Growth Substances; Mandava, N.;
ACS Symposium Series; American Chemical Society: Washington, DC, 1979.

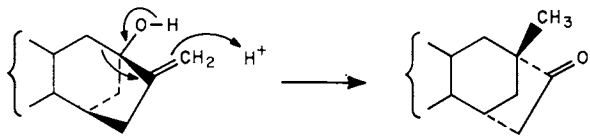


Figure 3. Acid-catalyzed Wagner–Merwein rearrangement of the C/D ring in 13-hydroxy GA's (5)

In Plant Growth Substances; Mandava, N.;
ACS Symposium Series; American Chemical Society: Washington, DC, 1979.

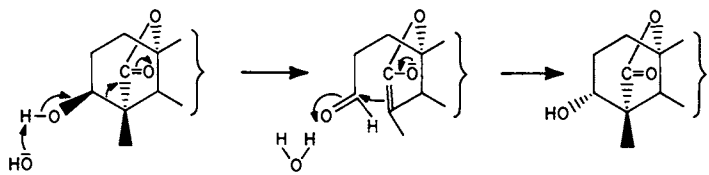


Figure 4. Proposed retro–aldol mechanism for the base-catalyzed epimerization of 3β-hydroxy GA's (9)

In Plant Growth Substances; Mandava, N.;
ACS Symposium Series; American Chemical Society: Washington, DC, 1979.

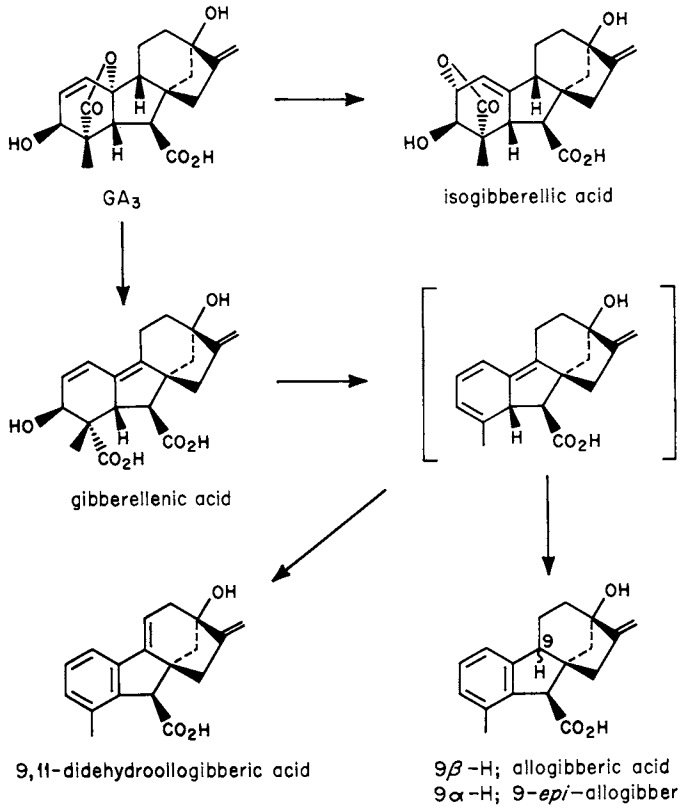


Figure 5. Proposed pathway for the decomposition of GA_3 in aqueous solution (12)

usually ethyl acetate. Some of the least polar GAs, particularly GA_9 and GA_{12}, and GA-glucosyl esters are also extracted in this fraction. Most GAs are extracted into ethyl acetate after adjustment of the pH to 3.0 with hydrochloric acid. Very polar GAs and GA-glucosides can be extracted with n-butanol. It is important that the acidic ethyl acetate and butanol extracts be washed with water before being concentrated to dryness. Otherwise traces of acid (phosphoric acid if phosphate buffer was used) will be concentrated leading to rearrangement or hydration of any GAs present.

Many methods have been used for additional purification of the acidic extracts. If the weight of the extract is not too large then thin-layer chromatography, either adsorption chromatography on silica gel or partition chromatography on kieselguhr, is most convenient, although the separation obtained by this method is poor. Better resolution has been obtained using partition chromatography on columns of silicic acid (17,18,19) or sephadex (17,20). Recently high performance liquid chromatography has been used with GAs and gives excellent separation (21).

The disadvantage of chromatographic methods is that they separate the GAs from each other as well as from other components in the extract. Thus numerous fractions are generated, each of which has to be analyzed separately for GAs. The most satisfactory method available for identifying microgram or less quantities of GAs in a complex mixture is combined gas chromatography-mass spectrometry (GC-MS), a technique which itself contains a separation step. Therefore, ideally, purification methods prior to GC-MS analysis should separate the GAs as a group from other components. Short columns of charcoal-celite (22), gel filtration chromatography on sephadex (23), and anion-exchange chromatography (24,25) have been used for this purpose. Insoluble PVP is often used to remove phenolic compounds (26) and can be added to the aqueous extract before partition against organic solvent. The use of affinity chromatography in which antibodies specific to GA_3 were bound to sepharose has been investigated (27) and could provide a rapid method for purifying GAs if antibodies to all GAs could be developed.

Gibberellin glucosyl ethers and esters are difficult to analyze by GC-MS although they can be gas chromatographed as trimethylsilyl (TMS) ethers (28,29). The conjugates are generally hydrolyzed enzymatically in the crude extract and the free GAs subsequently purified and analyzed. Commercial cellulase (30) or pectinase (31) have been used for the enzyme hydrolysis with varying success. Acid or base hydrolysis is also possible but may lead to rearrangement of the GAs. This complicates the identification unless the rearranged products for each GA are available for comparison (14).

Identification. Combined GC-MS has advanced plant hormone research greatly in recent years and with the introduction of

In Plant Growth Substances; Mandava, N.;
ACS Symposium Series; American Chemical Society: Washington, DC, 1979.

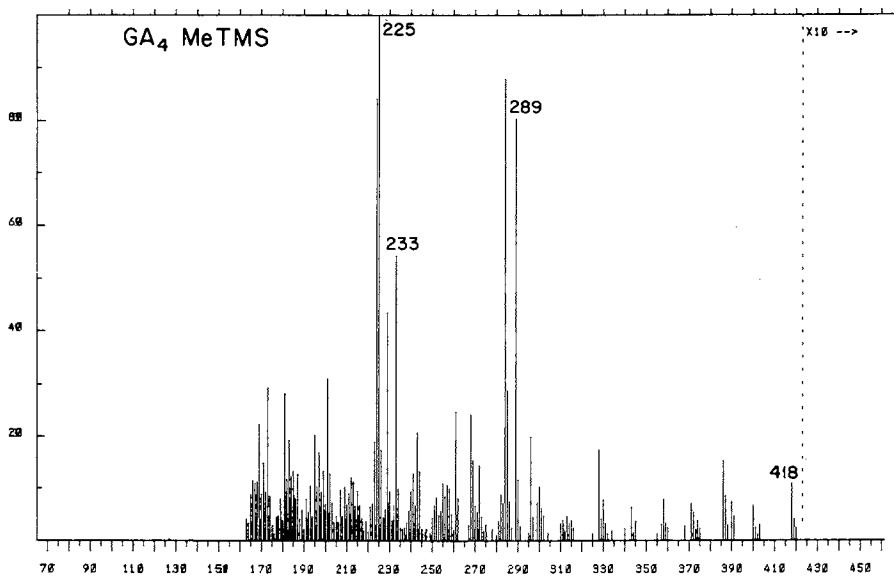
computerized systems (32) the sensitivity and versatility of this technique have been increased still further. The general methodology for GA analysis by GC-MS has been reviewed recently (33, 34). The volatility of GAs is increased prior to GC by forming the methyl esters with diazomethane. Hydroxylated GAs are often converted to trimethylsilyl (TMS) ethers after methylation. The mass spectra of GA methyl esters TMS ethers frequently contain intense molecular ions and characteristic fragmentation patterns, which are easier to interpret than those of the free hydroxy compounds. When recovery of GAs is required after GC, GA TMS ether esters are a convenient derivative since the free GA can easily be recovered after hydrolysis in water.

Combined GC-MS-computer systems with repetitive scanning can lead to the identification of GAs as minor components of complex extracts at levels down to 10^{-11} g. In such cases mass fragmentograms can be constructed in which the distribution of ions of particular m/e values are plotted throughout a GC-MS run. Thus if the presence of a particular GA is suspected, characteristic ions in the mass spectrum of the derivatized GA are plotted. An identification can be made if the ions peak at the same retention time as the GA and have the same relative intensity as in the mass spectrum of the authentic compound (see Figure 6). In this way GAs can be detected which are masked in the GC trace by other compounds of similar retention time (cf. 33). In order for an identification to be made from mass fragmentograms sufficient ions (at least six) must be scanned. It is sometimes possible to obtain a full, interpretable mass spectrum in such cases by background subtraction. The scan (spectrum) in which the ions due to the compound of interest are at a maximum is determined from the mass fragmentograms. This spectrum is then "cleaned up" by subtraction of those ions contributed by the contaminant. It is also possible to compare the background-subtracted spectrum directly with authentic spectra stored in the instrument.

The sensitivity of GC-MS can be increased still further by selective ion current monitoring (SICM) whereby only a limited number of characteristic ions in the mass spectrum of the compound are monitored. Therefore the time for which each ion is monitored, and hence the sensitivity, is increased so that the amount of an identifiable GA is reduced to 10^{-14} g. As in mass fragmentometry, sufficient ions must be monitored for an identification to be made.

Quantitation. Combined GC-SICM has been used mainly for quantitation. For a particular GA the absolute intensity of a characteristic ion in its mass spectrum is related to the amount of GA present, using standards to calibrate the instrument. Frydman et al. (35) used this "external standard method" to measure the levels of a number of GAs throughout the development of pea seeds. An alternative and preferable approach employs

In Plant Growth Substances; Mandava, N.;
ACS Symposium Series; American Chemical Society: Washington, DC, 1979.



In Plant Growth Substances; Mandava, N.;
ACS Symposium Series; American Chemical Society: Washington, DC, 1979.

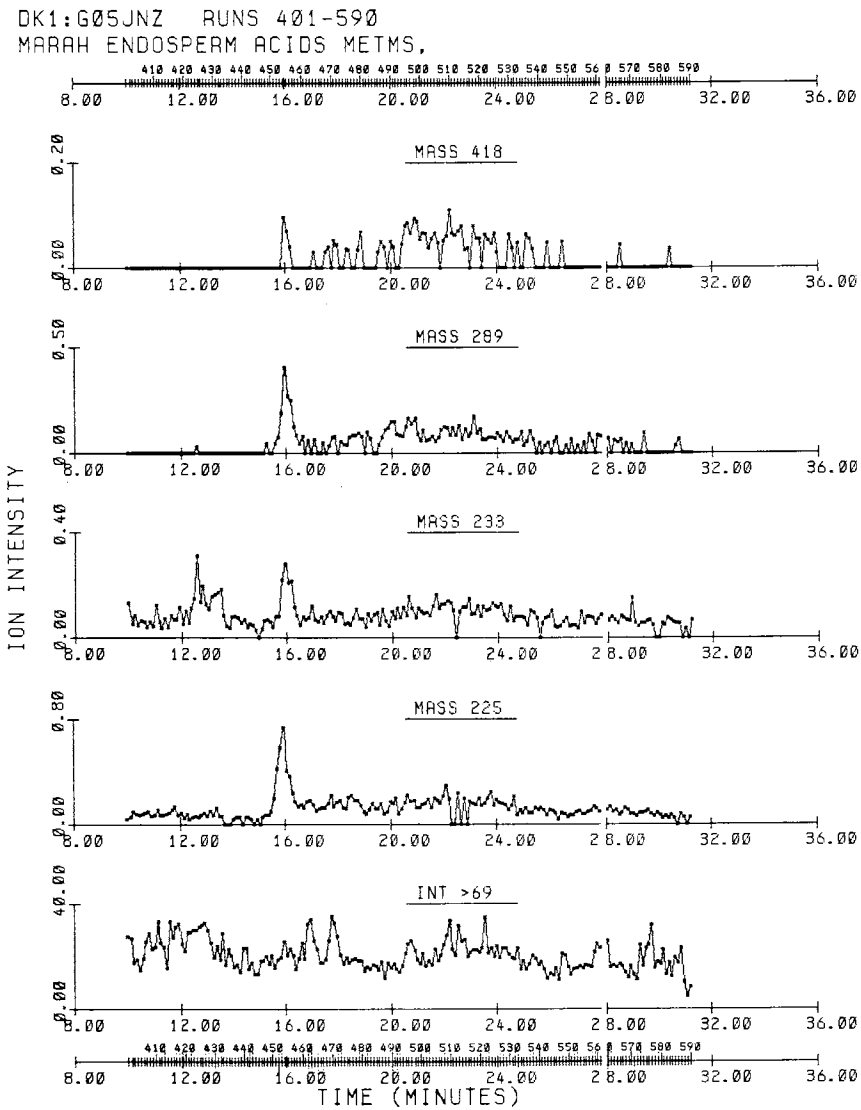


Figure 6. Mass fragmentograms of the acidic fraction from an extract of Marah macrocarpus *endosperm. Ions in the mass spectrum of* GA_4 *Me TMS (*shown above*) were examined. The extract was run as the Me TMS derivative on a MS 902 spectrometer coupled to a Varian 2700 GC via a membrane separator. GC–MS conditions—3% OV-17 on 100–120-mesh Gas Chrom Q in a 2 m × 0.2 cm i.d. column. Temperature—200°C for 5 min; then programmed at 4°C/min. Helium flow—16.5 cm³/min. Electron energy—70 eV; accelerator potential—2.9 KV; separator temperature—226°C; source temperature—250°C.*

In Plant Growth Substances; Mandava, N.;
ACS Symposium Series; American Chemical Society: Washington, DC, 1979.

the use of an internal standard such as an isotopically-labeled analog of the GA being quantitated. A known amount of the standard is added to the plant extract at any early stage of the purification procedure so that account is taken for losses, which can be quite considerable. The most conveniently prepared standards are deuterated GAs (see later). The natural and deuterated GA have almost the same GLC retention time and the group of ions in the region of the molecular ion (M^+) of the natural GA are monitored throughout the mass peak (see Figure 7). The relative intensities of the ions at m/e M and M + X, where X is the number of deuterium atoms in the standard, are calculated and, after correction for natural heavy isotopes in the M + X ions, the relative amounts of the natural and deuterated GAs are determined.

Sponsel and MacMillan (36,25) have illustrated the use of GAs labeled with deuterium and tritium to quantitate GAs and also to follow their metabolism. In one experiment (25) they injected a measured amount of $[^2H][^3H]GA_{29}$ (both species labeled at the same position) into immature pea seeds. Some seeds were extracted immediately and others at regular time intervals thereafter so that the metabolism of GA_{29} could be studied. The $[^3H]$ label was present to determine how much of the added GA_{29} remained unmetabolized after a particular time. Then by comparison of the relative amounts of the natural and deuterated GA_{29} by mass spectrometry, the amount of endogenous GA_{29} was calculated. Using this method they also compared the rates of metabolism of exogenous and endogenous GA_{29}.

Structure determination for new GAs. Mass spectrometry is a useful tool for identifying GAs whose structures have been previously determined, in which case comparison of mass spectra is sufficient. In contrast, the characterization of GAs of unknown structure is a much more difficult and time-consuming task. In these cases mass spectrometry can give information such as molecular weight and some indications of structure. For instance in the mass spectra of the methyl esters TMS ethers a strong ion at m/e 129 indicates that the A ring contains a single hydroxyl group at the 1 or 3 positions. 13-Hydroxy GAs produce ions at m/e 207/208 and vicinal alcohol functions, such as in GA_8, result in a strong ion at m/e 147 (37).

If the structure of a GA can be inferred from its mass spectrum the suspected compound may be synthesized and its mass spectrum compared with that of the unknown. Thus the structures of GA_{46} and GA_{47} were confirmed by the partial synthesis of their methyl esters from GA_4 (38). This synthesis will be discussed in detail later. GA_{45} was identified in immature seeds of Pyrus communis (pear) in an analogous manner by comparison of its mass spectrum with that of a product obtained from incubating ent-15α-hydroxykaurenoic acid with a mutant of the fungus G. fujikuroi (29).

In Plant Growth Substances; Mandava, N.;
ACS Symposium Series; American Chemical Society: Washington, DC, 1979.

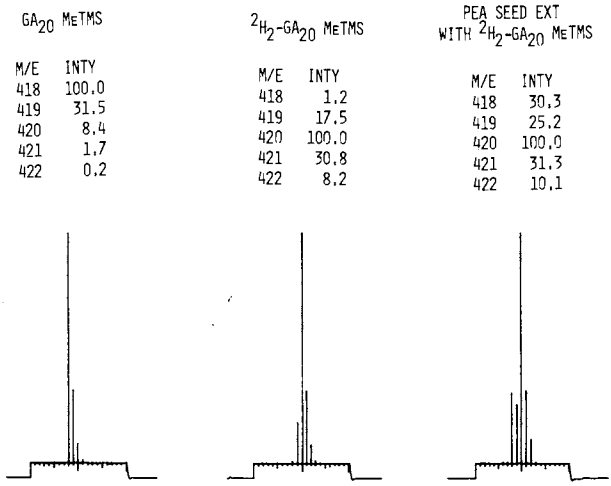


Figure 7. An example of the use of deuterated GA's as internal standards for the quantitation of GA's in plant extracts. [2α-$^2H_1$]GA_{29} was added to an extract of young pea seeds in order to quantitate GA_{29} (cf. 25). Ions in the region of the molecular ion were scanned. The acidic fraction from the pea seed extract was run as the Me TMS derivative on a MS 30 coupled to a Pye 104 GC via a single-stage silicone membrane separator (34).

In Plant Growth Substances; Mandava, N.;
ACS Symposium Series; American Chemical Society: Washington, DC, 1979.

Where it has been possible to obtain sufficient material, GAs of previously unknown structure have been fully characterized and their structure determined by a combination of chemical and spectroscopic methods. Proton Nuclear Magnetic Resonance (NMR) spectroscopy provides a great deal of structural information (4). ^{13}C NMR promises to be a very powerful technique for both structure determination and metabolism studies of GAs (40,41). Yamaguchi et al. (42) used a combination of proton and ^{13}C NMR to determine the structure of GA_{40} (2α-hydroxy GA_9), a minor metabolite of G. fujikuroi.

The assigned structures are confirmed by relating them chemically to GAs or GA-derivatives of known structure. For example, GA_{40} methyl ester was converted to the known compound, deoxy GA_5 methyl ester (43), by formation of the toluene-p-sulfonate and treatment of this with boiling collidine (42). Thus the structures of C_{19} GAs are ultimately related to that of GA_3, whose structure was originally determined by degradation (44) and absolute stereochemistry has been confirmed by X-ray diffraction (45,46,47,48). Recently the total synthesis of GA_3 has been completed by Corey and co-workers (49).

The structures of C_{20} GAs were related ultimately to ent-kaurene via 7β-hydroxykaurenolide (50). However, the two classes of GAs have now been related directly by the oxidative decarboxylation of GA_{13} to give GA_4 (51,52). Bearder and MacMillan (51) treated GA_{13} with lead tetra-acetate to obtain a mixture of GA_4 and the isomeric 20,4 lactone (Figure 8). Murofushi et al. (52) employing a more lengthy procedure, decarboxylated the dimethyl ester of GA_{13} with lead tetra-acetate and lactonized the resulting olefin with iodine (Figure 8). The total syntheses of GA_{15} (53) and GA_{12} (54,55) have also been reported.

The Preparation of Less-readily Available GAs

The isolation of significant quantities of many of the less-accessible GAs from plant tissues is usually impractical. GAs are required as standards, both for qualitative and quantitative analysis, as substrates for metabolism studies (often isotopically labeled) and for biological assays. The most practical methods for preparing these compounds are the chemical or biological conversion to the more available GAs or ent-kaurenoids. Gibberella fujikuroi produces a number of GAs (56), of which GA_3, GA_4, GA_7, GA_{13} and GA_{14} can be obtained in relatively large amounts. These fungal GAs are the starting point for the partial synthesis of less-accessible GAs by relatively simple chemical procedures. Also microbiological methods have been developed to convert GAs and GA-precursors (or analogs of these) of both fungal and higher plant origin to useful products.

In Plant Growth Substances; Mandava, N.;
ACS Symposium Series; American Chemical Society: Washington, DC, 1979.

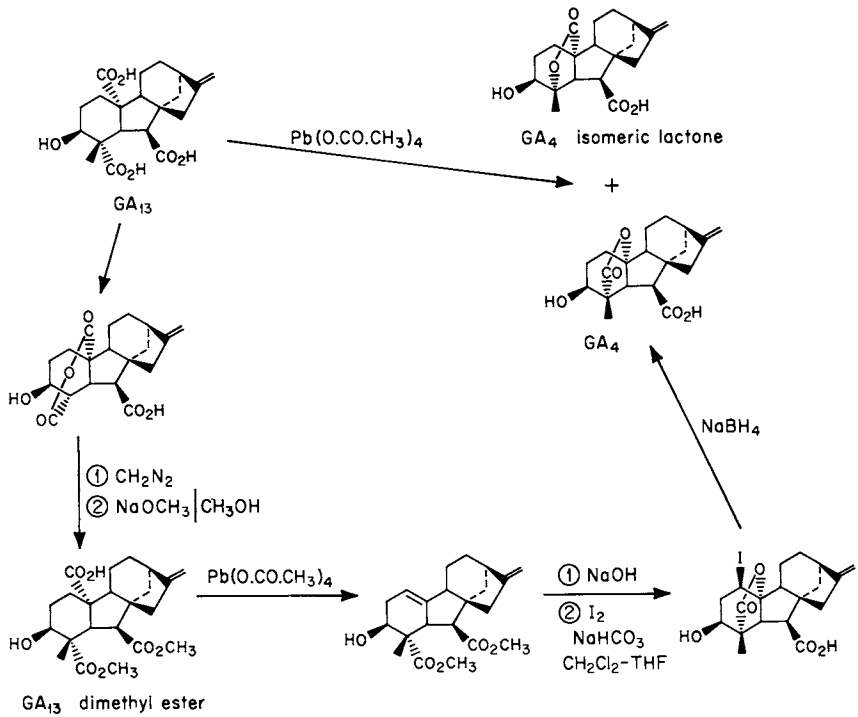
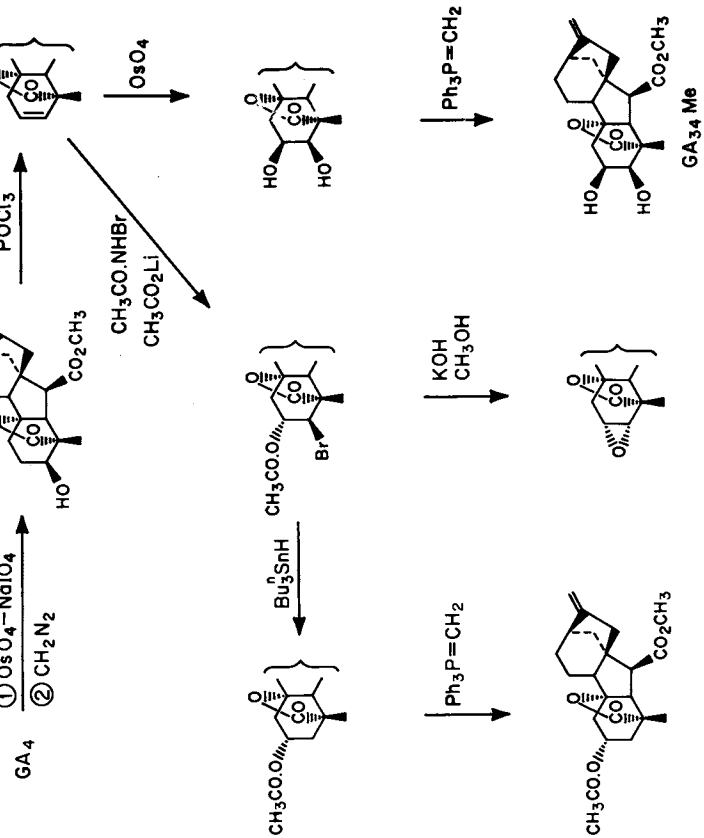


Figure 8. Methods for the oxidative decarboxylation of GA_{13} to GA_4 (51, 52)

In Plant Growth Substances; Mandava, N.;
ACS Symposium Series; American Chemical Society: Washington, DC, 1979.


GA4
① OsO4–NaIO4
② CH2N2
POCl3
OsO4
CH3CO.NHBr
CH3CO2Li
Bu3SnH
KOH
CH3OH
Ph3P=CH2
Ph3P=CH2
GA34 Me

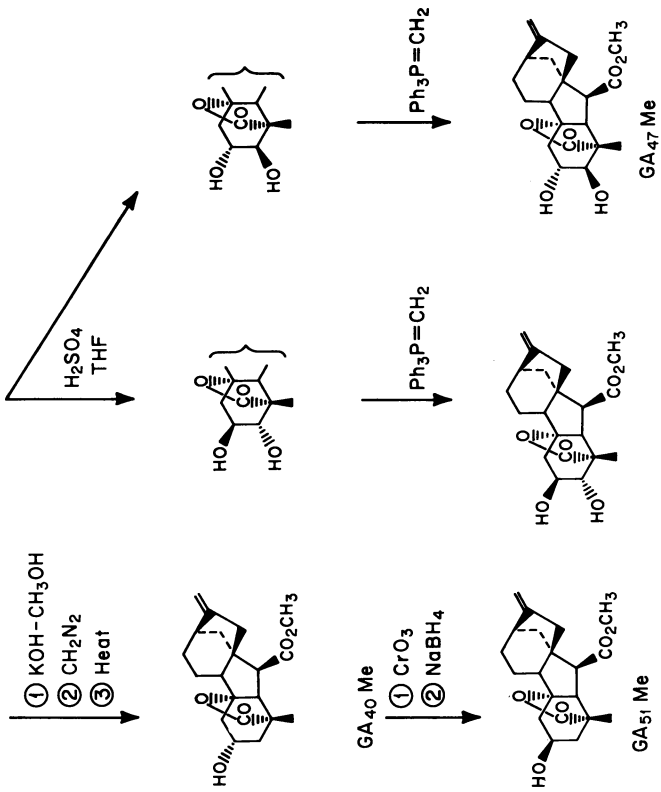


Figure 9. Chemical methods for the partial syntheses of the methyl esters of GA_{34}, GA_{40}, GA_{47}, and GA_{51} from GA_4 (38, 42)

Chemical Methods. As an example, Figure 9 shows a scheme for the preparation of four 2-hydroxy GAs from GA_4. Two of the products are less abundant fungal GAs (GA_{40} and GA_{47}) and the others (GA_{34} and GA_{51}) occur in higher plants. The preparation of the methyl esters of GA_{40}, GA_{47} and GA_{34} was described by Beeley and MacMillan (38) and the conversion of GA_{40} to GA_{51} by Yamaguchi *et al.* (42). The starting point was a mixture of GA_4 and GA_7, GAs which are not easily separated. This mixture was treated with OsO_4 and $NaIO_4$ to convert GA_4 to the 17-nor-16-ketone which is easily separated from the GA_7 product in which the 1,2-double bond is also oxidized. The exocyclic double bond is thus protected as the norketone and can be restored later by the Wittig reaction. This reaction also gives an opportunity to introduce a label at the 17 position (see later). To prevent complications due to the presence of a free carboxylic acid group, the GA_4 norketone was methylated and subsequent reactions were carried out on the methyl esters. Demethylation is achieved for the 3-deoxy products by methanolic NaOH treatment (57) and for the 3-hydroxy GAs using the method described by Nagata *et al.* (53). The reduction of GA_{40}-ketone with $NaBH_4$ gives a mixture of the 2α (GA_{40}) and 2β (GA_{51}) alcohols which can be separated by preparative TLC (42). A similar series of reactions has been used (38) to convert the C_{20} GA, GA_{13}, to GA_{43} and GA_{46}, both of which occur in the Cucurbitaceae (58).

The synthesis of GA-6β-aldehydes is of particular interest since GA_{12}-aldehyde is the immediate product of ring B contraction in GA biosynthesis (59). GA_{12}-aldehyde also appears to be a substrate for hydroxylation in *G. fujikuroi* and at least one higher plant (60). GA_{12}-aldehyde was first synthesized from 7β-hydroxykaurenolide, a metabolite of *G. fujikuroi*, by treating the 7α-toluene-*p*-sulfonate with KOH in methanol (61). The yield was poor and the methyl ester was obtained as product. The yield has been improved using *t*-butanol as solvent (59) with a small amount of water (62). Still higher yields were obtained with *p*-bromobenzenesulfonate as the leaving group (63). The aldehydes of GA_{14} and GA_{53} have also been prepared from 3β,7β- and 7β,13-dihydroxykaurenolides, respectively, using the same basic method (64,65). The proposed mechanism for ring contraction of the kaurenolide is shown in Figure 10; the reaction requires an antiperiplanar relationship for the migrating 5,6-bond and the leaving group. Thus the 7β-alcohol of the kaurenolide must be epimerized to the α position before formation of the sulfonate ester. The initial product is the 6α-aldehyde but epimerization occurs under the basic conditions of the reaction to give the thermodynamically-favored 6β-aldehyde. The γ-lactone is not essential for the reaction and the opened hydroxy acid will also undergo ring contraction on treatment with base (collidine or sodium hydride). The reaction involves abstraction of the 6-hydroxy proton but requires the free 19-oic acid. It was proposed that the proton was abstracted from the sterically-

In Plant Growth Substances; Mandava, N.;
ACS Symposium Series; American Chemical Society: Washington, DC, 1979.

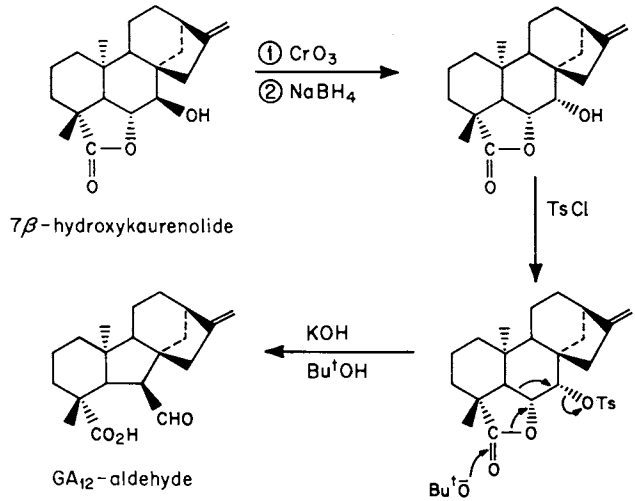


Figure 10. The conversion of 7β-hydroxykaurenolide to GA_{12}-aldehyde showing the proposed mechanism of the reaction (59). Ts = toluene-p-sulfonyl.

In Plant Growth Substances; Mandava, N.;
ACS Symposium Series; American Chemical Society: Washington, DC, 1979.

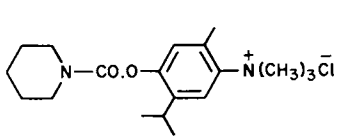
hindered 6α-hydroxy group by internal attack of the carboxyl anion (62). Node et al. (66) in a detailed investigation of the mechanism of ring contraction concluded that opening of the lactone precedes bond migration so the reaction is not concerted.

Microbiological Methods. The low substrate specificity of many of the enzymes involved in GA biosynthesis in Gibberella fujikuroi has been utilized for the preparation of higher plant GAs. Suitable analogs of the natural GA-precursors are converted by the fungus to the corresponding GA analogs. It is usual to prevent the synthesis of the natural GAs in order to facilitate purification of the unnatural products. A mutant strain, B1-41a, in which GA biosynthesis is blocked early in the pathway (67) (between ent-kaurenal and ent-kaurenoic acid) has been used. It is also possible to block GA synthesis chemically using inhibitors such as AMO-1618 (68), or the quaternary ammonium iodide compound in Figure 11 (69,70), which block ent-kaurene formation.

Steviol, the 13-hydroxy analog of ent-kaurenoic acid, occurs naturally as the glucoside, stevioside, in leaves of the shrub, Stevia rebaudiana. Steviol is converted by B1-41a to a number of 13-hydroxylated GAs (71). Although 13-hydroxylation is a normal process in fungal GA biosynthesis, it is the final step in the pathway so that the end product, GA_3, is normally the only 13-hydroxy GA formed in large amounts. When steviol is incubated with B1-41a the major products are GA_1 (equivalent to the natural fungal metabolite, GA_4) and GA_{18} (equivalent to GA_{14}). Other GAs such as GA_{53}, GA_{19}, and GA_{20} are also produced. There is no GA_3 because the presence of a 13-hydroxy group inhibits formation of the 1,2 double bond. When steviol acetate was fed the major GA-products were the acetates of GA_{20} and GA_{17} (72) since the presence of a 13-acetoxyl group prevents 3β-hydroxylation. Thus it was possible to predetermine which products were obtained. Relative yields of products could also be manipulated by changing the concentration of the substrate. The B1-41a mutant was found also to metabolize ent-15α-hydroxykaurenoic acid to a number of 15β-hydroxylated GAs (J.R. Bearder & K. Kybird, unpublished information), one of which, 15β-hydroxy GA_9, was subsequently identified as a new GA, GA_{45}, in seeds of Pyrus communis (39).

Other fungi have also proved useful for preparing GAs. GA_9 methyl ester was hydroxylated by Rhizopus nigricans to a number of products among which the methyl esters of GA_{40} (2α-hydroxylation), GA_{20} (13-hydroxylation), GA_{45} (15β-hydroxylation) and a 12-hydroxy GA_9 of undetermined stereochemistry were identified (73). Interestingly the free acid was metabolized only to GA_{10} by hydration of the 16,17 double bond. While the individual yields in the above conversions were not high, another species, R. Arrhizus, 13-hydroxylates GAs and GA-precursors in very high yield (65).

In Plant Growth Substances; Mandava, N.;
ACS Symposium Series; American Chemical Society: Washington, DC, 1979.



AMO-1618

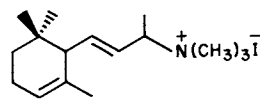


Figure 11. Inhibitors of GA biosynthesis: AMO-1618 (2′-isopropyl-4′-(trimethylammonium chloride)-5′-methylphenylpiperidine-1-carboxylate) and N, N, N-*trimethyl-1-methyl-(2′,6′,6′-trimethylcyclohex-2′-en-1′-yl)prop-2-enylammonium iodide.*

In Plant Growth Substances; Mandava, N.;
ACS Symposium Series; American Chemical Society: Washington, DC, 1979.

The Preparation of Labeled GAs

Isotopically-labeled GAs and GA-precursors are required for metabolic studies and as internal standards for quantitation by mass spectrometry. Numerous chemical methods have been used for the synthesis of labeled GAs from readily-available GAs. Labeled GAs have also been prepared biochemically using either fungal cultures or cell-free preparations from higher plants.

Chemical Methods. ^{14}C has been introduced by chemical means exclusively at carbon-17 by the Wittig reaction. The compound to be labeled is oxidized to the 17-nor-16-ketone with osmium tetroxide and sodium metaperiodate. The ketone is then reacted with the labeled ylid, [^{14}C-methylene]triphenylphosphorane. There are many examples of the use of this method in which the ylid was generated using [^{14}C-methyl]triphenylphosphonium iodide and n-butyl lithium as base (e.g. 59). Alternative methods which give higher yields have been published recently by Bearder et al. (72,73). They used potassium t-butoxide as base or salt-free ylid prepared from methyltriphenylphosphonium bromide and excess sodium hydride in tetrahydrofuran. The Witting reaction has been used also to introduce ^{3}H into GAs by reaction of the norketones with [$^{3}H_2$-methylene]triphenylphosphorane. ^{3}H has an advantage over ^{14}C in being less expensive and obtainable with a higher specific radioactivity. Bearder et al. (72) labeled the methylphosphonium bromide by exchange with $^{3}H_2O$ in tetrahydrofuran containing triethylamine. In the final product there is scrambling of label between the 17 and 15 positions. The strongly basic conditions required in the Wittig reaction necessitate the protection of base-labile groups. Thus the 3- and 13-hydroxy groups can be converted to trimethylsilyl ethers (38) or tetrahydropyranyl ethers which are then easily removed by mild acid treatment.

Tritiated GAs of very high specific radioactivity have been prepared by catalytic reduction. The 1,2 double bond of GA_3 can be selectively reduced, using a partially poisoned palladium catalyst, to give [1,2-$^{3}H_2$]GA_1 (74,75,76), although some reduction of the 16,17 double bond and the lactone also occurs (76). Introduction of ^{3}H at sites other than carbon atoms 1 and 2 has also been found (76). [^{3}H]GA_4 has been prepared from GA_7 by a similar method (77). [^{3}H]GA_1 was converted to [^{3}H]GA_5 by elimination of the 3-toluene-p-sulfonate (78,79).

Murofushi et al. (8) protected the 16,17 double bond of GA_5 methyl ester by forming the epoxide with metachloroperbenzoic acid. After catalytic reduction of the 2,3 double bond they restored the exomethylene group by treatment with a mixture of sodium iodide, sodium acetate and zinc and hydrolyzed the methyl ester to obtain [2,3-$^{3}H_2$]GA_{20}. Yakota et al. (81) prepared [2,3-$^{3}H_2$]GA_9 from GA_4 by an analogous method via 2,3-dehydro GA_9. Selective catalytic reduction of the 3-methane-

In Plant Growth Substances; Mandava, N.;
ACS Symposium Series; American Chemical Society: Washington, DC, 1979.

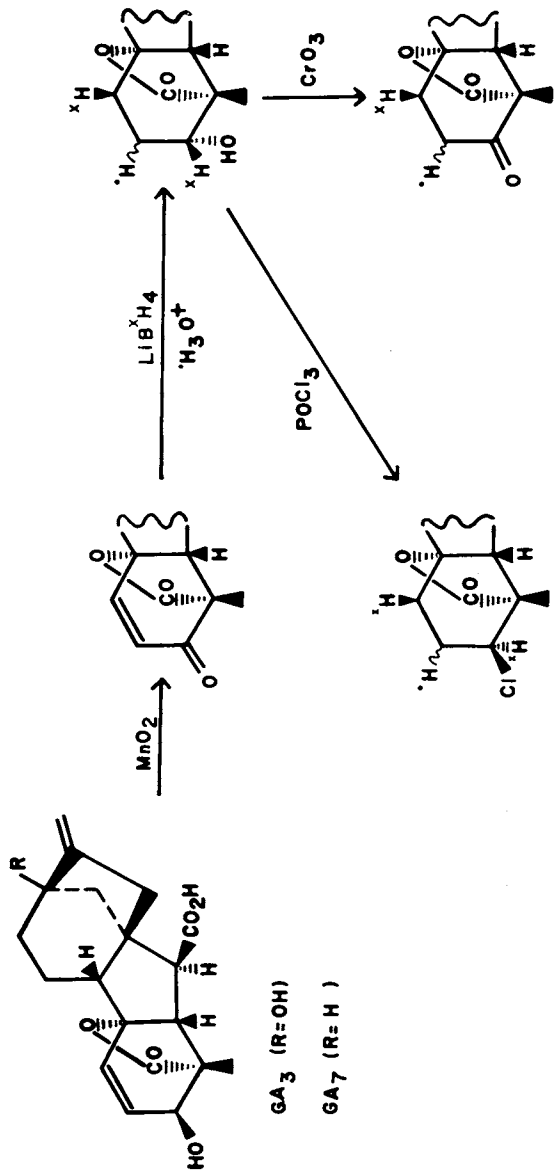
sulfonate of GA_3 methyl ester was used by Murofushi *et al.* to prepare [1-$^3H_1$]GA_5, from which [1-^3H]GA_8 was obtained by treatment with osmium tetroxide (80).

A convenient method for the specific introduction of ^2H or ^3H (or both) into a molecule is by ketone reduction with labeled metal hydride. Beale and MacMillan (10) have utilized this method for the preparation of GAs labeled at the 1, 2 or 3 positions from GA_3 or GA_7 (Figure 12). One point of interest is the lithium borohydride reduction of the enone formed by manganese dioxide oxidation of GA_3 or GA_7. When the reaction is carried out in anhydrous tetrahydrofuran it proceeds in two steps. Initially the lithium enolate is formed which incorporates a proton at carbon-2 from the acid used in the work-up, forming the 3-ketone. This ketone is reduced to the 3α-alcohol by the borohydride which is decomposed more slowly than is the lithium enolate. Thus it is possible to introduce two different labels in a single reaction.

Acid or base exchangeable protons can be easily labeled with ^3H or ^2H. Bearder *et al.* (67) labeled the 15 and 17 positions of ent-kaurene by treatment with $CF_3CO^3H(^2H)$. A mixture of the 16,17 and 15,16 double bond isomers is obtained and they are separated by $AgNO_3$ TLC. This method could be used with some 13-deoxy GAs although separation of the resulting isomers would be more difficult than for ent-kaurene. The 6-hydrogen in GA_{12}-aldehyde and GA_{14}-aldehyde has been labeled by treatment with $MeO^3H(^2H)$ or $^3H_2(^2H_2)O$ and sodium methoxide (62,64).

Biological Methods. Microbiological methods have been used in conjunction with chemical synthesis to convert chemically-labeled precursors to labeled GAs. Hanson and Hawker (82) prepared [17-^{14}C]GA_3 by incubating chemically-synthesized [17-^{14}C] GA_{12}-7-alcohol with *G. fujikuroi* cultures. In this case the product was diluted by endogenous GA_3. The method could be improved by using cultures in which the endogenous GA levels are reduced, either by mutation (B1-41a) or with inhibitors of GA biosynthesis (70). Bearder *et al.* (72) used the *G. fujikuroi* mutant, B1-41a, to prepare [17-$^3H_2$]GA_{20} with high specific radioactivity by feeding [17-$^3H_2$] steviol acetate. This method has the potential for the preparation of a number of labeled 13-hydroxy GAs (see 71,72).

Cell-free systems provide a rapid and convenient method for preparing GA and GA-precursors with high specific radioactivity, although on a relatively small scale. [^{14}C]-labeled ent-kaurenoid precursors of GAs have been obtained from [2-^{14}C] mevalonic acid by incubating with cell-free systems from endosperm of *Marah macrocarpus* (83) or *Cucurbita maxima* (84,85). The *C. maxima* system can be used also to prepare labeled C_{20} GAs from [^{14}C]mevalonic acid (86).



In Plant Growth Substances; Mandava, N.;
ACS Symposium Series; American Chemical Society: Washington, DC, 1979.

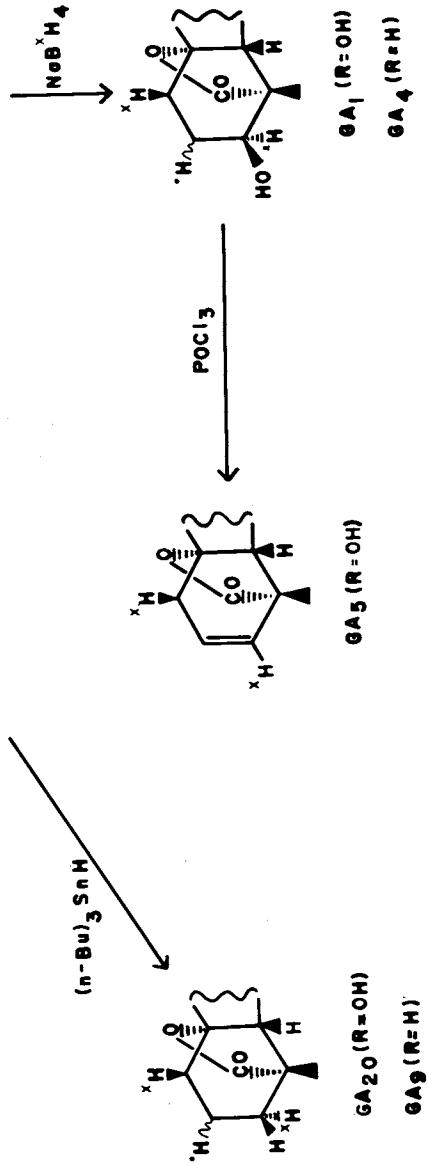


Figure 12. Methods for the preparation of GA's labeled with ^{2}H or ^{3}H at positions 1, 2, or 3 by metal hydride reduction (10)

Structure-Activity Relationships

Gibberellins vary greatly in the degree of response they elicit in biological assays, and indeed, of the 53 naturally occurring GAs, less than half have appreciable bio-activity in the standard assays. The relationship between the structure of a GA and its bio-activity has attracted considerable attention and the available information has been extensively reviewed (13,87,88).

Two explanations for the different responses of plants to a particular GA have been suggested (87). The first assumes that the GA must bind to a specific receptor site to give a response, the degree of response being related to the binding efficiency. Variations in the structure of the receptor from one plant species to another would then explain the differences in the bio-activity of a GA in different assays. The second possibility is that the bio-activity of some GAs is due to their metabolism to active products by the assay plants. The presence or absence of bio-activity in an assay would then reflect the ability of the assay to metabolize the applied GA. The structure of the GA-receptor and the ability of the assay plant to metabolize the applied GA probably both influence the result of a bioassay, and it is in fact difficult to distinguish between these possibilities. Thus those C_{20} GAs which show bio-activity may do so because they are converted to C_{19} GAs. However, C_{20} GAs with the 19,20 δ-lactone are probably active *per se* since they are protected from further oxidation at carbon-20 and therefore probably from conversion to C_{19} GAs.

It has been pointed out by several workers that it is often misleading to compare bioassay data from different laboratories since the sensitivity of a response can be very dependent on the bioassay technique used (13,88). Therefore the publications of Brian *et al.* (89), who compared the bio-activities of 134 GA-related compounds in four bioassays under the same conditions, and of Crozier *et al.* (90), who compared the bio-activities of 26 GAs in nine bioassays, are very useful. Thus it is possible to make some general observations about the structural features of the GA molecule which are necessary for high biological activity. A free carboxyl group on the B-ring appears to be essential. The γ-lactone characteristic of C_{19} GAs is required for high bio-activity but substantial, although reduced, activity is exhibited by C_{20} GAs with an aldehyde at carbon-20 or with the δ-lactone. GAs with a methyl or carboxyl group at carbon-20 have little activity. Reeve and Crozier (87) suggested that the δ-lactone and δ-lactol formed from the C-20 aldehyde might mimic the γ-lactone of the C_{19} GAs. The isomeric 20,4-lactone of GA_4 was found to have activity equal to GA_4 in some assays and only slightly reduced activity in others (57). It was concluded that the lactone was necessary only because of the shape it conferred on the molecule. The slight change in the position

In Plant Growth Substances; Mandava, N.;
ACS Symposium Series; American Chemical Society: Washington, DC, 1979.

of the lactone in GA_3 from 19,10 to 19,2 has no effect on bio-activity (89,91).

In most bioassays a 3β-hydroxyl group increases bio-activity as does a 13-hydroxyl group. An exception is the cucumber hypocotyl assay in which 13-hydroxy GAs have lower activity than the equivalent 13-deoxy compounds. In general the most active GAs have both 3β- and 13-hydroxyl groups and a 1,2 double bond or some combination of these. Interestingly, when the 3β-hydroxyl group is epimerized to the 3α position bio-activity is virtually eliminated (89). The effect of hydroxylation at positions other than 3β or 13, with the exception of the 2β-position, is difficult to assess because of insufficient examples.

2β-Hydroxylation causes loss of bio-activity and is quite possibly a deactivating process in higher plants (60). The deactivation is fairly stereospecific since 2α-hydroxylation, while reducing bio-activity, does not eliminate it (57). The idea that higher plants may use 2β-hydroxylation as a deactivation mechanism has led to methods for producing GA-derivatives with very high biological activity. 2β-Methyl GA_4 has been synthesized in an attempt to prevent 2β-hydroxylation from taking place (M. Beale and J. MacMillan, personal communication). This compound was found to have higher bio-activity than GA_4, especially when the duration of the bioassay was increased (J. MacMillan *et al.*, unpublished information). A more dramatic effect was seen with 2,2-dimethyl GA_4 which, in the *dwarf-5* maize assay, is a hundred times more active than GA_3. The enhanced activity is much less marked in short-duration bioassays. The unexpected result that dimethyl GA_4 is more active than the monomethyl compound complicates the interpretation and more derivatives need to be tested. However the preliminary results indicate that blocking the 2 position leads to higher (or prolonged) bio-activity. 2β-Methoxylation, as in 2β-methoxy GA_9, reduces activity as effectively as hydroxylation (J. MacMillan *et al.*, unpublished information).

Conclusions

Since the first attempts to determine the structure of GA_3, the chemistry of GAs has been the subject of a large number of publications. Chemically, the GAs have proved to be difficult compounds to work with, a consequence of the high number and arrangement of functional groups in the molecule. GA_3 is particularly labile and it is only recently that its total synthesis has been completed (49), more than twenty years after its structure was established.

Total chemical synthesis is not a feasible method for preparing useful quantities of GAs; GA_3 and some of the other GAs produced by *Gibberella fujikuroi* are more practically obtained from cultures of this fungus. However, preparatively useful chemical methods have been developed for the partial synthesis

In Plant Growth Substances; Mandava, N.;
ACS Symposium Series; American Chemical Society: Washington, DC, 1979.

of some less-accessible GAs from more abundant precursors, such as the fungal GAs. Microbiological conversion, using G. fujikuroi and other fungi, is also a promising method for obtaining higher-plant GAs from readily-available substrates.

The identification and quantitation of GAs in plant extracts are particularly difficult problems due both to the very low amounts of GAs present in plant tissues and to the large number of different GA structures that can be encountered. When only a limited number of GAs were known, identification was often based on co-chromatography of the unknown with standards on thin-layer plates. It is now realized that comparison of chromatographic behaviour with that of standards in any system is not sufficient basis for identification. Furthermore bioassays have proved to be very unreliable methods for GA-quantitation. Combined gas chromatography-mass spectrometry has the advantage of giving conclusive identification on very low amounts of components in complex mixtures. It is therefore being used increasingly for the detection of GAs as well as other plant hormones. It also provides an accurate means for quantitation.

The mechanism of action of GAs at the molecular level still eludes plant physiologists. There have been reports of stereospecific binding of GAs to protein (92,93) and other cell fractions (94) but it has not been demonstrated that the binding is associated with a physiological response. However, correlations of the biological activities of GAs with their structures are one possible method for obtaining information on the site of action. Furthermore, a possibly valuable "spin-off" from structure-activity studies is the design of GA-like molecules which, because of increased bio-activity or specific physiological properties, may have important agricultural applications.

Acknowledgments

The preparation of this article was supported by a grant from the N.S.F. to B. O. Phinney. The author wishes to thank Professor B. O. Phinney for helpful comments on the manuscript.

Literature Cited

1. MacMillan, J., Takahashi, N. Nature, 1968, 217, 170-171.
2. Graebe, J.E., Hedden, P. In "Biochemistry and Chemistry of Plant Growth Regulators"; Schreiber, K., Schütte, H.R., Sembdner, G., Ed. Institute of Plant Biochemistry: Halle, G.D.R. 1974, pp. 1-16.
3. Harrison, D.M., MacMillan, J. J. Chem. Soc. (C), 1971, 631-636.
4. MacMillan, J., Pryce, R.J. In "Phytochemistry"; Miller, L.P., Ed. Van Nostrand-Reinhold: New York, 1973, pp. 283-326.

5. Grove, J.F., MacMillan, J., Mulholland, T.P.C., Turner, W.B. J. Chem. Soc. 1960, 3049-3057.
6. Grove, J.F. J. Chem. Soc. 1961, 3545-3547.
7. Hanson, J.R. Tetrahedron. 1966, 22, 701-703.
8. Bearder, J.R., MacMillan, J. J.C.S. Perkin Trans. 1. 1973, 2824-2830.
9. MacMillan, J., Pryce, R.J. J. Chem. Soc. (C), 1967, 740-742.
10. MacMillan, J. Pure & Appl. Chem. 1978, 50, 995-1004.
11. Pryce, R.J. Phytochemistry. 1973, 12, 507-514.
12. Pryce, R.J. J.C.S. Perkin Trans. 1. 1974, 1179-1184.
13. Graebe, J.E., Ropers, H.-J. In "Phytohormones and Related Compounds: A Comprehensive Treatise. Volume 1." Lethem, D.S., Goodwin, P.B., Higgins, T.J.V., Ed. Elsevier/ North Holland Biomedical Press: Amsterdam, Oxford, New York. 1978, pp. 107-203.
14. Gaskin, P., MacMillan, J. Phytochemistry. 1975, 14, 1575-1578.
15. Yamaguchi, T., Yokota, T., Murofushi, N., Takahashi, N., Ogawa, V. Agric. Biol. Chem. 1975, 39, 2399-2403.
16. McComb, A.J. Nature. 1961, 192, 575-576.
17. Pitel, D.W., Vining, L.C., Arsenault, G.P. Can. J. Biochem. 1971, 49, 185-193.
18. Durley, R.C., Crozier, A., Pharis, R.P., McLaughlin, G.E. Phytochemistry. 1972, 11, 3029-3033.
19. Rao, T.P., Nagor, P.K. J. Expt. Botany. 1973, 24, 412-417.
20. MacMillan, J., Wels, C.M. J. Chromatog. 1973, 87, 271-276.
21. Reeve, D.R., Crozier, A. In "Isolation of Plant Growth Substances." Hillman, J.R., Ed. Cambridge Univ. Press, 1978, pp. 41-77.
22. Durley, R.C., MacMillan, J., Pryce, R.J. Phytochemistry. 1971, 10, 1891-1908.
23. Crozier, A., Aoki, H., Pharis, R.P. J. Exp. Botany. 1969, 20, 786-795.
24. Browning, G., Saunders, P.F. Nature, 1977, 265, 375-377.
25. Sponsel, V.M., MacMillan, J. Planta. 1978, 144, 69-78.
26. Glenn, J.L., Kuo, C.C., Durley, R.C., Pharis, R.P. Phytochemistry. 1972, 11, 345-351.
27. Fuchs, Y., Gertman, E. Plant Cell Physiol. 1974, 15, 629-633.
28. Schneider, G., Jänicke, S., Sembdner, G. J. Chromatog. 1975, 109, 409-412.
29. Yokota, T. Hiraga, K., Yamane, H., Takahashi, N. Phytochemistry. 1975, 14, 1569-1574.
30. Knöfel, H.-D., Müller, P., Sembdner, G. In "Biochemistry and Chemistry of Plant Growth Regulators." Schreiber, K., Schütte, H.R., Sembdner, G., Ed. Institute of Plant Biochemistry: Halle, G.D.R. 1974, pp. 121-124.
31. Frydman, V.M., MacMillan, J. Planta. 1975, 14, 1575-1578.

In Plant Growth Substances; Mandava, N.;
ACS Symposium Series; American Chemical Society: Washington, DC, 1979.

32. MacMillan, J. In "Plant Growth Substances 1970." Carr, D.J., Ed. Springer: Berlin, Heidelberg, New York. 1972, pp. 790-797.
33. Gaskin, P., MacMillan, J. In "Isolation of Plant Growth Substances." Hillman, J.R., Ed. Cambridge Univ. Press. 1978, pp. 79-95.
34. Hedden, P. In "Proceedings of the Plant Growth Regulator Working Group." Abdel-Rahman, M., Ed. Plant Growth Regulator Working Group: Syracuse, New York. 1978, pp. 33-44.
35. Frydman, V.M., Gaskin, P., MacMillan, J. Planta. 1974, 118, 123-132.
36. Sponsel, V.M., MacMillan, J. Planta. 1977, 135, 129-136.
37. Binks, R., MacMillan, J., Pryce, R.J. Phytochemistry. 1969, 8, 271-284.
38. Beeley, L.J., MacMillan, J. J.C.S. Perkin Trans. 1. 1976, 1022-1028.
39. Bearder, J.R., Dennis, F.G., MacMillan, J., Martin, G.C., Phinney, B.O. Tet. Letters. 1975, 669-670.
40. Yamaguchi, I., Takahashi, N., Fujita, K. J.C.S. Perkin Trans. 1. 1975, 992-996.
41. Evans, R., Hanson, J.R., Siverns, M. J.C.S. Perkin Trans. 1. 1975, 1514-1517.
42. Yamaguchi, I., Mujamoto, M., Yamane, H., Murofushi, N., Takahashi, N., Fujita, K. J.C.S. Perkin Trans. 1. 1975, 996-999.
43. Murofushi, N., Yokota, T., Watanabe, A., Takahashi, N. Agric. Biol. Chem. 1973, 37, 1101-1113.
44. Grove, J.F. Quart. Rev. (Chem. Soc. London) 1961, 15, 56-70.
45. Hartsuck, J.A., Lipscomb, W.N. J. Am. Chem. Soc. 1963, 85, 3414-3419.
46. McCapra, F., McPhail, A.T., Scott, A.I., Sim, G.A., Young, D.W. J. Chem. Soc. (C). 1966, 1577-1585.
47. Kutschabsky, L., Reck, G., Adam, G. Tetrahedron, 1975, 31, 3065-3068.
48. Höhne, E., Schneider, G., Schreiber, K. J. Prakt. Chem., 1970, 312, 816-822.
49. Corey, E.J., Danheiser, R.L., Chandrasekaran, S., Keck, G.E., Gopalan, B., Larsen, S.D., Siret, P., Gras, J.-L. J. Am. Chem. Soc. 1978, 100, 8034-8036.
50. Gross, B.E., Galt, R.H.B., Hanson, J.R. J. Chem. Soc. 1963, 2944-2961.
51. Bearder, J.R., MacMillan, J. J.C.S. Chem. Comm. 1976, 421.
52. Murofushi, N., Yamaguchi, I., Ishigooka, H., Takahashi, N. Agric. Biol. Chem. 1976, 40, 2471-2474.
53. Nagata, W., Wakabayashi, T., Hayase, Y., Narisada, M., Kamata, S. J. Am. Chem. Soc. 1970, 92, 3202-3203.
54. Mori, K., Takemoto, I., Matsui, M. Tetrahedron. 1976, 32, 1497-1502.

In Plant Growth Substances; Mandava, N.;
ACS Symposium Series; American Chemical Society: Washington, DC, 1979.

55. Nakata, T., Tahara, A. Tet. Letters. 1976, 1515-1518.
56. MacMillan, J., Wels, C.M. Phytochemistry 1974. 13, 1413-1417.
57. Sponsel, V.M., Hoad, G.V., Beeley, L.J. Planta. 1977, 135, 143-147.
58. Beeley, L.J., Gaskin, P., MacMillan, J. Phytochemistry. 1975, 14, 779-783.
59. Gross, B.E., Norton, K., Stewart, J.C. J. Chem. Soc. (C), 1968, 1054-1063.
60. Hedden, P., MacMillan, J., Phinney, B.O. Ann. Rev. Plant Physiol. 1978, 29, 149-192.
61. Galt, R.H.B., Hanson, J.R. J. Chem. Soc. 1965, 1565-1570.
62. Bearder, J.R., MacMillan, J., Phinney, B.O. Phytochemistry. 1973, 12,, 2173-2179.
63. Hanson, J.R., Hawker, J. Tet. Letters 1972, 4299-4302.
64. Hedden, P., MacMillan, J., Phinney, B.O. J.C.S. Perkin Trans. 1. 1974, 587-592.
65. Down, G. Ph.D. Thesis. 1978, University of Bristol, England.
66. Node, M., Hori, H., Fujita, E. J.C.S. Perkin Trans. 1. 1976, 2144-2149.
67. Bearder, J.R., MacMillan, J., Wels, C.M., Chaffey, M.B., Phinney, B.O. Phytochemistry. 1974, 13, 911-917.
68. Dennis, D.T., Upper, C.D., West, C.A. Plant Physiol. 1965, 40, 948-952.
69. Haruta, H., Yagi, H., Iwata, T., Tamura, S. Agric. Biol. Chem. 1974, 38, 417-422.
70. Hedden, P., Phinney, B.O., MacMillan, J., Sponsel, V.M. Phytochemistry. 1977, 16, 1913-1917.
71. Bearder, J.R., MacMillan, J., Wels, C.M., Phinney, B.O. Phytochemistry. 1975, 14, 1741-1748.
72. Bearder, J.R., Frydman, V.M., Gaskin, P., MacMillan, J., Wels, C.M., Phinney, B.O. J.C.S. Perkin Trans. 1. 1976, 173-178.
73. Bearder, J.R., Frydman, V.M., Gaskin, P., Hatton, I.K., Harvey, W.E., MacMillan, J., Phinney, B.O. J.C.S. Perkin Trans. 1, 1976, 178-183.
74. Kende, H. Plant Physiol. 1967, 42, 1612-1618.
75. Pitel, D.W., Vining, L.C. Can. J. Biochem. 1970, 48, 259-263.
76. Nadeau, R., Rappaport, L. Phytochemistry. 1974, 13, 1537-1545.
77. Durley, R.C., Pharis, R.P. Planta. 1973, 109, 357-361.
78. Musgrave, A., Kende, H. Plant Physiol. 1970, 45, 56-61.
79. Durley, R.C., Railton, I.D., Pharis, R.P. Phytochemistry. 1973, 12, 1609-1612.
80. Murofushi, N., Durley, R.C., Pharis, R.P. Agric. Biol. Chem. 1974, 38, 475-476.
81. Yokota, T., Reeve, D.R., Crozier, A. Agric. Biol. Chem. 1976, 40, 2091-2094.

In Plant Growth Substances; Mandava, N.;
ACS Symposium Series; American Chemical Society: Washington, DC, 1979.

82. Hanson, J.R., Hawker, J. Phytochemistry. 1973, 12, 1073-1075.
83. Dennis, D.T., West, C.A. J. Biol. Chem. 1967, 242, 3293-3300.
84. Graebe, J.E. Planta. 1969, 85, 171-174.
85. Graebe, J.E., Hedden, P., MacMillan, J. In "Plant Growth Substances 1973," Hirokawa: Tokyo. 1974, pp. 260-266.
86. Graebe, J.E., Hedden, P., Gaskin, P., MacMillan, J. Phytochemistry. 1974, 13, 1433-1440.
87. Reeve, D.R., Crozier, A. J. Exp. Botany. 1974, 25, 431-445.
88 Reeve, D.R., Crozier, A. In "Gibberellins and Plant Growth." Krishnamoorthy, H.N., Ed. Wiley Eastern: New Delhi, 1975, pp. 35-64.
89. Brian, P.W., Grove, J.F., Mulholland, T.P.C. Phytochemistry. 1967, 6, 1475-1499.
90. Crozier, A., Kuo, C.C., Durley, R.C., Pharis, R.P. Can. J. Bot., 1970, 48, 867-877.
91. Hoad, G.V., Pharis, R.P., Railton, I.D., Durley, R.C. Planta. 1976, 130, 113-120.
92. Stoddart, J., Breidenbach, W., Nadeau, R., Rappaport, L. Proc. Nat. Acad. Sci. U.S.A. 1974, 71, 3255-3259.
93. Konjevic, R., Grubisic, D., Morkovic, R., Petrovic, J. Planta. 1976, 131, 125-128.
94. Jelsema, C.L., Ruddat, M., Norre, D.J., Williamson, F.A. Plant Cell Physiol. 1977, 18, 1009-1019.

RECEIVED June 19, 1979.

Gibberellin Biosynthesis in the Fungus *Gibberella fujikuroi* and in Higher Plants

BERNARD O. PHINNEY

Department of Biology, University of California, Los Angeles, CA 90024

The gibberellins (GAs)[1] were originally identified as secondary metabolites of the fungus Gibberella fujikuroi Saw Wr. (Fusarium moniliforme Sheld.). While these compounds have no apparent role[2] in the fungus, they have been found to elicit a variety of responses in higher plants (seed plants) including shoot elongation, sex expression, fruit growth, and seed germination (1,2). The early descriptions of GA-induced elongation, especially those associated with genetic dwarfism (3,4), led to the idea that GAs might be naturally-occurring in normal, non-dwarf strains of higher plants. The first evidence for the presence of gibberellin-like substances in higher plants came from the fact that semipurified extracts from such material would mimic a GA-induced growth response when applied to genetic dwarfs (5,6,7). This evidence was soon followed by the isolation and chemical identification of GAs from higher plants (8,9,10). Since then 53 GAs have been identified as naturally occurring, 22 of them being found in the fungus G. fujikuroi, and 40 of them in higher plants including members of the Gymnospermae (e.g. pines) and the Angiospermae (flowering plants) (1,11). Although gibberellin-like substances have also been obtained from other groups of plants such as algae, other fungi, bacteria, mosses and ferns, these substances have yet to be identified chemically as GAs. It seems likely that GAs will be found to occur universally in the plant kingdom.

All GAs are tetracyclic diterpene acids; they can be divided into two types, the C_{20}-GAs and the C_{19}-GAs (Figure 1).

The unraveling of the details of the biosynthetic origin of the GAs has proven to be less difficult than the diversity of structures would suggest. Some of the simplifying factors are (1) that the origin of the GAs in both the fungus and higher plants is through a single terpenoid pathway leading to the common GA-precursor, GA_{12}-aldehyde; (2) that 20 of the 22 fungal GAs have now been biosynthetically related to each other via two pathways; and (3) that, although 40 GAs have been identified from higher plants, less than 15 have been found in any one

0-8412-0518-3/79/47-111-057$05.50/0

In Plant Growth Substances; Mandava, N.;
ACS Symposium Series; American Chemical Society: Washington, DC, 1979.

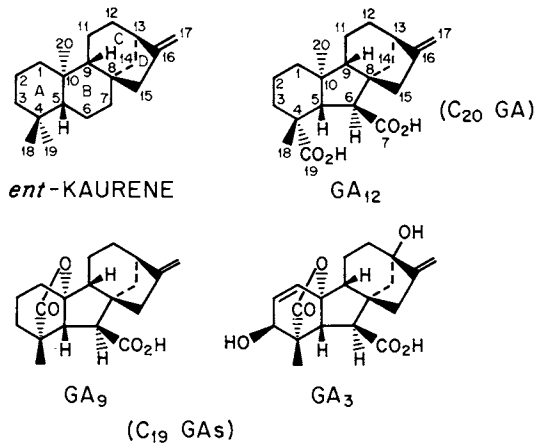


Figure 1. Numbering system for ent-*kaurene and the GA's (GA_{12}) and the structures of* ent-*kaurene, GA_{12}, GA_9, and GA_3*

In Plant Growth Substances; Mandava, N.;
ACS Symposium Series; American Chemical Society: Washington, DC, 1979.

species of plant.

This review will present an overall picture of GA biosynthesis in plants. In addition the role of GA biosynthesis in relation to GA-induced shoot elongation will be briefly discussed, as will the correlation of levels of endogenous GA-like substances with elongation growth in higher plants. All biosynthetic steps relating GAs to each other and to GA-precursors presented here are based on feeds of radiolabeled GAs and their precursors to plant preparations, including intact plants, tissue sections, and cell free homogenates The evidence for the specific steps described here has recently been reviewed in detail by Hedden et al. (11) and by Graebe and Ropers (1). The latter also includes a critical evaluation of information on the physiology of the GAs. The details of the biochemistry of polyisoprenoid biosynthesis has recently been reviewed by Beytia and Porter (12) and Goodwin (13).

From MVA to GA_{12}-aldehyde in the Fungus and in Higher Plants (Figure 2)

The earliest steps (MVA to GGPP) for polyisoprenoid biosynthesis are identical for all plants and animals (12,13). They involve the well-known diterpene pathway, MVA ⟶ MVAP ⟶ MVAPP ⟶ IPP + DMAPP ⟶ GPP ⟶ FPP ⟶ GGPP. The enzymes catalyzing these steps have been studied extensively, especially from animals (liver) and yeast, and to a more limited extent from higher plants. In some cases the enzymes have been purified to homogeneity; most have been only partially purified. In both plants and animals a major branch at FPP leads to the production of squalene and the steroids. In plants, three major branches occur at GGPP, of which one leads to the carotenoids via phytoene, a second to the phytyl group of chlorophyll, and a third to the GAs.

The steps in the pathway from GGPP to GA_{12}-aldehyde are unique to plants and involve the reactions GGPP ⟶ ent-kaurene ⟶ ent-kaurenol ⟶ ent-kaurenal ⟶ ent-kaurenoic acid ⟶ ent-7α-hydroxy kaurenoic acid ⟶ GA_{12}-aldehyde. The two step reaction, GGPP ⟶ CPP ⟶ ent-kaurene, is catalyzed by ent-kaurene synthetase (15). This enzyme or enzyme complex is responsible for (1) the proton-initiated cyclization to form the A and B rings of the bicyclic intermediate CPP (A activity), and (2) the loss of pyrophosphate, cyclization and rearrangement of the resulting carbonium ion, and loss of H^+ from carbon-17 to produce ent-kaurene (B activity). ent-Kaurene is the first committed intermediate in the biosynthetic pathway leading to the GAs, and it has been suggested that the A activity may be a limiting step in GA biosynthesis (16). The enzymes catalyzing the steps from MVA to ent-kaurene are soluble.

After production of ent-kaurene, carbon-19 is sequentially oxidized to give ent-kaurenol, ent-kaurenal, and ent-kaurenoic

In Plant Growth Substances; Mandava, N.;
ACS Symposium Series; American Chemical Society: Washington, DC, 1979.

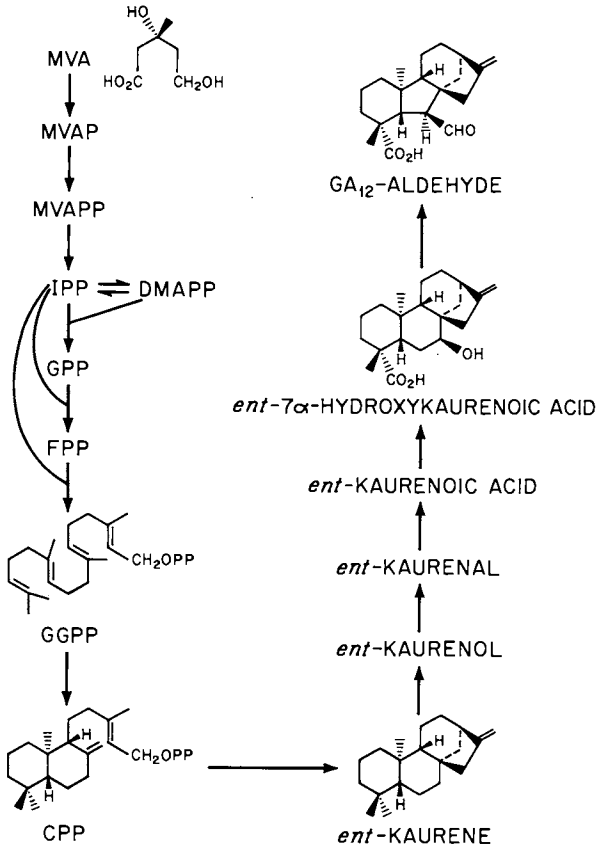


Figure 2. The GA biosynthetic pathway from MVA to GA_{12}-aldehyde. This pathway is found in the fungus Gibberella fujikuroi *and higher plants*

In Plant Growth Substances; Mandava, N.;
ACS Symposium Series; American Chemical Society: Washington, DC, 1979.

acid. This is followed by a hydroxylation on carbon-7 to give ent-7α-hydroxykaurenoic acid. There is some evidence that the enzymes for the pathway from MVA to ent-7α-hydroxykaurenoic acid are present in subcellular organelles - proplastids and chloroplasts (14,16,17,18). The characteristics of the enzymes catalyzing the steps between ent-kaurene and ent-7α-hydroxykaurenoic acid have been studied in a cell-free preparation obtained from the endosperm of wild cucumber seed (Marah macrocarpus = Echinocystis macrocarpa). The system requires oxygen and NADPH, the activity being found in the high-speed pellet (105,000 × g). The enzymes appear to be cytochrome P450 mixed-function oxygenases with electron transfer components similar to those found in liver (19,20).

The subsequent conversion of ent-7α-hydroxykaurenoic acid to GA_{12}-aldehyde is catalyzed by a single enzyme which is also found in the high-speed pellet. The reaction involves contraction of ring B from a 6 to a 5 membered ring with extrusion of carbon-7 as an aldehyde group. The evidence for this mechanism comes from a cell-free system obtained from the endosperm of young pumpkin seed (Cucurbita maxima) (21). In feeds of doubly-labeled ent-7α-hydroxykaurenoic acid (i.e. ^{14}C-labeled in which the hydrogen atoms on carbon-6 were also labeled with tritium), the ent-6α-hydrogen has been shown to be lost during the formation of GA_{12}-aldehyde. Ring contraction would then occur by migration of the 7,8 bond to carbon-6. On the other hand, the mechanism for the ring contraction step in the fungus is still unresolved, and the evidence in the literature is inconclusive. It was suggested from feeds of doubly-labeled GPP that ring contraction in the fungus is initiated by loss of the ent-7β-hydrogen atom rather than the ent-6α-hydrogen atom (22). However, this evidence was based on extremely low incorporation (~ 0.005%).

GA_{12}-Aldehyde and the Gibberellins

As previously stated, the immediate precursor common to both fungal and higher plant GAs is GA_{12}-aldehyde. The pathways that stem from this compound vary depending on the species of plant under study. They differ from each other in the extent, position, and sequence of ring hydroxylations.

The Fungus. The most complete evidence for details of the biosynthesis of GAs beyond GA_{12}-aldehyde is from the fungus G. fujikuroi (11). This organism is particularly suitable for metabolic studies since it is easily grown on a synthetic medium, and it produces large amounts of GAs within 4-6 days following inoculation. These GAs accumulate in the growth medium, a fact which simplifies their extraction and purification prior to GC and GC-MS. The current knowledge of the biosynthetic pathways in the fungus (Figure 3) was greatly enhanced by the use of a

In Plant Growth Substances; Mandava, N.;
ACS Symposium Series; American Chemical Society: Washington, DC, 1979.

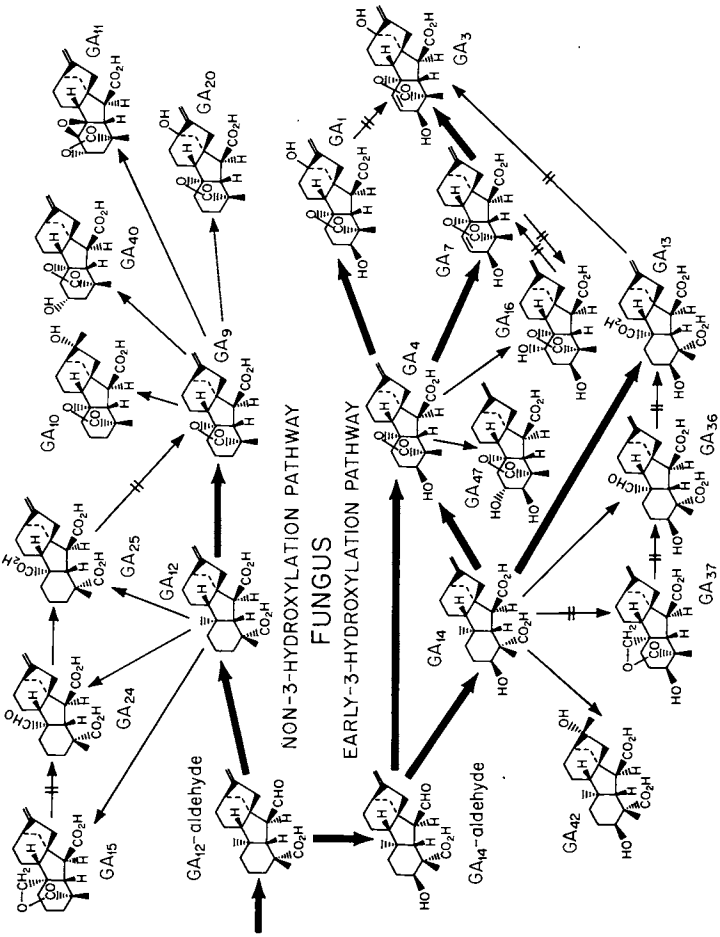


Figure 3. The GA biosynthetic pathway after GA_{12}-aldehyde for the fungus G. fujikuroi. *Heavy arrows indicate steps connecting major metabolites. Cross hatches indicate the absence of observed conversions.*

In Plant Growth Substances; Mandava, N.;
ACS Symposium Series; American Chemical Society: Washington, DC, 1979.

GA-less mutation (B1-41a), which blocks the conversion of ent-kaurenal to ent-kaurenoic acid (23). Feeding studies revealed that this mutant would metabolize substrates after the block to the same GAs present in the normal GA-producing strain from which the mutant originated (24). Thus it has been possible to feed radio-labeled GAs and their precursors to this mutant and recover the radio-labeled metabolites without the added complication of endogenous synthesis. From these and other investigations, it now appears that there are two main pathways in the fungus, both of which originate from GA_{12}-aldehyde. The first is a non-3-hydroxylating pathway which leads to an array of GAs including GA_9. The second is an early-3-hydroxylating pathway which leads to the well-known GA_3 (gibberellic acid) and other 3β-hydroxylated GAs.

In the non-3-hydroxylation pathway, the initial step from GA_{12}-aldehyde is an enzyme-catalyzed oxidation of carbon-7 which yields the acid, GA_{12}. This is followed by the loss of carbon-20 to give GA_9. The mechanism for the loss of carbon-20 is still unresolved. There are several lines of evidence which eliminate a number of possible mechanisms. For instance the two oxygens in the lactone of GA_9 have been shown to have their origin from the 19-oic acid group of the C_{20} precursor (25). The lactone is therefore not due to esterification of the 19-oic acid with an alcohol function on carbon-10. It suggests that lactone formation involves direct attack on the carboxylate ion on carbon-10, perhaps concomitant with the loss of carbon-20. It is still not clear at which oxidation state carbon-20 is lost, and feeding studies are inconclusive. In feeds of GA_{12}, metabolites were identified that contain carbon-20 at all levels of oxidation, i.e. GA_{15}, GA_{24} and GA_{25} (24,26); these GAs also occur as natural products in the fungus (27,28). However, none of the three GAs were metabolized to GA_9 when re-fed. In support of a decarboxylation mechanism is the report that $^{14}CO_2$ was released when ent-[^{14}C]kaurene, labeled at carbon-20, was fed to the fungus (29). The possibility has yet to be discounted that carbon-20 is lost at a lower oxidation level with the subsequent liberation of CO_2. Clarification of this mechanism will simplify the non-hydroxylation pathway and relate one or more of the oxidation levels of carbon-20 (GA_{15}, GA_{24}, GA_{25}) to steps between GA_{12} and GA_9. GA_9 has also been shown to be metabolized in trace amounts to the four GAs, GA_{10}, GA_{40}, GA_{11} and GA_{20} (30). They have not been re-fed to the fungus and they are indicated in Figure 3 as single step branches from GA_9.

The early-3-hydroxylation pathway is initiated from GA_{12}-aldehyde by a 3-hydroxylation to give GA_{14}-aldehyde. The order of the subsequent reactions, loss of carbon-20 and oxidation of carbon-7 to give GA_4, is not known. GA_4 may thus originate from GA_{12}-aldehyde, through GA_{14}, or via a hypothetical intermediate such as GA_4-aldehyde. Although GA_{14} is a major metabolite of GA_{14}-aldehyde, it is metabolized to C_{19}-GAs more slowly than is

In Plant Growth Substances; Mandava, N.;
ACS Symposium Series; American Chemical Society: Washington, DC, 1979.

GA_{14}-aldehyde (31). Thus the question of whether GA_{14} is on the direct pathway to C_{19} GAs is unresolved. The answer may require the development of cell-free systems that will catalyze these steps. Again, the three higher oxidation levels of the carbon-20 of GA_{14} are found in native fungal GAs (GA_{37}, GA_{36}, GA_{13}) (32,33), none of which are further metabolized. Two of them, GA_{36} and GA_{13}, have been shown to be metabolites from radio-labeled feeds of GA_{14} to the fungus (24). Thus the mechanism for the loss of carbon-20 is also unresolved in the early-3-hydroxylation pathway.

GA_4 is 13-hydroxylated to give the terminal GA_1, or dehydrogenated at the 1,2-position to give GA_7 which is in turn 13-hydroxylated to GA_3. GA_4 may also be hydroxylated at carbon-1 to GA_{16} and at carbon-2 to give GA_{47}. GA_{16} is not metabolized (further) to GA_7 nor is GA_7 a precursor to GA_{16}. GA_{47} is present in trace amounts only.

Higher Plants. In higher plants evidence for the steps beyond GA_{12}-aldehyde is only beginning to appear in the literature and this information is fragmentary and scattered amongst a number of plant species. Meaningful data have been difficult to obtain because of problems in recovering metabolites from radio-labeled feeds; these problems are due in part to the low rates of metabolism which in turn are a reflection of the relatively low levels of endogenous GAs. As a result, most studies in higher plants have used developing seed which are known to have relatively high levels of native GAs. It would appear that the two pathways occurring in the fungus also operate in some higher plant species, although GA_{14}, a possible intermediate in the early-3-hydroxylated pathway, has yet to be identified as native to higher plants. In addition there is evidence for an early-13-hydroxylation pathway which is absent in the fungus. Finally certain details of the pathways in higher plants differ significantly from those in the fungus. For instance 2β-hydroxylation, which results in the loss of biological activity, appears to be widespread in higher plants (34) and lacking in the fungus. Also many species of higher plants conjugate GAs as glucosyl ethers and/or glucosyl esters, GA-derivatives that are absent in the fungus. These conjugates are either low or lacking in biological activity, and their physiological role in the plant is uncertain. While the variety of GAs present in higher plants suggests the presence of more than 2 major pathways from GA_{12}-aldehyde, this is by inference only and confirmation must come from metabolic studies using radio-labeled GAs and their precursors. The choice of substrates becomes critical for studies with higher plants, since it has been shown that many of the GA-hydroxylating enzymes in the fungus are non-specific in terms of substrate. For example (Figure 4) the fungal mutant B1-41a will metabolize ent-13-hydroxy kaurenoic acid, a non-fungal analogue of ent-kaurenoic acid, to the non-fungal-13-hydroxylated

In Plant Growth Substances; Mandava, N.;
ACS Symposium Series; American Chemical Society: Washington, DC, 1979.

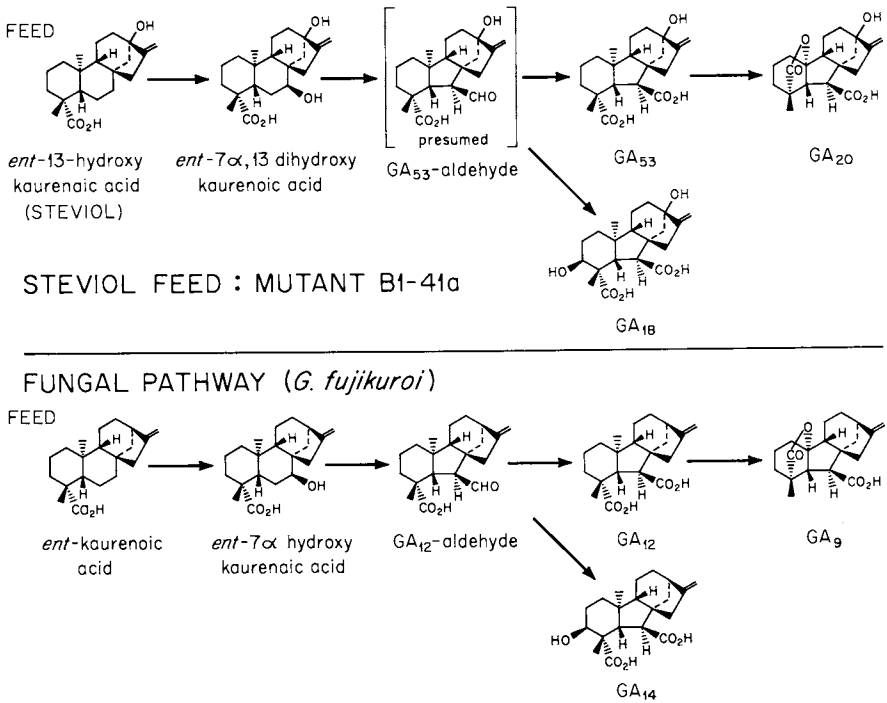


Figure 4. Metabolites from feeds of steviol (ent-*13-hydroxykaurenoic acid) and* ent-*kaurenoic acid to the GA mutant B1-41a of* G. fujikuroi. *Steviol is found as a glucoside in the higher plant* Stevia rebaudiana; *it is not found in the fungus.*

In Plant Growth Substances; Mandava, N.;
ACS Symposium Series; American Chemical Society: Washington, DC, 1979.

analogues of ent-7α-hydroxy kaurenoic acid, GA_{12}, GA_9, GA_{14} and other products (35). Two of these GAs are found only in some higher plants and not in the fungus. There are now several examples of this type of non-specificity (11). The information becomes particularly useful with the realization that the same non-specificity probably holds for hydroxylation steps in higher plants. Thus it follows that extreme care should be taken in the choice of substrates to be used in feeding studies with higher plants. Unless the GA to be used is native to the plant being studied, the probability is high that a pathway based on such feeds and re-feeds may in fact have little to do with the actual (natural) pathway for that particular plant.

In higher plants, the most complete understanding of a pathway beyond GA_{12}-aldehyde comes from studies with cell-free systems obtained from the endosperm of developing pumpkin seed (C. maxima) (36,37,38). Radio-labeled GA_{12}-aldehyde has been fed to this system, the metabolites identified, and re-fed with subsequent identification of metabolites. What appears is a metabolic grid in which the series GA_{12}-aldehyde ⟶ GA_{12} ⟶ GA_{24} ⟶ GA_{25} is connected via any of three 3β-hydroxylation steps to the series GA_{14}-aldehyde ⟶ GA_{14} ⟶ GA_{36} ⟶ GA_{13}. GA_{13} is further 2β-hydroxylated to GA_{43}. On the basis of relative rates, a single "preferred pathway" has been suggested, namely GA_{12}-aldehyde ⟶ GA_{12} ⟶ GA_{24} ⟶ GA_{36} ⟶ GA_{13} (see heavy arrows in Figure 5). More definitive evidence for this single pathway must await studies on the isolation and properties of the enzymes involved in these steps.

In beans (Phaseolus vulgaris and P. coccineus), 14 GAs and 8 GA-conjugates have been identified as natural products and 5 of these GAs have been fed as radio-labeled substrates to developing seed. The metabolites from these feeds suggest the presence of two pathways (Figure 6). The first is a late 13-hydroxylating pathway leading to GA_{20}, GA_1 and the 2β-hydroxylated GAs, GA_8 and GA_{29}; the second is an early-3-hydroxylation pathway leading to GA_4, GA_1 and GA_8. Since radio-labeled GA_1 was not observed from GA_5 feeds, nor was GA_5 found from GA_1 and GA_{20} feeds, the biosynthetic relationship of GA_5 to the other GAs is unresolved. It is interesting that all five GAs used in the feeds were metabolized to conjugates, of which two are not native to beans. These conjugates could be an artifact of the feed, i.e. they are consequences of the loss of compartmentalization and not a measure of the natural pathway in beans (58). A soluble 2β hydroxylase (GA_1 ⟶ GA_8) has been partially purified from P. vulgaris cotyledons (39,40). The enzyme has properties similar to the soluble hydroxylases found in pumpkin.

In peas (Pisum sativum) the complete pathway has been demonstrated from MVA through ent-kaurene and GA_{12}-aldehyde (see Figure 2) to 13-hydroxylated C_{20} GAs (Figure 7) (41,42). The pathway was established using cell-free systems obtained

In Plant Growth Substances; Mandava, N.;
ACS Symposium Series; American Chemical Society: Washington, DC, 1979.

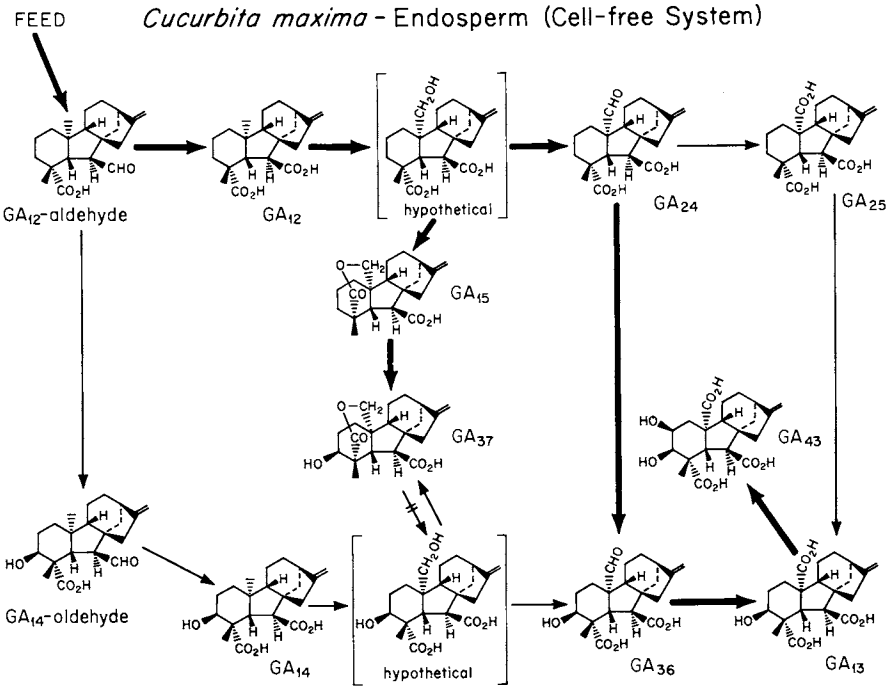


Figure 5. The metabolic pathway beyond GA_{12}-aldehyde for pumpkin endosperm (Cucurbita maxima). *Heavy arrows represent the preferred pathway based on relative rates of conversion and levels of metabolites.*

In Plant Growth Substances; Mandava, N.;
ACS Symposium Series; American Chemical Society: Washington, DC, 1979.

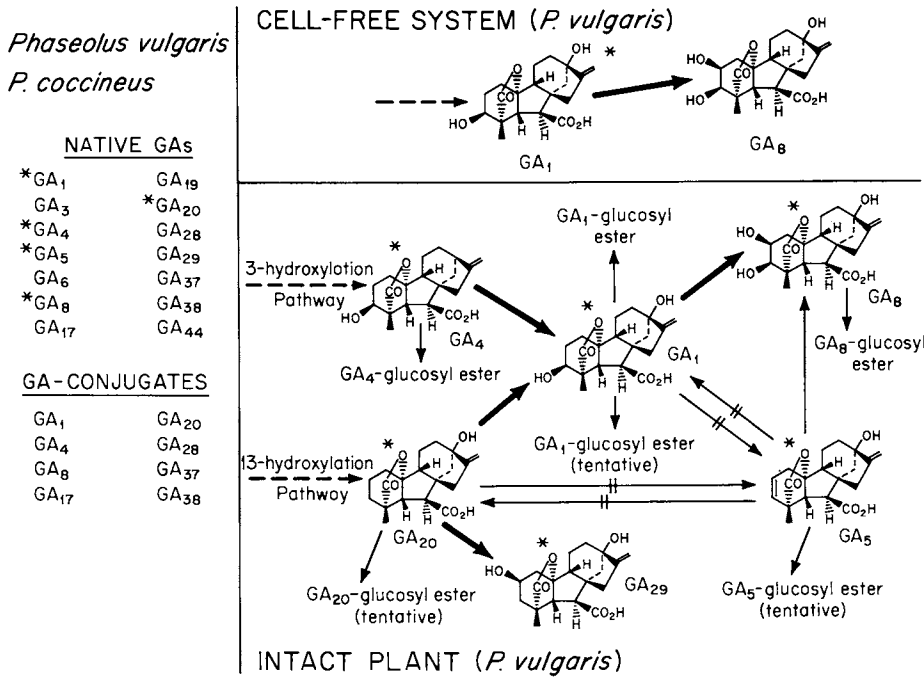


Figure 6. GA-biosynthetic steps for the bean Phaseolus vulgaris. *Asterisks represent substrates fed to the system (58).*

In Plant Growth Substances; Mandava, N.;
ACS Symposium Series; American Chemical Society: Washington, DC, 1979.

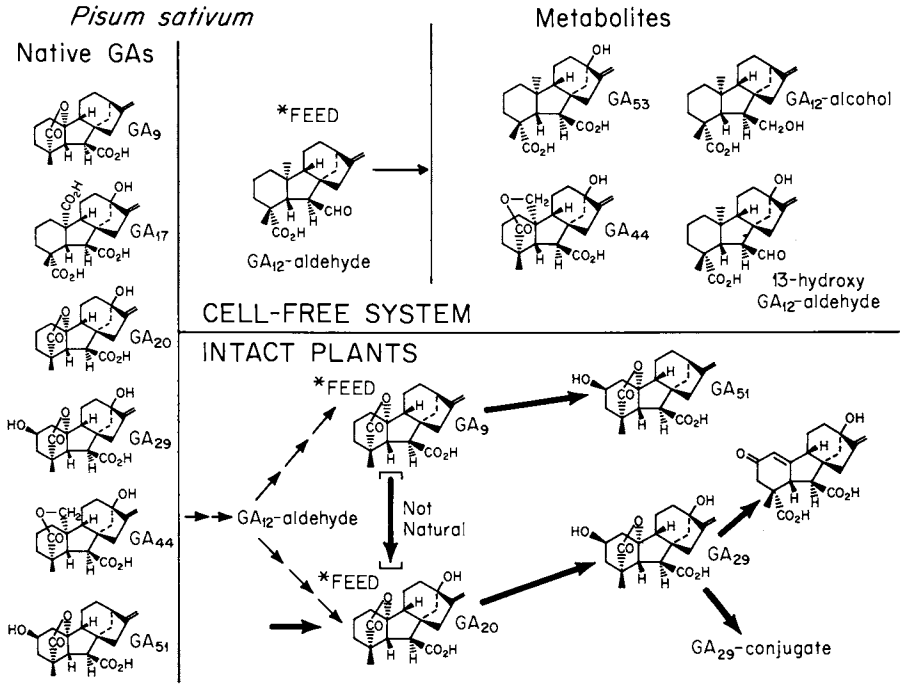


Figure 7. GA-biosynthetic steps for the pea, Pisum sativum. *The metabolites from the feed of GA_{12}-aldehyde to cell-free systems have not been fed back to that system. The identification of 13-hydroxy GA_{12}-aldehyde by GC–MS is from unpublished data (56).*

from very young seed. However, there is limited information on the steps beyond GA_{12}-aldehyde. Six GAs have been identified as native to peas and their interrelationship has been examined by radio-labeled feeds of GAs to developing seed and seedlings (42,43,44,45,46). These show the presence of two apparently unrelated pathways involving the 2β-hydroxylation steps, $GA_9 \longrightarrow GA_{51}$ and $GA_{20} \longrightarrow GA_{29}$. GA_{29} was converted to its conjugate and to a 2-oxo-derivative. The formation of the 2-oxo-derivative, which is also endogenous to pea seeds and shoots, may be the first step in GA degradation which is initiated by 2β-hydroxylation of GA_{20}. An interesting aspect of the studies with peas involves the correlation of metabolism with developmental stages of pea seed. At first glance, the evidence from radio-labeled feeds to pea seed suggested the pathway $GA_9 \longrightarrow GA_{20} \longrightarrow GA_{29}$. However, when the evidence is considered in light of the native GAs present at different stages of seed development, it was found that the ability to 13-hydroxylate GA_9 occurs only at a stage in development where native GA_9 and GA_{20} are absent. When GA_9 and GA_{20} are present (in older seed), GA_9 is 2β-hydroxylated to GA_{51} with no evidence for 13-hydroxylation to GA_{20}. This information has been interpreted to mean that GA_9 and GA_{20} originate from separate pathways from GA_{12}-aldehyde and that the observed metabolism of GA_9 to GA_{20} is an artifact due to the non-specific 13-hydroxylation of a substrate not normally present at early stages of development.

GAs and Shoot Elongation

While there are innumerable examples of enhanced shoot growth (elongation) in response to exogenously applied GAs, the evidence for a positive correlation of such growth with the endogenous level of identified GAs is yet to be documented. The limited evidence in the literature is based on apparent levels of extractable GA-like substances, for which there are examples of both negative and positive correlations with growth. An example of a negative correlation is found in *Agrostemma githago* (corn cockle), a plant which grows as a tight rosette in short days, and bolts (elongates) when transferred to long days. The resultant elongated stem can be ten to twenty times the height of its rosetted counterpart. Rosetted plants maintained in short day can be induced to bolt by treatment with exogenous GA. Since this GA-induced elongation mimics that initiated by long-day, one might suspect that plants photoperiodically induced to bolt would have higher levels of native GAs than rosetted members. However, this turns out not to be the case; rosetted plants have levels of extractable GA-like substances equal to or greater than bolting plants (J.A.D. Zeevaart, personal communication). There are a number of possible explanations for negative correlations of this type. For instance, the native GAs may be compartmentalized in the cell and not available for the

In Plant Growth Substances; Mandava, N.;
ACS Symposium Series; American Chemical Society: Washington, DC, 1979.

initiation of growth until released from these compartments (organelles). This release could be the basis for the observed photoperiodic-dependent bolting. Total extraction of GA-like substances would not provide the critical data, and so the significance of this and other similar types of negative correlations (47,48) remains unresolved.

The dwarf-5 mutant of Zea mays is an example of a positive correlation of the level of GA-like substances with growth. This particular single gene mutant grows to one fifth of the height of normals, and its dwarf habit of growth is visible from the seedling stage to maturity (49). Dwarf-5 seedlings respond to exogenously applied GAs by normal-type growth, and with continued treatment this dwarf will become phenotypically indistinguishable from normals (4). While evidence for GA-like substances has been obtained from seedlings of normal maize, no GA-like biological activity has been found from extracts of the d_5 mutant (50). Thus the mutant gene could be controlling GA-levels through GA-biosynthesis. Since this particular dwarf mutant gives a normal type growth response to feeds of a number of GA-precursors including ent-kaurene and its oxidation products (51), it was concluded that the mutant gene was blocking an early step in the GA-biosynthetic pathway. Information has recently appeared (52) which strongly suggests that this mutant gene (d_5) controls the B activity of ent-kaurene synthetase (Figure 8). In cell-free systems obtained from normal and dwarf seedlings, normals were found to synthesize ent-kaurene from radio-labeled feeds of MVA, CPP and GGPP; in contrast the dwarfs synthesized ent-kaurene at levels one fifth that of the normals. Concomitant with this reduced level of ent-kaurene synthesis is the production by the dwarf system of relatively large amounts of the ent-kaurene isomer, ent-isokaurene. This isomer is not metabolized to iso-GAs, at least by the fungus G. fujikuroi (53). It would appear then that the absence of GA-like substances in the mutant is due to a low level of ent-kaurene biosynthesis. Since the native GAs of maize have recently been identified (GA_{53}, GA_{44}, GA_{19}, GA_{17}, GA_{20}, B.O. Phinney, P. Hedden, J. MacMillan, unpublished information), the stage is now set for the quantification of GA levels in normal and dwarf-5 plants.

In conclusion it can be said that the most significant contributions on the role of GAs in growth and development are yet to be made, especially at the chemical and biochemical level. It is clear that there is still only limited information from higher plants on the detailed steps of GA biosynthesis beyond GA_{12}-aldehyde and it is not really known whether one or more GAs are physiologically active per se. The significance of compartmentalization in GA biosynthesis and release has yet to be clarified, and while there is some information on the transport of GA-like substances in the plant (1,2) virtually nothing is known about this subject at the chemical level. Finally, as is

In Plant Growth Substances; Mandava, N.;
ACS Symposium Series; American Chemical Society: Washington, DC, 1979.

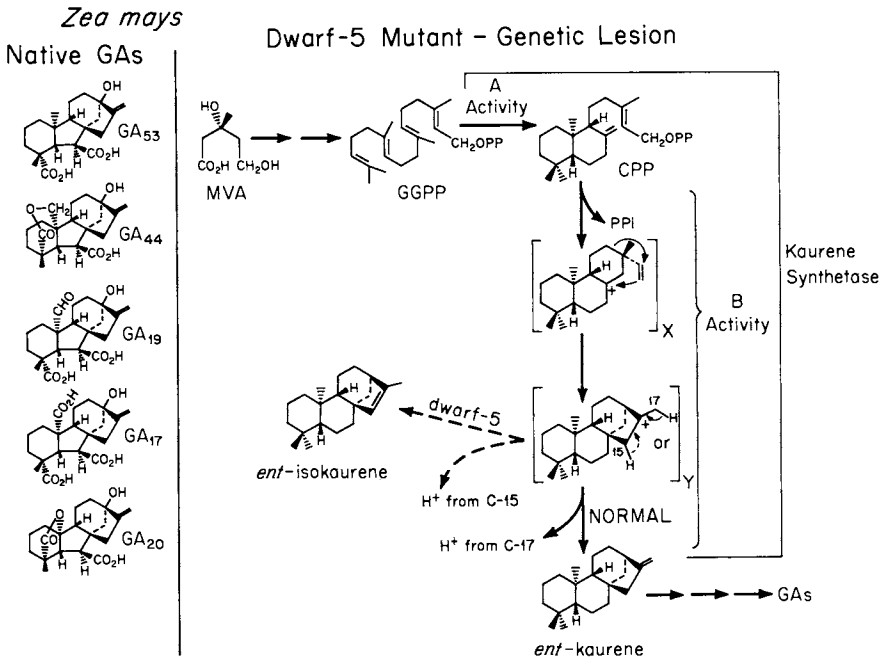


Figure 8. The ent*-kaurene synthetase system and the* d_5 *lesion of maize,* Zea mays. *Apparently the* d_5 *lesion favors the loss of the proton from carbon-15 rather than from carbon-17 as is the case for normal maize. Compounds in parentheses are hypothetical intermediates. The GA's identified by GC–MS were obtained from young maize tassels (57).*

In Plant Growth Substances; Mandava, N.;
ACS Symposium Series; American Chemical Society: Washington, DC, 1979.

also true for the other classes of plant hormones (54) the mechanism of action of GAs remains enigmatic.

---

[1]Abbreviations. CPP (copalyl pyrophosphate), DMAPP (dimethylallylpyrophosphate), FPP (farnesylpyrophosphate, GA(s) (gibberellin(s)), GA_x (gibberellins$_{1-x}$, individual gibberellins named as GA_{1-x}), GC-MS (combined gas-liquid chromatography-mass spectrometry), GC-RC (combined gas-liquid chromatography-radio counting), GGPP (geranylgeranyl pyrophosphate), GPP (geranylpyrophosphate), GC (gas-liquid chromatography), IPP (isopentenyl pyrophosphate), MVA (mevalonic acid), TLC (thin-layer chromatography).

[2]Gibberellin-producing strains of the fungus were originally associated with a rice disease, called ine-bakanae-byo in Japan (literally translated, rice foolish-seedling disease). Among the pathological effects of this disease was an excessive elongation of the shoots of seedlings and young plants. The result was either death of the seedlings or the absence of fruit in the mature plants. The early studies that led to the discovery of GAs by the Japanese were motivated by the need for a better understanding of this disease which, in fact, could reduce the rice yield by 40% or more. It was a plant pathologist, Kurosawa, who first coined the name gibberellin in 1933 for a purified fraction of the fungus that would greatly stimulate shoot elongation in rice seedlings. Gibberellins were first isolated by Yabuta in 1933; the chemical identification of gibberellic acid (GA_3) was first accomplished by Cross et al. in 1958. An interesting historical account of the discovery of GAs has been published by Stodola (55).

Acknowledgments

The author wishes to thank Dr. Peter Hedden for helpful discussions in preparing this manuscript. The preparation of this report was supported by a grant from the N.S.F.

Literature Cited

1. Graebe, J.E., Ropers, H.-J. "The gibberellins," in "Phytohormones and related compounds: A comprehensive treatise. Volume 1." Lethem, D.S., Goodwin, P.B., Higgins, T.J.V., Ed. Elsevier/North Holland Biomedical Press, Amsterdam, Oxford, New York, 1978, pp. 107-203.

2. Jones, R. "Gibberellins; their physiological role." Ann. Rev. Plant Physiol. 1978, 29, 149-192.

3. Brian, P.W., Hemming, H.G. "The effect of gibberellic acid on shoot growth of pea seedlings." Physiol. Plantarum, 1955, 86, 669-681.

4. Phinney, B.O. "The growth response of single gene dwarf mutants of Zea mays to gibberellic acid." Proc. Natl. Acad. Sci. USA, 1956, 42, 185-189.

5. West, C.A., Phinney, B.O. "Properties of gibberellin-like factors from extracts of higher plants." Pl. Physiol. (suppl.), 1956, 31, xx.

6. Radley, M. "Occurrence of substances similar to gibberellic acid in higher plants." Nature (London), 1956, 178, 1070-1071.

7. Phinney, B.O., West, C.A., Ritzel, M.B., Neely, P.M. "Evidence for gibberellin-like substances from flowering plants." Proc. Natl. Acad. Sci. USA, 1957, 43, 398-404.

8. MacMillan, J., Suter, P.J. "The occurrence of gibberellin A_1 in higher plants: isolation from the seed of runner bean (Phaseolus multiflorus)." Naturwissen. 1958, 45, 46.

9. West, C.A., Phinney, B.O. "Gibberellins from flowering plants. I. Isolation and properties of a gibberellin from Phaseolus vulgaris L." J.A.C.S., 1959, 81, 2424-2427.

10. Sumiki, Y., Kawarada, A. "Occurrence of gibberellin A_1 in the water sprouts of Citrus," in "Plant Growth Regulation," IVth International Conference on Plant Growth Regulation, Klein, R.M., Ed., The Iowa State University Press, 1961, 483-487.

11. Hedden, P., MacMillan, J., Phinney, B.O. "The metabolism of the gibberellins." Ann. Rev. Plant Physiol., 1978, 29, 149-192.

12. Beytia, E.D., Porter, J.W. "Biochemistry of polyisoprenoid biosynthesis." Ann. Rev. Biochem., 1976, 45, 113-142.

13. Goodwin, T.W. "Biosynthesis of terpenoids." Ann. Rev. Plant Physiol., 1979, 30, 369-404.

14. Buggy, M.J., Britton, G., Goodwin, T.W. "Terpenoid biosynthesis by chloroplasts isolated in organic solvents." Phytochemistry, 1974, 13, 125-129.

In Plant Growth Substances; Mandava, N.;
ACS Symposium Series; American Chemical Society: Washington, DC, 1979.

15. Schechter, I., West, C.A. "Biosynthesis of gibberellins. IV. Biosynthesis of cyclic diterpenes from trans-geranyl-geranyl pyrophosphate." J. Biol. Chem., 1969, 244, 3200-3209.

16. Simcox, P.D., Dennis, D.T., West, C.A. "Kaurene synthetase from plastids of developing plant tissues." Biochem. Biophys. Res. Commun., 1975, 66, 166-172.

17. Murphy, G.J.P., Briggs, D.E. "Metabolism of ent-kaurenol-17-^{14}C, ent-kaurenal-17-^{14}C and ent-kaurenoic acid-17-^{14}C by germinating Hordeum distichon grains." Phytochemistry, 1975, 14, 429-433.

18. Moore, T.C., Coolbaugh, R.C. "Conversion of geranyl-geranyl pyrophosphate to ent-kaurene in enzyme extracts of sonicated chloroplasts." Phytochemistry, 1976, 15, 1241-1247.

19. Hasson, E.P., West, C.A. "Properties of the system for the mixed function oxidation of kaurene and kaurene derivatives in microsomes of the immature seed of Marah macrocarpus. Cofactor requirements." Plant Physiol., 1976, 58, 473-478.

20. Hasson, E.P., West, C.A. "Properties of the system for the mixed function oxidation of kaurene and kaurene derivatives in microsomes of the immature seed of Marah macrocarpus. Electron transfer components." Plant Physiol., 1976, 58, 479-484.

21. Graebe, J.E., Hedden, P., MacMillan, J. "The ring contraction step in gibberellin biosynthesis." J.C.S. Chem. Commun., 1975, 161-162.

22. Hanson, J.R., Hawker, J. "The ring contraction stage in gibberellin biosynthesis." J.C.S. Chem. Commun., 1971, 208.

23. Bearder, J.R., MacMillan, J., Wels, C.M., Chaffey, M.B., Phinney, B.O. "Position of the metabolic block for gibberellin biosynthesis in mutant B1-41a of Gibberella fujikuroi." Phytochemistry, 1974, 13, 911-917.

24. Bearder, J.R., MacMillan, J., Phinney, B.O. "Fungal products. Part XIV. Metabolic pathways from ent-kaurenoic acid to the fungal gibberellins in mutant B1-41a of Gibberella fujikuroi." J.C.S. Perkin Trans. 1975, 1, 721-726.

25. Bearder, J.R., MacMillan, J., Phinney, B.O. "Origin of the oxygen atoms in the lactone bridge of C_{19}-gibberellins." J.C.S. Chem. Commun., 1976, 834-835.

In Plant Growth Substances; Mandava, N.;
ACS Symposium Series; American Chemical Society: Washington, DC, 1979.

26. Evans, R., Hanson, J.R. "Studies in terpenoid biosynthesis. Part XIII. The biosynthetic relationship of the gibberellins in Gibberella fujikuroi." J.C.S. Perkin Trans., 1975, 1, 663-666.

27. Hanson, J.R. "New metabolites of Gibberella fujikuroi - XII Gibberellin A_{15}." Tetrahedron, 1967, 23, 733-735.

28. Harrison, D.M., MacMillan, J. "Two new gibberellins, A_{24} and A_{25} from Gibberella fujikuroi; their isolation, structure, and correlations with gibberellin A_{13} and A_{15}." J. Chem. Soc. C. 1971, 631-636.

29. Dockerell, B., Hanson, J.R. "The fate of C-20 in C_{19}-gibberellin biosynthesis." Phytochemistry, 1978, 17, 701-704.

30. Bearder, J.R., Frydman, V.M., Gaskin, P., MacMillan, J., Wels, C., Phinney, B.O. "Fungal products. Part XVII. Microbiological hydroxylation of gibberellin A_9 and its methyl esters." J.C.S. Perkin Trans. 1976, 1, 178-183.

31. Hedden, P., MacMillan, J., Phinney, B.O. "Fungal products. Part XII. Gibberellin A_{14}-aldehyde, an intermediate in gibberellin biosynthesis in Gibberella fujikuroi." J.C.S. Perkin Trans., 1974, 1, 587-592.

32. Bearder, J.R., MacMillan, J. "Fungal products. Part IX. Gibberellins A_{16}, A_{36}, A_{37}, A_{41} and A_{42} from Gibberella fujikuroi." J.C.S. Perkin Trans., 1973, 1, 2824-2830.

33. Galt, R.H.B. "New metabolites of Gibberella fujikuroi, Part IX, Gibberellin A_{13}." J. Chem. Soc., 1965, 3143-3151.

34. Rappaport, L., Davies, L., Lavee, S., Nadeau, R., Patterson, R., Stolp, C.F. "Significance of metabolism of $(^3H)GA_1$ for plant regulation. In "Plant Growth Substances." Tokyo: Hirokawa, 1973, pp. 314-324.

35. Bearder, J.R., MacMillan, J., Wels, C.M., Phinney, B.O. "The metabolism of steviol to 13-hydroxylated ent-gibberellanes and ent-kauranes." Phytochemistry, 1975, 14, 1741-1748.

36. Graebe, J.E., Hedden, P. "Biosynthesis of gibberellins in a cell-free system." In "Biochemistry and Chemistry of Plant Growth Regulators," Schreiber, K., Schütte, H.R., Sembdner, G., Ed. Halle (Saale), G.D.R.: Acad. Sci., German Democratic Republic, Inst. Plant Biochem. 1974, pp. 1-16.

In Plant Growth Substances; Mandava, N.;
ACS Symposium Series; American Chemical Society: Washington, DC, 1979.

37. Graebe, J.E., Hedden, P., Gaskin, P., MacMillan, J. "Biosynthesis of gibberellins A_{12}, A_{15}, A_{24}, A_{36}, and A_{37} by a cell-free system from Cucurbita maxima." Phytochemistry, 1974, 13, 1433-1440.

38. Graebe, J.E., Hedden, P., Gaskin, P., MacMillan, J. "The biosynthesis of a C_{19}-gibberellin from mevalonic acid in a cell-free system from a higher plant." Planta, 1974, 120, 307-309.

39. Patterson, R.J., Rappaport, L. "The conversion of gibberellin A_1 to gibberellin A_8 by a cell-free enzyme system." Planta, 1974, 119, 183-191.

40. Patterson, R., Rappaport, L., Breidenbach, R.W. "Characterization of an enzyme from Phaseolus vulgaris seeds which hydroxylates GA_1 to GA_8." Phytochemistry, 1975, 14, 363-368.

41. Ropers, H.-J., Graebe, J.E., Gaskin, P., MacMillan, J. "Gibberellin biosynthesis in a cell-free system from immature seeds of Pisum sativum." Biochem. Biophys. Res. Commun., 1978, 80, 690-697.

42. Railton, I.D., Durley, R.C., Pharis, R.P. "Metabolism of tritiated gibberellin A_9 by shoots of dark-grown dwarf pea, cv. Meteor." Plant Physiol., 1974, 54, 6-12.

43. Railton, I.D., Murofushi, N., Durley, R.C., Pharis, R.P. "Interconversion of gibberellin A_{20} to gibberellin A_{29} by etiolated seedlings and germinating seeds of dwarf Pisum sativum." Phytochemistry, 1974, 13, 793-796.

44. Frydman, V.M., MacMillan, J. "The metabolism of gibberellins A_9, A_{20} and A_{29} in immature seeds of Pisum sativum cv. Progress No. 9. Planta, 1975, 125, 181-195.

45. Sponsel, V.M., MacMillan, J. "Further studies on the metabolism of gibberellins (GAs) A_9, A_{20}, A_{29} in immature seeds of Pisum sativum cv. Progress No. 9." Planta, 1977, 135, 129-136.

46. Sponsel, V.M., MacMillan, J. "Metabolism of gibberellin A_{29} in seeds of Pisum sativum cv. Progress No. 9; use of [^{2}H] and [^{3}H] GAs, and the identification of a new GA catabolite." Planta, 1978, 144, 69-78.

47. Cleland, C.F., Zeevaart, J.A.D. "Gibberellins in relation to flowering and stem elongation in the long day plant Silene armeria." Plant Physiol., 1970, 46, 392-400.

In Plant Growth Substances; Mandava, N.;
ACS Symposium Series; American Chemical Society: Washington, DC, 1979.

48. Zeevaart, J.A.D. "Effects of photoperiod on growth rate and endogenous gibberellins in the long-day rosette plant spinach." Plant Physiol., 1971, 47, 821-827.

49. Emerson, R.A., Beadle, C.W., Fraser, A.C. "Linkage studies in maize." N.Y. State Agr. Coll. Expt. Sta., Geneva, N.Y. 1935, Mem. 39, 1-83.

50. Phinney, B.O. "Dwarfing genes in Zea mays and their relation to the gibberellins." In Plant Growth Regulation, Klein, R.M., Ed. Iowa State Univ. Press, Ames, Iowa, U.S.A. 1961, pp. 489-501.

51. Katsumi, M., Phinney, B.O., Jefferies, P.R., Henrick, C.A. "Growth response of the d_5 and an-1 mutants of maize to some kaurene derivatives." Science, 1964, 144, 849-850.

52. Hedden, P., Phinney, B.O. "Comparison of ent-kaurene and ent-isokaurene synthesis in cell-free systems from etiolated shoots of normal and dwarf-5 maize seedlings." Phytochemistry, 1979, 18, in press.

53. Hedden, P., Phinney, B.O., MacMillan, J., Sponsel, V.M. "Metabolism of kaurenoids by Gibberella fujikuroi in the presence of the plant growth retardant, N,N,N-trimethyl-1-methyl-(2',6',6'-trimethylcyclohex-2'-en-1'-yl)prop-2-enylammonium iodide." Phytochemistry, 1977, 16, 1913-1917.

54. Kende, H., Gardner, G. "Hormone binding in plants." Ann. Rev. Plant Physiol. 1976, 27, 267-290.

55. Stodola, F.H. "Source Book on Gibberellins, 1828-1957." Agr. Res. Serv., U.S.D.A., Washington, D.C. 1958, pp. 1-138.

56. MacMillan, J. personal communication.

57. Hedden, P., Phinney, B. O., MacMillan, J. unpublished information.

58. Yamane, H., Murofushi, N., Osada, H., and Takahashi, N. Metabolism of Gibberellins in Early Immature Bean Seeds, Phytochemistry, 1977, 16, pp. 831-835.

RECEIVED June 19, 1979.

Anticytokinins as Probes of Cytokinin Utilization

SIDNEY M. HECHT[1]

Department of Chemistry, Massachusetts Institute of Technology, Cambridge, MA 02139

The term cytokinin refers to substances that promote cell division and growth in certain plant tissues, and that are involved in the cell differentiation phenomenon and numerous other physiological events in plants, including regulation of organ formation, enhancement of flowering and seed germination and resistance to aging (1,2,3). Although the existence of a cell division factor was first postulated many years ago (4), the chemical nature of such species was unknown until 1955 when Miller et al (5,6,7) isolated kinetin from both old and autoclaved (pH 4.3, 120°, 30 min) preparations of DNA. The factor was identified as 6-furfurylaminopurine (1), which had presumably arisen by dehydration and rearrangement of deoxyadenosine moieties in the DNA (8,9). While the formation of kinetin from DNA is of uncertain physiological significance, its structural identification prompted the preparation of a remarkable variety of analogs, which served to define the structural requirements for cytokinin activity (10, 11,12,13). Among these was 6-benzylaminopurine (2) (14,15,16), a compound that has found wide utility in studies of cytokinin activity, and 6-(3-methyl-2-butenylamino)purine (3) (17), which was subsequently isolated as a growth factor from the plant pathogens Corynebacterium fascians (18,19) and Agrobacterium tumefaciens (20). Additionally isolated from Corynebacterium were several other cytokinin-active species, including cis and trans 6-(4-hydroxy-3-methyl-2-butenylamino)purine (21,22), the trans isomer of which (4) had been isolated earlier from young sweet corn (Zea mays) and named zeatin (23,24,25,26). Zeatin has also been found to occur in plums (27) and in the fungus Rhizopogan roseolus (28); its (-)-dihydro derivative is present in the immature seeds of

[1]Current address: Departments of Chemistry and Biology, University of Virginia, Charlottesville, Virginia 22901.

0-8412-0518-3/79/47-111-079$05.00/0

Lupinus luteus (29,30). Cytokinins also occur naturally at the ribonucleoside and ribonucleoside phosphate levels (31,32,33) and exogenously applied cytokinins have been shown to undergo metabolism to glucosyl derivatives (34,35,36,37,38).

Cytokinin-Active Nucleosides are Components of Transfer RNA's

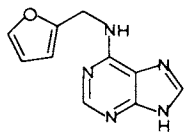
In 1966, Zachau and his coworkers reported the sequences of $tRNA_1^{Ser}$ and $tRNA_2^{Ser}$ from brewer's yeast; these tRNA's were both found to contain the cytokinin-active nucleoside N^6-(3-methyl-2-butenyl)adenosine (5) in the positions adjacent to the 3'-end of the anticodon triplet (39,40). Intensive investigation of the cytokinin content of a variety of tRNA's from plants, animals and microorganisms revealed that virtually every one contained at least one type of cytokinin-active nucleoside (2). Chemically, the species isolated included N^6-(3-methyl-2-butenyl)adenosine (5) (39,40,41) and N^6-(4-hydroxy-3-methyl-2-butenyl)adenosine (6; trans isomer shown) (42,43,44) as well as the corresponding 2-methylthio derivatives of these species (7 and 8) (45,46,47,48). For 6 and 8, both geometrical isomers have been shown to occur as tRNA constituents (49,50). It is interesting that the tRNA's from some natural sources, such as yeast (42), immature corn kernels (43) and human liver (44), contain only a single cytokinin-active nucleoside while multiple species have been detected in the tRNA's from other sources (49,50,51,52). As was true for yeast $tRNA^{Ser}$'s (39,40), all of sequenced tRNA's containing cytokinins have these species in the anticodon-adjacent position. Additionally, it may be noted that cytokinins occur exclusively in those tRNA's that respond to codons beginning with uridine. A possible function for these nucleosides in protein biosynthesis has been suggested (53), although it is not clear that their presence in tRNA's is related to their growth-promoting properties in plants.

Tobacco Explants are Used for the Bioassay of Cytokinins

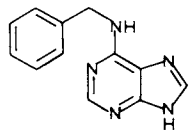
Cytokinin activity can be assayed conveniently with several plant tissues, including soybean (54) and tobacco (55,56) callus, radish leaf discs (11,12) and lettuce seed (57). The first two of these are probably the most useful since they are not excessively sensitive to light or temperature (58,59) and reflect both cell enlargement and division (60).

Bioassay of our test compounds has been carried out by Professor Folke Skoog at the University of Wisconsin. Aqueous solutions of the compounds to be tested are filter sterilized or treated with dimethylsulfoxide, and added at several concentrations to replicate cultures, each containing three pieces of tobacco callus grown on agar; the synthetic medium contains all essential nutrients including the auxin indole-3-acetic acid (61,62,63). The cultures are allowed to grow in continuous, diffuse light at about 28° for five weeks and then harvested and used to

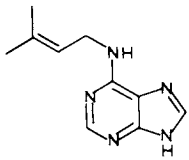
In Plant Growth Substances; Mandava, N.;
ACS Symposium Series; American Chemical Society: Washington, DC, 1979.



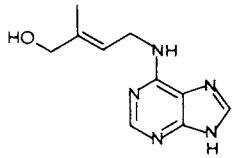
1



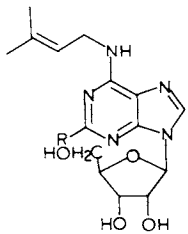
2



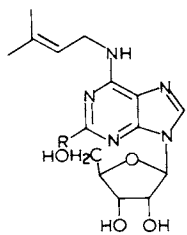
3



4



5 R=H
7 R=SCH$_3$



6 R=H
8 R=SCH$_3$

In Plant Growth Substances; Mandava, N.;
ACS Symposium Series; American Chemical Society: Washington, DC, 1979.

record fresh weight yields. The results of individual experiments are expressed as plots of fresh weight yields vs the logarithm of cytokinin concentration (see, e.g. ref. 13, Fig. 3). Also, since a nearly linear relationship is obtained starting essentially at the concentration at which activity is first detectable and continuing nearly to the point of maximum yield, this linear range may be determined from several experiments and the average values presented as a bar graph.

Probing the Cytokinin Receptor Site(s)

Shortly after the identification of kinetin as 6-furfurylaminopurine, Strong (10) demonstrated that the activity was limited to 6-substituted purines and this finding was confirmed independently (11,12). Investigation by Skoog et al of about 70 potential cytokinins revealed that the length of the N^6-substituent was important; activity was greatest for those compounds having a substituent with 4-7 carbon atoms (13). Replacement of the exocyclic N-atom with O,S or C generally afforded analogs having somewhat lower cytokinin activity (13,64), while alterations within the heterocyclic nucleus typically resulted in drastically lower activity (13,65). Substitution at C-2 or C-8 had relatively little effect on cytokinin activity (66,67). Substituents at the N^6-, 7- or 9-positions tended to lower activity and those at N-1 and N-3 greatly lowered activity (13).

Unsaturation in the N^6-substituent was found to greatly enhance cytokinin activity, the most striking example being that of 6-(3-methyl-2-butenylamino)purine which was not only much more active than its saturated derivative, 6-isopentylaminopurine, but also ten times as active as kinetin in the region of highest sensitivity (13,60,68). Even more remarkable was the observation that geometrical isomers had markedly different cytokinin activities (69,70). 6-(4-Hydroxy-3-methyl-*trans*-2-butenylamino)purine (4), for example, was found to be about 50 times more active than the *cis*-isomer (70), consistent with the results obtained earlier for the corresponding ribosylated derivatives (71,72). Moreover, Matsubara et al (73) found that while R-(+) and S-(-)-dihydrozeatins (9 and 10, respectively) had quite similar activities in the tobacco bioassay, the former was substantially more active in promoting lettuce seed germination and an increase in the fresh weight yield of excised radish cotyledons, as well as inhibiting the degradation of chlorophyll in senescing radish cotyledons. Differences in cytokinin activities were also observed for several enantiomeric N-(purin-6-yl)amino acid methyl esters (74).

A Rationale for the Preparation of Specific Anticytokinins

The observations that cytokinins have activity in the tobacco bioassay detectable at concentrations as low as 10^{-11} M, and that the promotion of such activity can depend on structural parameters

In Plant Growth Substances; Mandava, N.;
ACS Symposium Series; American Chemical Society: Washington, DC, 1979.

as subtle as geometrical and absolute configuration, suggested that there must be one or more cellular receptor sites for such molecules. On the additional assumption that the activity of individual cytokinins is not proportional to their affinity for the receptor site(s), it seemed reasonable to attempt to prepare compounds with little or no cytokinin activity per se, but having a high enough affinity for the postulated receptor site(s) to block the binding of active cytokinins, and hence the expression of cytokinin activity. It was anticipated that the design of such inhibitors would bear at least formal analogy to the preparation of inhibitors of other biochemical processes, such as enzymatic transformations. In this context it was of interest that, e.g., allopurinol (4-hydroxypyrazolo[3,4-d]pyrimidine) was found to inhibit oxidation of the isomeric compound hypoxanthine by xanthine oxidase (75,76) and that incorporation of ATP into polydeoxynucleotides by DNA-dependent RNA polymerase was inhibited by the isomeric formycin (7-amino-3-(β-D-ribofuranosyl)pyrazolo[4,3-d]-pyrimidine)5'-triphosphate (77). Consistent with the hypothesis that similar structural alterations of cytokinins might afford species that inhibited cytokinin utilization was the finding, discussed above, that changes in the heterocyclic nucleus of N^6-substituted purines greatly lowered their activity as cytokinins (13, 65).

Substituted Pyrazolo[4,3-d]pyrimidines as Potential Anticytokinins

Four substituted pyrazolo[4,3-d]pyrimidines (11-14) were prepared for testing as cytokinins and anticytokinins. By analogy with results obtained in the purine series it was anticipated that the compounds having the isopentenyl substituent (11,12) would be more active as cytokinins than the respective compounds with isopentyl substituents and that the presence of an additional methyl substituent (12,14) would also diminish activity. These compounds were assayed for cytokinin activity in the tobacco bioassay and the results are indicated in Fig. 1. As shown in the Figure, 7-(3-methyl-2-butenylamino)pyrazolo[4,3-d]pyrimidine (11) was the most active of the four compounds tested, eliciting maximal growth response at 2 μM concentration. As expected on the basis of the 10-fold lower activity obtained upon formal saturation of the substituent in 3, the isopentyl analog of 11 (13) produced maximal growth response only at 20 μM concentration. Similarly, the methylated analog of 11 (12) was several-fold less active than the parent compound, as also was observed for analogous substitutions in the purine series. Compound 14, having both 7-isopentyl and 3-methyl substituents, lacked detectable cytokinin activity. Thus the order of cytokinin activities among the substituted pyrazolo-[4,3-d]pyrimidines was precisely as predicted based on structure-activity relationships in the purine series (although 11-14 were about 200-fold less active than the corresponding purines), consistent with the postulated interaction of all compounds

In Plant Growth Substances; Mandava, N.;
ACS Symposium Series; American Chemical Society: Washington, DC, 1979.

at the same cellular receptor site(s).

Compound 14, having the structural and growth-promoting characteristics expected of a potential anticytokinin, was tested for its ability to block the promotion of cell division and growth by other compounds having significant cytokinin activity. As shown in Fig. 2, when added to replicate cultures of tobacco callus grown on 6-benzylaminopurine (BAP), compound 14 diminished the fresh weight yield of callus obtained after a growth period of five weeks (78,79). For the cultures containing optimal (2.7-8.1 x 10^{-2} μM) 6-benzylaminopurine, application of compound 14 in approximately 100-fold molar excess completely inhibited the growth of the tobacco callus. Similar results were obtained using 6-(3-methyl-2-butenylamino)purine (3), although slightly greater concentrations of 14 were required to inhibit this more potent cytokinin. Consistent with the hypothesis that 14 acts as a specific cytokinin antagonist, the inhibition caused by this compound was found to be reversible by added cytokinin. As shown in the Figure, e.g., at concentrations of 6-benzylaminopurine and 14 that afforded incomplete inhibition of growth, the addition of a greater concentration of the cytokinin resulted in greater net growth after a period of five weeks, while the addition of 14 at higher concentrations gave more inhibition. When pieces of tobacco callus were transferred between media lacking or containing compound 14 at 0.1 μM concentration (but always containing 1 x 10^{-2} μM 3), inhibition of the rate of growth of the callus was also observed (80).

Tobacco callus grown on low concentrations of 14 could be transferred to a fresh growth medium at the conclusion of the five-week test period and grew as well as tissue never treated with the anticytokinin. However, tissue that had undergone marked growth reduction in the presence of 14 did not grow properly when subsequently transferred to a cytokinin-containing medium. This was especially true of tissue grown on media containing > 1 μM 14, which darkened during the course of the initial assay. In the belief that the antagonist might simply be interferring more generally in purine metabolism when utilized at high concentrations, experiments were carried out to determine the effect of adenine on the inhibition of cytokinin-induced growth by compound 14. It was found that adenine, supplied at 150 μM concentration, had no effect on tobacco callus grown in the presence of low concentrations of 14, or in its absence, but doubled the concentration range over which 3 could reverse the inhibition caused by 14 (79).

Reversal of inhibition caused by 14 was assayed quantitively using compound 3, 6-benzylaminopurine and diphenylurea, the latter two of which are about 1/10 and 1/1000 as active as 3, respectively, in promoting the growth of tobacco callus (13,81). Not unexpectedly, 6-benzylaminopurine was only about 1/3 as potent as 3 in reversing inhibition by 14 and diphenylurea was only 1/500 as effective. Thus, the ability of the cytokinins to reverse inhibition paralleled their activity as cytokinins, consistent with the

In Plant Growth Substances; Mandava, N.;
ACS Symposium Series; American Chemical Society: Washington, DC, 1979.

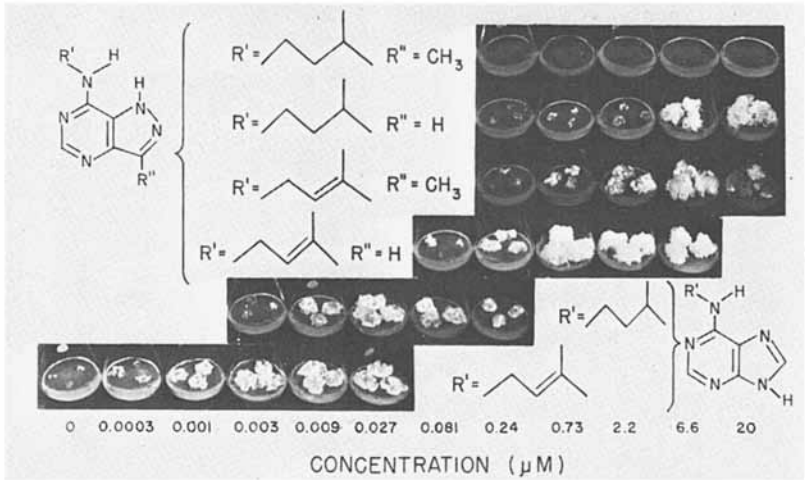


Figure 1. Cytokinin activities in the tobacco bioassay of 6-(3-methyl-2-butenylamino)purine (3), 6-isopentylaminopurine, and four (3-methyl-)7-alkylaminopyrazolo[4,3-d]pyrimidine (11–14)

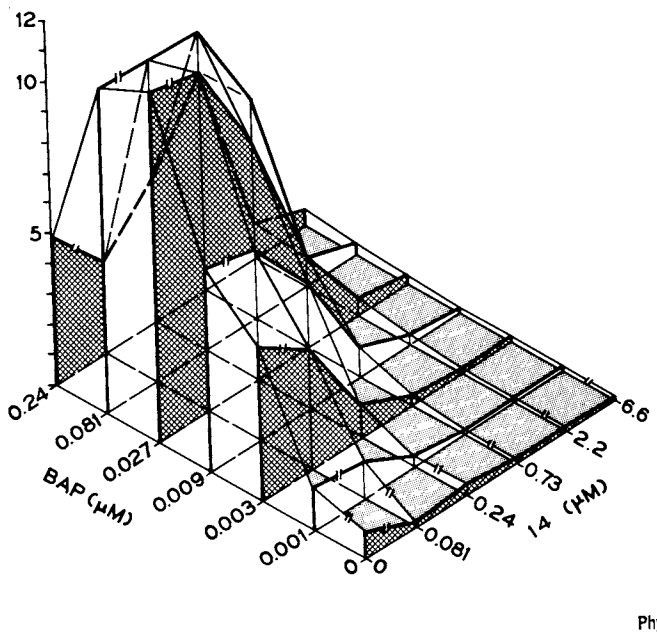


Figure 2. The effect on the fresh weight yield of tobacco callus of 6-benzylaminopurine (2) and 3-methyl-7-(3-methylbutylamino)pyrazolo[4,3-d]pyrimidine (14)

assertion that 14 acts by blocking cytokinin utilization.

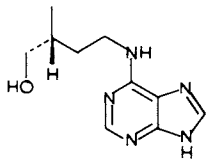
An additional assumption implicit in the preparation of 14 as a potential cytokinin antagonist was that the 7-substituent should be about the same size as those in the best cytokinins, to facilitate binding of the antagonist to the cytokinin receptor site(s). The bioassay results obtained for 14 encouraged us to prepare additional 7-substituted pyrazolo[4,3-d]pyrimidines, which permitted us to verify the validity of this assumption. As shown in Table 1, the compounds having 7-substituents with 4-7 carbon atoms were the most active as antagonists, in parallel with structure-activity results observed for N^6-substituted purines having intense cytokinin activity. Those compounds lacking an alkyl substituent at the 7-position were inactive as anticytokinins.

Also investigated as potential anticytokinins were four 7-alkylamino-3-(β-D-ribofuranosyl)pyrazolo[4,3-d]pyrimidines (15-18) (82). Compound 15 was found to be a weak cytokinin, having activity in the tobacco bioassay intermediate between those of compounds 12 and 13. In comparison, compounds 16-18 inhibited the growth of callus cultured on a medium containing compound 3 and the inhibition could be reversed by added cytokinin. However, unlike the results obtained for the 7-substituted 3-methylpyrazolo-[4,3-d]pyrimidines, testing of the analog of 16-18 lacking the 7-substituent (formycin; 7-amino-3-(β-D-ribofuranosyl)pyrazolo[4,3-d]pyrimidine) revealed that this species also inhibited the growth of tobacco tissue and that the inhibition could not be counteracted by added cytokinin.

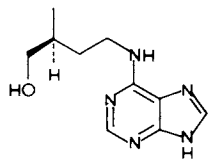
Substituted Pyrrolo[2,3-d]pyrimidines as Potential Anticytokinins

The apparent anticytokinin activity of certain 7-substituted pyrazolo[4,3-d]pyrimidines prompted a search for other structural series of compounds with similar biological activity. The best results to date have been obtained with 2,4-disubstituted pyrrolo-[2,3-d]pyrimidines. Skoog et al (83), for example, reported on the preparation and testing for cytokinin and anticytokinin activity of a number of (2-methylthio-) 4-alkylaminopyrrolo[2,3-d]-pyrimidines. Compounds 19 and 20, lacking a 2-substituent, were found to be weak cytokinins, while a number of related analogs (e.g., 21-24) containing a 2-methylthio group lacked activity as cytokinins in the tobacco bioassay but were found to be potent anticytokinins. The best of these was 4-cyclopentyl-2-methylthio-pyrrolo[2,3-d]pyrimidine (23) which gave detectable inhibition of tobacco callus grown on 3 x 10^{-3} μM 3 when applied to the cultures in three-fold molar excess relative to that cytokinin (i.e., at 9 x 10^{-3} μM concentration). Virtually complete inhibition of cytokinin-promoted growth was achieved using 23 at 2.4 x 10^{-2} μM concentration. Efficient reversal of the inhibition obtained with 23 could be achieved with cytokinin 3 for concentrations of the antagonist as high as 2.2 μM; at any given concentration of 23, increasing amounts of 3 increasingly counteracted growth inhibi-

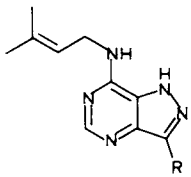
In Plant Growth Substances; Mandava, N.;
ACS Symposium Series; American Chemical Society: Washington, DC, 1979.



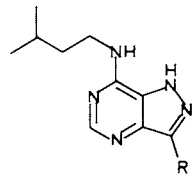
9



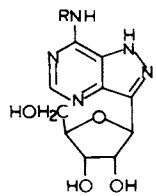
10



11 R=H
12 R=CH_3



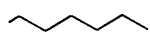
13 R=H
14 R=CH_3



15 R =

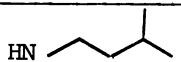
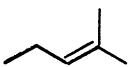
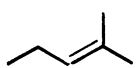
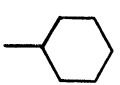
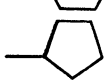
16 R =

17 R =



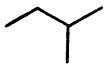
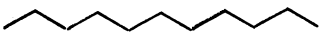
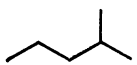
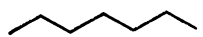
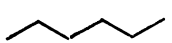
18 R =

Table I. Biological Activity of Substituted 7-Aminopyrazolo[4,3-d]pyrimidines[a] (79)

R"	R'	Range[b] of conc. tested (μM)	Cytokinin activity min. conc. (μM) for Detection	Cytokinin activity min. conc. (μM) for Maximum growth	Growth inhibition, min. conc. (μM) for Detection	Growth inhibition, min. conc. (μM) for Complete inhibition[c]
H		0.009-20	1.0	20	NA	---
H		0.001-20(3)	0.08	1.0	NA	---
CH_3	OH	0.08-20	NA	---	NA	---
CH_3	S	0.24-20	NA	---	NA	---
CH_3		0.08-20(4)	0.24	6.6	NA	---
CH_3		0.24-20	NA	---	NA	---
CH_3		0.73-20	NA	---	NA	---
CH_3		0.73-20	NA	---	6.6	NR

In Plant Growth Substances; Mandava, N.;
ACS Symposium Series; American Chemical Society: Washington, DC, 1979.

Table I (con'd)

CH_3 HN 	0.24-20	2.2	7[d]	6.6	NR
CH_3 HN 	0.73-20	NA	---	2.2	NR
CH_3 HN 	0.24-20	NA	---	3.0	NR
CH_3 HN 	0.03-6.6	NA	---	0.2	2.2
CH_3 HN 	0.03-6.6	NA	---	0.1	0.73
CH_3 HN 	0.009-20(4)	NA	---	0.1	0.73
CH_3 HN 	0.009-20	NA	---	0.03	0.5
CH_3 HN 	0.009-20(3)	NA	---	0.03	0.2

[a]Abbreviations: NA, not active; NR, not reached.

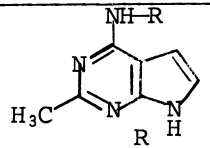
[b]All values averages of two tests except as indicated by numbers in parentheses.

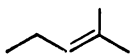
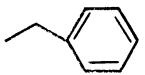
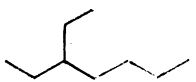
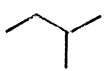
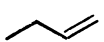
[c]Only slight growth stimulation

[d]In presence of 0.003 μM i^6Ade.

In Plant Growth Substances; Mandava, N.;
ACS Symposium Series; American Chemical Society: Washington, DC, 1979.

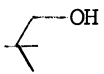
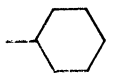
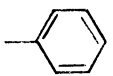
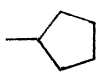
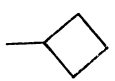
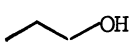
Table II. Effects of 4-Substituted-2-methylpyrrolo[2,3-d]pyrimidines on Tobacco Callus Growth (84)



	Cytokinin activity maximum response (μM)	Anticytokinin activity I_{50} (μM) against 0.05 μM kinetin
	4.0	
	10.0	
	10.0	
	10.0	2.0
		40.0
		12.5
		20.9
		6.0

In Plant Growth Substances; Mandava, N.;
ACS Symposium Series; American Chemical Society: Washington, DC, 1979.

Table II (con'd)

	3.0
	2.0
	0.9
	0.5
	0.3
	0.07
	0.06
	NA[a]

[a]Not active enough to estimate I_{50} value within the concentration range tested (∿ 40 μM).

In Plant Growth Substances; Mandava, N.;
ACS Symposium Series; American Chemical Society: Washington, DC, 1979.

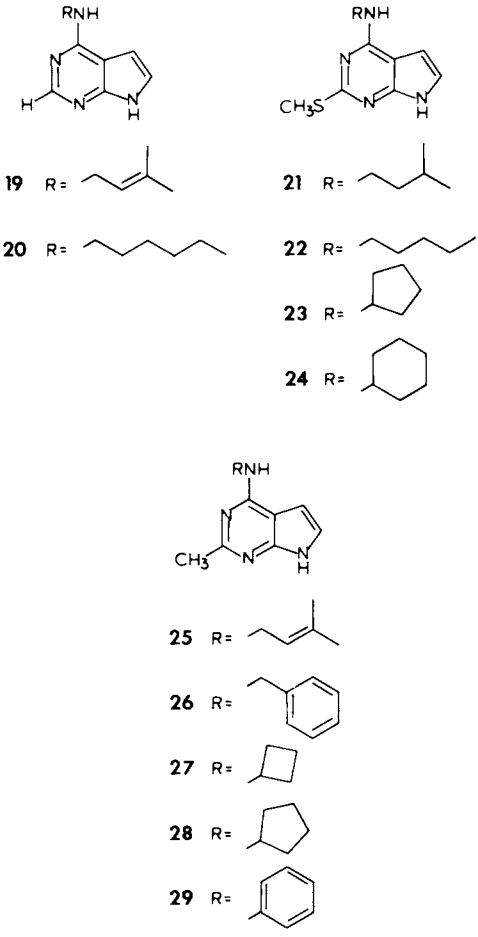
tion. Importantly, growth inhibition (by antagonist 24) was shown not to be reversed by indole-3-acetic acid or by gibberellic acid and the pyrrolo[2,3-d]pyrimidine lacking the 4-alkyl group (4-amino-2-methylthiopyrrolo[2,3-d]pyrimidine) was completely inactive as a cytokinin or anticytokinin.

Recently, Iwamura et al (84) have prepared a number of 4-alkylamino-2-methylpyrrolo[2,3-d]pyrimidines and studied their activities in three bioassay systems. As was true for each of the structural series of potential antagonists studied previously, some of the analogs (e.g. 25 and 26) had weak cytokinin activity in the tobacco callus bioassay while others (e.g. 27-29) inhibited cytokinin-induced growth. The most potent anticytokinins in this assay were 4-cyclobutylamino- and 4-cyclopentylamino-2-methylpyrrolo[2,3-d]pyrimidines (27 and 28), which caused 50% inhibition of the fresh weight yield of tobacco callus grown on 5 x 10^{-2} μM kinetin when utilized at concentrations of 6 and 7 x 10^{-2} μM, respectively (Table 2). The analogs were also tested in the lettuce seed germination assay and for their ability to affect the production of betacyanin in explants of Amaranthus caudatus. Compounds 25 and 26 were the most active of the analogs tested in promoting betacyanin synthesis, although several other compounds (including four that were anticytokinins in the tobacco assay) also had a stimulatory effect in this assay. Interestingly, 4-hydroxyethyl-2-methylpyrrolo[2,3-d]pyrimidine, which had little activity in the tobacco bioassay, was fairly effective in suppressing betacyanin synthesis elicited by compound 3. In the lettuce seed germination assay, compounds 25 and 26 had a strongly stimulatory effect, as did compound 29.

The accumulated assay results obtained with the 4-alkyl-2-methylpyrrolo[2,3-d]pyrimidines are of special interest. Compounds 25 and 26 exhibited significant cytokinin activity in all assays utilized. On the other hand, compound 27 was a potent antagonist in the tobacco bioassay but had little activity in the other two assay systems, while 29 acted as an anticytokinin in the tobacco bioassay and a cytokinin in the lettuce seed germination assay, but was without activity in the Amaranthus test system. This illustrates a principle noted previously (83), namely that there would seem to be distinct cytokinin sites for individual cytokinin-mediated functions, the cytokinin requirements of which (in structural terms) are related but not identical. In a practical sense, it is not unreasonable to anticipate that compounds can be prepared which are capable of promoting or antagonizing single cytokinin-dependent functions.

In addition to the substituted pyrrolo[2,3-d]pyrimidines discussed above, several 4-substituted-7-(β-D-ribofuranosyl)pyrrolo-[2,3-d]pyrimidines were also prepared and tested in the tobacco bioassay (85,86); most of the analogs inhibited the growth of tobacco callus cultured on kinetin. It is not clear from the published data, however, to what extent inhibition could be reversed by higher concentrations of kinetin. In addition, the analog

In Plant Growth Substances; Mandava, N.;
ACS Symposium Series; American Chemical Society: Washington, DC, 1979.


RNH
H
N
N
N
H
19 R=
20 R=
RNH
CH3S
N
N
N
H
21 R=
22 R=
23 R=
24 R=
RNH
CH3
N
N
H
25 R=
26 R=
27 R=
28 R=
29 R=

lacking the 4-substituent (tubercidin) was found to be essentially equally as inhibitory in the test system utilized (see, however, ref. 82), although it may be noted that 4-methylamino-7-(β-D-ribofuranosyl)pyrrolo[2,3-d]pyrimidine was without inhibitory activity.

Anticytokinins Elicit Responses in Several Plant Bioassays

Aside from their activities in the systems used to screen for anticytokinins, the analogs described above have been found to promote certain other physiological responses. For example, antagonist 14 was shown to inhibit the growth of a cytokinin-autonomous strain of tobacco callus in the same fashion as the cytokinin-dependent strain utilized routinely in the tobacco bioassay and the observed inhibition was also reversed by added cytokinin. Although without significant effect on seed germination, the same compound inhibited root initiation and development in both wheat and radish seedlings, as well as Coleus cuttings. It should be noted that similar effects were also obtained with compounds 2 and 3 (79).

Cytokinins are known to retard senescence and it was of special interest to determine the ability of the antagonists to counteract this property. Tomato seedlings grown on a nutrient medium containing 14 (12-120 μM) underwent severe wilting; while young tomato and tobacco plants were generally unaffected by spraying with solutions of 14, there was one instance in which some yellowing of lower leaves (typical of senescence) was observed (79). Also, while intact sweetcorn plants were not affected by the antagonist (R. M. Bock, personal communication), excised discs from the mature leaves of such plants occasionally exhibited enhanced senescence when placed in contact with a solution of 14 (79). Compounds 1 and 3 are known to decrease ethylene production in avocado and apple slices and the antagonist was found to oppose this effect of the cytokinins in apple slices (M. Lieberman, personal communication).

As discussed above, the potential anticytokinins prepared for study may be regarded as structural analogs of cytokinins, a concept reinforced by the weak cytokinin activity of many of the synthesized compounds. Not surprisingly, some of the compounds found to oppose the action of the cytokinins in the promotion of cell division and growth in the tobacco bioassay were actually found to reinforce the cytokinins in other senses. For example, compounds 23 and 24 enhanced cytokinin-promoted budding of tobacco callus in the presence of compound 3 and two weak cytokinins in the 4-alkyl-2-methylpyrrolo[2,3-d]pyrimidine series gave analogous results in the absence of any other exogenous cytokinin (84).

In Plant Growth Substances; Mandava, N.;
ACS Symposium Series; American Chemical Society: Washington, DC, 1979.

Literature Cited

1. Hall, R. H. Prog. Nucl. Acid Res. Mol. Biol., 1970, 10, 57.
2. Skoog, F.; Armstrong, D. J. Annu. Rev. Plant Physiol., 1970, 21, 359.
3. Leonard, N. J. Recent Adv. Phytochem., 1974, 7, 21.
4. Wiesner, J. "Die Elementar Structur und Wachsthum der lebenden Substanz"; Holder: Vienna, 1892; p. 102 ff.
5. Miller, C. O.; Skoog, F.; von Saltza, M. H.; Strong, F. M. J. Am. Chem. Soc., 1955, 77, 1392.
6. Miller, C. O.; Skoog, F.; Okumura, F. S.; von Saltza, M. H. Strong, F. M. J. Am. Chem. Soc., 1955, 77, 2662.
7. Miller, C. O.; Skoog, F.; Okumura, F. S.; von Saltza, M. H.; Strong, F. M. J. Am. Chem. Soc., 1956, 78, 1375.
8. Leonard, N. J.; Henderson, T. R. J. Am. Chem. Soc., 1975, 97, 4990.
9. Scopes, D. I. C.; Zarnack, U.; Leonard, N. J.; Schmitz, R. Y.; Skoog, F. Phytochemistry, 1976, 15, 1523.
10. Strong, F. M. "Topics in Microbial Chemistry"; John Wiley: New York, 1958; Chapter III.
11. Okumura, F. S.; Enishi, N.; Itoh, H.; Masumura, M.; Kuraishi, S. Bull. Chem. Soc. Japan, 1959, 32, 886.
12. Kuraishi, S. Sci. Paper Coll. Gen. Educ., Univ. Tokyo, 1959, 9, 67.
13. Skoog, F.; Hamzi, H. Q.; Szweykowska, A. M.; Leonard, N. J.; Carraway, K. L.; Fujii, T.; Helgeson, J. P.; Loeppky, R. N. Phytochemistry, 1967, 6, 1169.
14. Skinner, C. G.; Shive, W. J. Am. Chem. Soc., 1955, 77, 6692.
15. Daly, J. W.; Christensen, B. E. J. Org. Chem., 1956, 21, 177.
16. Bullock, M. W.; Hand, J. J. J. Org. Chem., 1957, 22, 568.
17. Leonard, N. J.; Fujii, T. Proc. Natl. Acad. Sci. USA, 1964, 51, 73.
18. Klämbt, D.; Thies, G.; Skoog, F. Proc. Natl. Acad. Sci. USA, 1966, 56, 52.
19. Helgeson, J. P.; Leonard, N. J. Proc. Natl. Acad. Sci. USA, 1966, 56, 60.
20. Upper, C. D.; Helgeson, J. P.; Kemp, J. D.; Schmidt, C. J. Plant Physiol., 1970, 45, 543.
21. Scarbrough, E.; Armstrong, D. J.; Skoog, F.; Frihart, C. R.; Leonard, N. J. Proc. Natl. Acad. Sci. USA, 1973, 70, 3825.
22. Armstrong, D. J.; Scarbrough, E.; Skoog, F.; Cole, D. L.; Leonard, N. J. Plant Physiol., 1976, 58, 749.
23. Miller, C. O. Proc. Natl. Acad. Sci. USA, 1961, 47, 170.
24. Letham, D. S.; Shannon, J. S.; McDonald, I. R. Proc. Chem. Soc., 1964, 230.
25. Letham, D. S.; Miller, C. O. Plant Cell Physiol. (Tokyo), 1965, 6, 355.
26. Witham, F. H.; Miller, C. O. Physiol. Plantarum, 1965, 18, 1007.
27. Letham, D. S. Phytochemistry, 1966, 5, 269.

In Plant Growth Substances; Mandava, N.;
ACS Symposium Series; American Chemical Society: Washington, DC, 1979.

28. Miller, C. O. Science, 1967, 157, 1055.
29. Koshimizu, K.; Kusaki, T.; Mitsui, T.; Matsubara, S. Tetrahedron Lett., 1967, 1317.
30. Koshimizu, K.; Matsubara, S.; Kusaki, T.; Mitsui, T. Agr. Biol. Chem. (Tokyo), 1967, 31, 795.
31. Miller, C. O. Proc. Natl. Acad. Sci. USA, 1964, 54, 1052.
32. Letham, D. S. Life Sci., 1966, 5, 551.
33. Letham, D. S. Phytochemistry, 1973, 12, 2445.
34. Parker, C. W.; Letham, D.; Cowley, D; MacLeod, J. Biochem. Biophys. Res. Commun., 1972, 49, 460.
35. Yoshida, R.; Oritani, T. Plant Cell Physiol., 1972, 13, 337.
36. Deleuze, G.; McChesney, J. D.; Fox, J. E. Biochem. Biophys. Res. Commun. 1972, 48, 1426.
37. Fox, J. E.; Cornette, J.; Deleuze, G.; Dyson, W.; Giersak, C.; Niu, P.; Zapata, J.; McChesney, J. D. Plant Physiol., 1973, 52, 627.
38. Parker, C. W.; Wilson, M. M.; Letham, D. S.; Cowley, D. E.; MacLeod, J. K. Biochem. Biophys. Res. Commun., 1973, 55, 1370.
39. Zachau, H. G.; Dütting, D.; Feldmann, H. Angew Chem., 1966, 78, 392.
40. Biemann, K.; Tsunakawa, S.; Sonnenbichler, J.; Feldmann, H.; Dütting, D.; Zachau, H. G. Angew Chem., 1966, 78, 600.
41. Leonard, N. J.; Achmatowicz, S.; Loeppky, R. N.; Carraway, K. L.; Grimm, W. A. H.; Szweykowska, A.; Hamzi, H. Q.; Skoog, F. Proc. Natl. Acad. Sci. USA, 1966, 56, 709.
42. Hall, R. H.; Robins, M. J.; Stasiuk, L.; Thedford, R. J. Am. Chem. Soc., 1966, 88, 2614.
43. Hall, R. H.; Csonka, L.; David, H.; McLennan, B. Science, 1967, 156, 69.
44. Robins, M. J.; Hall, R. H.; Thedford, R. Biochemistry, 1967, 6, 1837.
45. Burrows, W. J.; Armstrong, D. J.; Skoog, F.; Hecht, S. M.; Boyle, J. T. A.; Leonard, N. J.; Occolowitz, J. Science, 1968, 161, 691.
46. Goodman, H. M.; Abelson, J.; Landy, A.; Brenner, S.; Smith, J. D. Nature, 1968, 217, 1019.
47. Harrada, F.; Gross, H. J.; Kimura, F.; Chang, S. H.; Nishimura, S.; RajBhandary, U. L. Biochem. Biophys. Res. Commun., 1968, 33, 299.
48. Hecht, S. M.; Leonard, N. J.; Burrows, W. J.; Armstrong, D. J.; Skoog, F.; Occolowitz, J. Science, 1969, 166, 1272.
49. Vreman, H. J.; Skoog, F.; Frihart, C. R.; Leonard, N. J. Plant Physiol., 1972, 49, 848.
50. Vreman, H. J.; Schmitz, R. Y.; Skoog, F.; Playtis, A. J.; Frihart, C. R.; Leonard, N. J. Phytochemistry, 1974, 13, 31.
51. Burrows, W. J.; Armstrong, D. J.; Kaminek, M.; Skoog, F.; Bock, R. M.; Hecht, S. M.; Dammann, L. G.; Leonard, N. J.; Occolowitz, J. Biochemistry, 1970, 9, 1867.
52. Swaminathan, S.; Bock, R. M. Biochemistry, 1977, 16, 1355.
53. Gefter, M. L.; Russell, R. L. J. Mol. Biol., 1969, 39, 145.

In Plant Growth Substances; Mandava, N.;
ACS Symposium Series; American Chemical Society: Washington, DC, 1979.

54. Miller, C. O. in Linskens, H. F.; Tracey, M. V., Eds. "Modern Methods of Plant Analysis"; vol. 6, Springer: Berlin, 1963; p. 194.
55. Murashige, T.; Skoog, F. Physiol. Plant, 1962, 15, 473.
56. Linsmaier, E. M.; Skoog, F. Physiol. Plant, 1965, 18, 100.
57. Miller, C. O. Plant Physiol., 1956, 31, 318.
58. Haber, A. H. Physiol. Plant, 1959, 12, 456.
59. Haber, A. H.; Luippold, H. J. Physiol. Plant, 1960, 13, 298.
60. Rothwell, K.; Wright, S. T. C. Proc. Roy. Soc., Ser. B., 1967, 167, 202.
61. van Overbeek, J. Science, 1966, 152, 721.
62. Helgeson, J. P. Science, 1968, 161, 974.
63. Letham, D. S. Bioscience, 1969, 19, 309.
64. Henderson, T. R.; Frihart, C. R.; Leonard, N. J.; Schmitz, R. Y.; Skoog, F. Phytochemistry, 1975, 14, 1687.
65. Skinner, C. G.; Shive, W. Plant Physiol., 1957, 32, 500.
66. Hecht, S. M.; Leonard, N. J.; Schmitz, R. Y.; Skoog, F. Phytochemistry, 1970, 9, 1173.
67. Dammann, L. G.; Leonard, N. J.; Schmitz, R. Y.; Skoog, F. Phytochemistry, 1974, 13, 329.
68. Fawcett, C. H.; Wright, S. T. C. Phytochemistry, 1968, 7, 1719.
69. Hecht, S. M.; Leonard, N. J.; Schmitz, R. Y.; Skoog, F. Phytochemistry, 1970, 9, 1907.
70. Schmitz, R. Y.; Skoog, F.; Playtis, A. J.; Leonard, N. J., Plant Physiol., 1972, 50, 702.
71. Hall, R. H.; Srivastava, B. I. S. Life Sci., 1968, 7, 7.
72. Leonard, N. J.; Hecht, S. M.; Skoog, F.; Schmitz, R. Y. Proc. Natl. Acad. Sci. USA, 1969, 63, 175.
73. Matsubara, S.; Shiojiri, S.; Fujii, T.; Ogawa, N.; Imamura, K.; Yamagishi, K.; Koshimizu, K. Phytochemistry, 1977, 16, 933.
74. Iwamura, H.; Yada, M.; Koshimizu, K.; Matsubara, S. Agr. Biol. Chem., 1978, 42, 1009.
75. Hitchings, G. H. Ann. Rheum. Dis., 1966, 25, 601.
76. Elion, G. B. Ann. Rheum Dis., 1966, 25, 608.
77. Ikehara, M.; Murao, K.; Harada, F.; Nishimura, S. Biochim. Biophys. Acta, 1968, 155, 82.
78. Hecht, S. M.; Bock, R. M.; Schmitz, R. Y.; Skoog, F.; Leonard, N. J. Proc. Natl. Acad. Sci. USA, 1971, 68, 2608.
79. Skoog, F.; Schmitz, R. Y.; Bock, R. M.; Hecht, S. M. Phytochemistry, 1973, 12, 25.
80. Helgeson, J. P.; Haberlach, G. T.; Hecht, S. M."Plant Growth Substances 1973"; Hirokawa Publ. Co., p. 485 ff.
81. McDonald, J. J.; Leonard, N. J.; Schmitz, R. Y.; Skoog, F. Phytochemistry, 1971, 10, 1429.
82. Hecht, S. M.; Frye, R. B.; Werner, D.; Hawrelak, S. D.; Skoog, F.; Schmitz, R. Y. J. Biol. Chem., 1975, 250, 7343.
83. Skoog, F.; Schmitz, R. Y.; Hecht, S. M.; Frye, R. B.; Proc. Natl. Acad. Sci. USA, 1975, 72, 3508.
84. Iwamura, H.; Masuda, N.; Koshimizu, K.; Matsubara, S. Phytochemistry, 1979, 18, 217.

In Plant Growth Substances; Mandava, N.;
ACS Symposium Series; American Chemical Society: Washington, DC, 1979.

85. Iwamura, H.; Ito, T.; Kumazawa, Z.; Ogawa, Y. Biochem Biophys. Res. Commun., 1974, 57, 412.
86. Iwamura, H.; Ito, T.; Kumazawa, Z.; Ogawa, Y. Phytochemistry, 1975, 14, 2317.

RECEIVED June 19, 1979.

Chemical and Biological Aspects of Abscisic Acid

JAN A. D. ZEEVAART

MSU–DOE Plant Research Laboratory, Michigan State University, East Lansing, MI 48824

Historical Background

Among the plant hormones discussed in this Symposium, the growth inhibitor abscisic acid is the most recently discovered one. Work in the 1950s and early 1960s by three groups, working on apparently unrelated problems, ultimately resulted in the isolation and identification of abscisic acid in each case (see reviews 1, 2, 3, 4): (a) The search for an abscission-promoting hormone by Addicott and associates at the University of California at Davis led to the isolation in crystalline form of an active compound, called abscisin II, from young cotton fruits (5, 6) for which in 1965 the correct structural formula was proposed (7). Abscisic acid (ABA) was later proposed as the trivial chemical name for abscisin II (8). (b) Wareing and coworkers at the University of Aberystwyth in Wales were attempting to isolate a dormancy-inducing substance from trees (9); the active chemical, isolated from leaves of Acer pseudoplatanus, turned out to be identical to ABA (10). (c) Rothwell and Wain (11) at Wye College in Kent, U. K., following earlier work by van Steveninck in New Zealand, had as objective the identification of a substance which stimulated flower and fruit drop in yellow lupin. This lupin-abscission factor was identified as ABA in 1966 in three different laboratories (12, 13, 14).

Other evidence suggesting the existence of growth inhibitors in plants came from analyses of acidic plant extracts by bioassay. This work indicated the presence of a zone in chromatograms with growth-inhibitory activity which was designated as inhibitor β. The most active component of inhibitor β was later shown to be ABA (15).

Although ABA was originally discovered as an abscission-accelerating and dormancy-inducing substance, it soon became clear that it has many other physiological effects in plants. When synthetic (+)-ABA became widely available, it was established that ABA is a potent inhibitor in various bioassays and

0-8412-0518-3/79/47-111-099$05.00/0

In Plant Growth Substances; Mandava, N.;
ACS Symposium Series; American Chemical Society: Washington, DC, 1979.

counteracts the effects of growth-promoting hormones (auxin, gibberellin, cytokinin) to which a particular organ or tissue responds (16). However, to what extent endogenous ABA functions as an inhibitor in intact plants is still not clear. More recent work indicates that ABA plays an important role in plants as a stress hormone (17, 18).

Properties of Abscisic Acid

ABA is a carboxylic acid which at pH 3.0 partitions readily into organic solvents such as diethyl ether and ethyl acetate. The molecule has one asymmetric carbon atom at C-1' and exhibits therefore optical activity. The naturally occurring enantiomer is dextrorotatory and has a sinister (S) configuration (Figure 1).

ABA absorbs in the ultraviolet, the maximum varying somewhat with the pH of the solution. The neutral methyl ester has a maximum absorption at 265 nm (E = 20,900) (2).

ABA shows exceptionally high optical activity with extrema at 289 and 246 nm ($[\alpha]289$ nm = +24,000°; $[\alpha]246$ nm = −69,000°). This property has been used to quantitatively measure the amounts of ABA present in purified plant extracts (15; see also Table 1).

In the side chain around C-2 the configuration can be either cis or trans. By convention 2-cis-ABA is simply called ABA (8). The isomer with the trans configuration is called 2-trans-ABA (t-ABA) (Figure 1). In solution light catalyzes the isomerization of the 2-cis double bond to establish a 1:1 ratio of ABA and t-ABA. Consequently, samples should be kept in darkness as much as possible during extraction and purification to avoid anomalous results.

In several growth inhibition assays the unnatural (-)-enantiomer was as active as (+)-ABA. However, (-)-ABA was much less active than (+)-ABA in closing stomata of detached barley leaves (see 3). When assayed in darkness (to avoid photoisomerization) t-ABA was completely inactive (2).

Methods for Detection and Measurement of Abscisic Acid

A variety of methods has been employed to detect and quantify ABA. During the early isolation procedures investigators were guided by measuring abscission-accelerating effects in the cotton explant abscission assay or growth inhibition in the wheat coleoptile or rice seedling assay (Table I). More sensitive bioassays such as inhibition of frond multiplication in Lemna grown under aseptic conditions (24), or stomatal closure in Commelina epidermal strips (25) have been developed more recently. The drawback of all bioassays is that they are laborious and time-consuming. Moreover, identification of ABA in bioassays is only tentative. Many other chemicals, besides

In Plant Growth Substances; Mandava, N.;
ACS Symposium Series; American Chemical Society: Washington, DC, 1979.

Table I. Various Methods Employed for Detection and Measurement of Abscisic Acid

Methods	Detection limits	References
Bioassays:		
Cotton explant abscission	5-10 ng per abscission zone	(5, 6, 19)
Wheat coleoptile growth	10-20 ng/ml	(20, 21, 22)
Rice seedling: growth of second leaf sheath	80 ng/ml	(23)
Lemna growth	0.02-0.03 ng/flask	(24)
Commelina stomatal aperture	0.02-0.1 ng/ml	(25)
Spectropolarimetry	200-500 ng/ml	(15, 26)
Gas chromatography* with flame ionization detector	5-50 ng per injection	(27, 28, 29, 30)
Gas chromatography* with electron capture detector	0.005-0.05 ng per injection	(27, 31, 32, 33)
Combined gas chromatography*-mass spectrometry	10-30 ng per injection	(20, 29, 33, 34)
High-performance liquid chromatography	1-2 ng per injection	(35, 36, 37)
Radioimmunoassay	0.1-0.3 ng per sample	(38, 39)

*ABA methylated or trimethylsilylated

In Plant Growth Substances; Mandava, N.;
ACS Symposium Series; American Chemical Society: Washington, DC, 1979.

ABA, when present in sufficiently high concentrations, can also cause (non-specific) inhibition of growth. Nowadays ABA is therefore preferably detected and measured by one of several methods which make use of certain unique properties of the ABA molecule. Spectropolarimetry is based on the large specific rotation in the ultraviolet (see above). This method is specific for ABA, but not very sensitive (Table I).

For gas chromatography volatile derivatives such as the methyl ester of ABA (27, 29, 30) or trimethylsilylated ABA (20, 28) must be prepared. ABA is a molecule with a high electron affinity so that the methyl ester can be measured with a gas chromatograph equipped with an electron capture detector. Injections of as little as 5 pg of Me-ABA cause a detector response (27). Metabolites of ABA such as phaseic and dihydrophaseic acid can also be measured by this method (33).

The most conclusive method to identify ABA is by combined gas chromatography-mass spectrometry (20, 29, 33). Little et al. (34) monitored the current of a single ion, viz. that of the base peak, m/e 190, in the mass spectrum of Me-ABA, for quantitative determinations of ABA in the cambium of Picea sitchensis. However, few laboratories are able to carry out analyses by this method on a routine basis.

The use of high performance liquid chromatography for measuring ABA has been reported by several workers (Table I). However, in view of the many substances in plant extracts that absorb in the ultraviolet region of the spectrum, this method cannot be considered as conclusive.

A radioimmunoassay which is highly specific for ABA, has been developed recently (38, 39). This method appears very attractive when large numbers of samples have to be analyzed routinely for ABA content.

It is obvious that unequivocal identification of small quantities of ABA can only be accomplished by combined gas chromatography-mass spectrometry. However, once this has been accomplished in a particular system, routine measurements will in the future probably mostly rely on gas chromatography with electron capture detector and on radioimmunoassay.

Occurrence

ABA is ubiquitous in higher plants. It has also been identified in gymnosperms, ferns, horsetails, lycopods and mosses, but not in liverworts (3, 39). In the latter group lunularic acid appears to take the place of ABA as a growth inhibitor (3).

In a recent report ABA was identified as a metabolite of the fungus Cercospora rosicola (40). This observation has been confirmed in our laboratory (Zeevaart, unpublished results). The availability of a microorganism that produces ABA, offers unique opportunities for biosynthetic and genetic studies of

In Plant Growth Substances; Mandava, N.;
ACS Symposium Series; American Chemical Society: Washington, DC, 1979.

ABA formation that cannot be readily conducted with higher plants. Similar studies with the fungus Gibberella fujikuroi, which produces gibberellins, have greatly advanced our knowledge of this group of hormones as reported by Phinney in this Symposium.

ABA has been detected in all organs of higher plants, as well as in phloem and xylem sap (3). However, the concentrations of ABA can greatly vary from organ to organ. Fruits, young seeds and buds usually have a high ABA content. In leaves the ABA content per unit weight is highest in the youngest leaves; it declines as the leaves expand (Figure 2).

Biosynthesis of Abscisic Acid

The incorporation of label from mevalonate into ABA, a sesquiterpenoid, has been demonstrated in different parts of plants (e.g. 41). This indicates that ABA can be synthesized throughout the plant. In addition to the direct incorporation of three isoprene units, derived from mevalonate, into ABA, an indirect biosynthetic pathway via carotenoids has been proposed. This idea stems from the finding that xanthophylls, in particular violaxanthin, can either photochemically or enzymatically be converted to the neutral inhibitor xanthoxin (42) (Figure 3). When labeled xanthoxin was fed in the transpiration stream to bean or tomato shoots, ca. 10% was converted to ABA over an 8-hr period (43). However, the importance of the biosynthetic route to ABA via xanthophylls and xanthoxin in normal metabolism remains to be established, and most of the evidence favors the direct synthesis route via a C_{15} precursor (see 2).

So far, only one in vitro system for ABA biosynthesis has been described (44). In this study chloroplasts isolated from ripening avocado fruits incorporated mevalonate into ABA. Upon lysing the cell-free system in dilute buffer, incorporation of label into ABA increased considerably, indicating that the chloroplast membrane was a major barrier to the penetration of mevalonate. Although these preliminary results demonstrate that ABA can be synthesized within the chloroplast, the possibility that synthesis can also take place outside the chloroplasts, can by no means be ruled out.

Metabolism of Abscisic Acid

ABA is metabolized via the unstable intermediate 6'-hydroxymethyl-ABA, more recently called hydroxyabscisic acid (HOABA) by Hirai et al. (45), to phaseic acid (PA). In certain plants the latter compound is further converted to 4'-dihydrophaseic acid (DPA) which accumulates as the end product. The ABA → PA → DPA pathway (Figure 4) operates in beans (46), in pea seedlings (47), in ash seeds (48), in castor bean (49), and

In Plant Growth Substances; Mandava, N.;
ACS Symposium Series; American Chemical Society: Washington, DC, 1979.

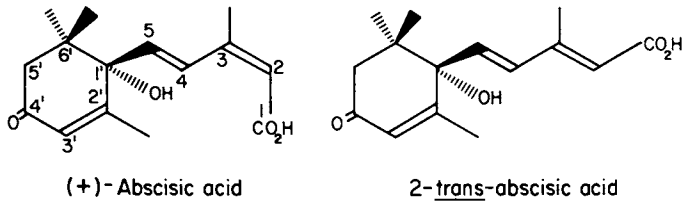


Figure 1. Structures of (+)-abscisic acid (ABA) and of 2-trans-*abscisic acid (t-ABA)*

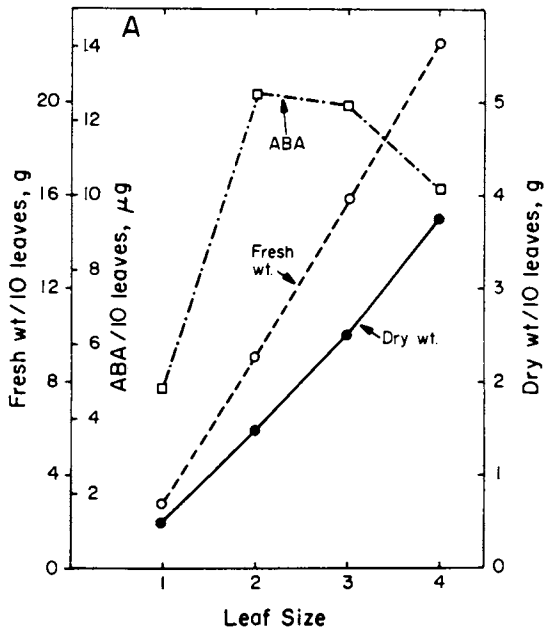
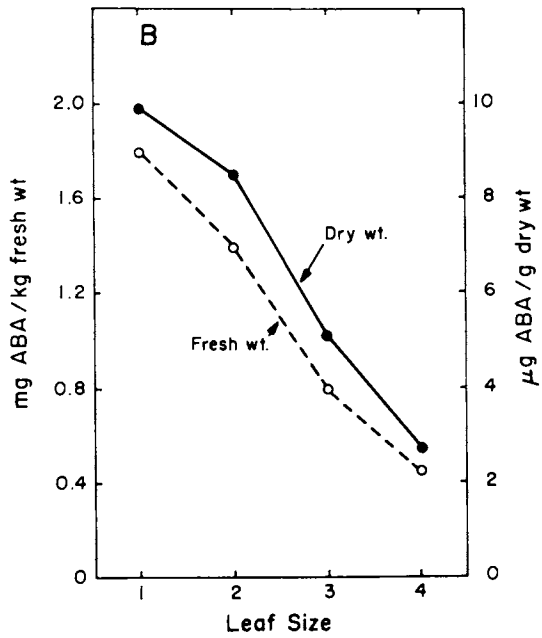


Figure 2A. Change in abscisic acid content of Xanthium *leaves with age. Fresh and dry weight and abscisic acid content of 10 leaves of different ages* (51).



Plant Physiology

Figure 2B. Change in abscisic acid content of Xanthium *leaves with age. Abscisic acid content of leaves of different ages expressed per unit fresh and dry weight* (51)

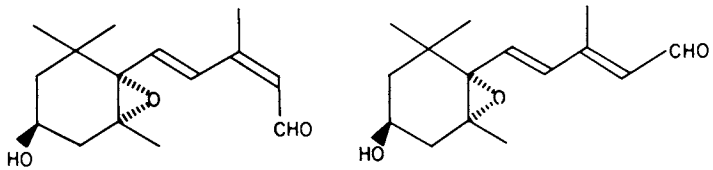


Figure 3. Structures of cis,trans-*xanthoxin and* trans,trans-*xanthoxin*

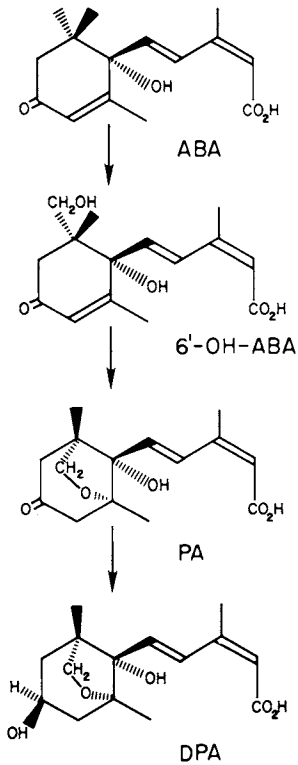


Figure 4. Metabolic pathway of abscisic acid (ABA) via the unstable intermediate 6′-hydroxymethyl-ABA to PA and DPA

In Plant Growth Substances; Mandava, N.;
ACS Symposium Series; American Chemical Society: Washington, DC, 1979.

in the endosperm of immature Echinocystis fruits (50). From this latter material a cell-free system has been prepared which converted ABA to PA and DPA. The enzyme preparation was separated by centrifugation into a particulate fraction with ABA-hydroxylating activity and a soluble PA-reducing activity (50).

Insignificant amounts of DPA were detected in leaves of Xanthium strumarium (51). This raises the possibility that in some plants conversion of PA to DPA is not the principal metabolic route for PA degradation.

When racemic ABA was fed to plants, only the (+)-enantiomer was metabolized to PA and DPA (2, 33). In addition, a conjugate with glucose, abscisyl-β-D-glucopyranoside was formed, identical to the glucose ester of ABA isolated from the fruits of yellow lupin (52). However, the natural glucose ester yielded exclusively (+)-ABA on hydrolysis, whereas the glucose ester produced after feeding of (±)-ABA gave predominantly (-)-ABA (2, 33). Thus, (+)-ABA can be both metabolized to PA and conjugated with glucose, whereas (-)-ABA is only conjugated. Likewise, t-ABA is only conjugated into the glucose ester (2, 53). Another conjugate of ABA, viz. β-hydroxy-β-methylglutarylhydroxyabscisic acid (HMG-HOABA) has recently been isolated from seeds of Robinia pseudoacacia (45).

Physiological Roles of Abscisic Acid

As discussed above, ABA was discovered as a substance that promotes abscission and as a substance associated with the onset of dormancy in woody perennials. Since then it has become clear that ABA is widely distributed in higher plants, but little evidence has accumulated that ABA, as an endogenous hormone, is involved in the regulation of either abscission or dormancy (see 2, 16, 54, 55). ABA is usually classified as an inhibitor that counteracts the effects of growth-promoting substances (16). While this appears to be true for growth substances applied to excised parts, it is not evident that this also pertains to the endogenous hormones in whole plants. For example, despite a very high ABA content (Figure 2), young leaves expand rapidly which argues against a role for ABA as an endogenous inhibitor. It is possible, of course, that in the absence of any ABA young leaves would expand more rapidly than they normally do, but without specific inhibitors for ABA synthesis, or ABA-deficient mutants, this idea cannot be tested at present.

In a recent review (16) of its physiological functions, evidence was presented that ABA plays a role in the geotropic response of roots and in tuber formation. It may have a role in other growth and developmental processes as well, but so far the evidence is inconclusive.

A most interesting development in work on ABA has been the

In Plant Growth Substances; Mandava, N.;
ACS Symposium Series; American Chemical Society: Washington, DC, 1979.

discovery that ABA plays an important role in stomatal closure and thus in reducing the loss of water from plants under stress. Consequently, ABA has been assigned a role as a stress hormone (17).

The Role of Abscisic Acid as a Stress Hormone. The role of ABA in water stress has been studied most extensively, although there is also evidence for involvement of ABA in other stresses such as salinity, mineral deficiency, high osmotic concentration, and water-logging. A review on the role of ABA in stress phenomena has appeared recently (18).

There are two basic observations with respect to the role of ABA in water stress:

(a) Exogenously applied (+)-ABA causes closure of the stomata in many plants, thus reducing transpiration. The response of detached leaves to (+)-ABA supplied via the transpiration stream was very rapid, the stomata of certain species starting to close 3 to 10 minutes after ABA was introduced in the irrigation water (56, 57, 58). Stomatal closure in bean, corn and rose leaves started when the ABA level reached approximately twice the normal endogenous concentration (57), but in Xanthium an increase in the ABA of the whole leaf by only 1-2% was sufficient to cause a stomatal response (58). Upon withdrawal of the (+)-ABA solution, the stomata started to open within 5 minutes (56). Thus, the response of stomata to (+)-ABA is both rapid and reversible. An effect on stomatal aperture can also be observed in isolated epidermal strips that are floating on a buffer solution; this phenomenon has been used as a very sensitive bioassay for ABA (Table I). When ABA was added to the solution, the stomatal aperture started to decline within a few minutes, due to the loss of K^+ ions from the guard cells, resulting in a decreased turgor in these cells (59).

(b) When plants start to wilt, there is a large accumulation of ABA in the leaves and also in other organs such as stems, apices, flowers, fruits, seeds, and roots (e.g. 18, 41, 60). An example of ABA accumulation in detached and wilted Xanthium leaves is given in Figure 5. Following wilting the ABA content increased over a 6-hr period from 200 to 1350 ng ABA per g fresh weight, and then leveled off. A 50% increase was discernable after 30 minutes. In bean seedlings a 1.5-fold increase in ABA has been observed 10 minutes after the onset of stress (61) which was correlated with increased leaf resistance (i.e. decreased stomatal aperture). However, such correlations have not always been found, and Walton et al. (62) have suggested that it is not the total ABA content that counts in determining stomatal aperture, but rather the rate of ABA synthesis.

Drought-induced ABA accumulation is common in mesophytes; it is less pronounced in hygrophytes, particularly in submerged leaves (18, 41). The rapid increase in ABA following stress occurs presumably by de novo synthesis, and not through release

In Plant Growth Substances; Mandava, N.;
ACS Symposium Series; American Chemical Society: Washington, DC, 1979.

from a conjugated form of ABA (26, 41).

A number of workers (63, 64, 65) have suggested that there is a critical threshold leaf water potential in the -10 to -12 bar range at which the ABA levels of leaves start to increase. However, more recent evidence indicates that zero turgor is the critical parameter at which the ABA content starts to increase (66, 67).

Following recovery from water stress the ABA levels of leaves decline rapidly (e.g. 61, 63, 68). This is illustrated in Figure 6 for Xanthium leaves. After detached leaves had been in the wilted state for 5 hours, the ABA content had increased to 3000 ng per g fresh weight. Upon submerging such wilted leaves in distilled water for 5 minutes to regain turgor, a significant drop in the ABA level was observed after 2 hours and after 4 hours it had essentially returned to the prestress value. This indicates that rapid ABA synthesis ceases as soon as turgor is regained while rapid ABA degradation continues until the excess ABA has been removed. This decrease in ABA is accompanied by a transient increase in PA as demonstrated in leaves of grapevine (69) and Xanthium (Zeevaart, unpublished results). Thus, following a period of drought rewatering will result in a rapid disappearance of the excess ABA. However, the stomata do not open for several more days and photosynthesis remains reduced (70). This so-called aftereffect of moisture stress was thought to be due to inhibition of photosynthesis by the accumulated PA (69), but this hypothesis has been disproven recently (see next section).

Research on ABA has practical implications for Agriculture since water is a limiting factor for crop production in many areas of the world. On an experimental scale ABA and certain derivatives have been applied to crop plants as "antitranspirants" (71, 72). In short-term experiments transpiration was considerably reduced without much effect on the rate of photosynthesis. Thus, applied ABA increased the water-use efficiency of plants.

Another development which may be useful is the finding that a drought-tolerant corn variety produced more ABA upon wilting than did two sensitive cultivars (73). The enhanced ability to accumulate ABA might be used as a marker for breeding drought-tolerant plants.

Roles of Metabolites of Abscisic Acid. Nothing is known about the physiological role of PA and DPA in plants, although these two metabolites of ABA have been tested in several bioassays recently. In the cotton explant abscission assay PA had one-tenth of the activity of ABA (19). PA and DPA were equally effective in inhibiting α-amylase secretion by barley aleurone layers treated with gibberellin A_3; DPA had approximately one-tenth of the activity of ABA in this system (74). The effect of PA on growth of bean embryos was negligible (75).

In Plant Growth Substances; Mandava, N.;
ACS Symposium Series; American Chemical Society: Washington, DC, 1979.

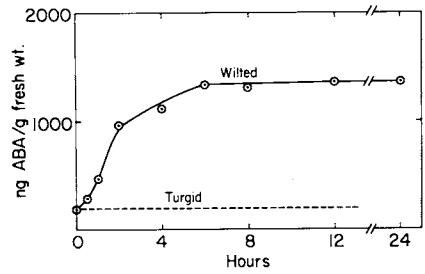


Figure 5. Accumulation of abscisic acid in detached mature leaves of Xanthium strumarium *after the fresh weight had been reduced by 10%*

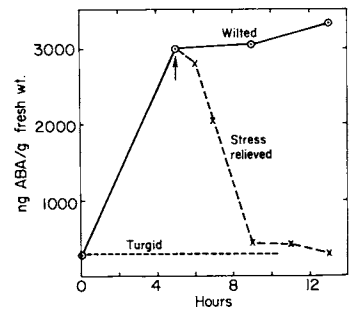


Figure 6. Decrease in abscisic acid content of wilted leaves of Xanthium *after stress was relieved. Detached leaves were wilted by reducing the fresh weight by 10%. Stress was relieved after 5 hr by submerging leaves into distilled water for 5 min.*

In Plant Growth Substances; Mandava, N.;
ACS Symposium Series; American Chemical Society: Washington, DC, 1979.

DPA did not cause stomatal closure in any of the species tested, while the effect of PA ranged from a response as rapid as that caused by ABA in Commelina, to a less rapid closure than after ABA treatment in Amaranthus, Hordeum, Xanthium, and Zea, to no response at all in Vicia (76).

Kriedemann et al. (69) have proposed that endogenous PA functions as an inhibitor of photosynthesis following relief of water stress when this metabolite accumulates. This proposal was based on the observation that plant extracts containing PA strongly inhibited photosynthesis in detached leaves of several species (69). However, subsequent work with crystallized PA has shown that it was not PA that inhibited photosynthesis, but rather impurities that were present in the solvents used (76). Thus, the physiological role of PA, if any, remains to be determined.

Concluding Remarks

Although much has been learned about the chemistry and physiology of ABA since its discovery in 1965, many unsolved problems remain. Degradation of ABA has been fairly well worked out, but biosynthesis is still poorly understood. Of particular importance would be to discover the sensing mechanism for stress-induced ABA accumulation. If turgor is the crucial factor, the plasma membrane might be involved, since at zero turgor this organelle is no longer pressed against the cell wall. On the other hand a certain amount of ABA is always produced in fully turgid cells, thus suggesting that there may be two different mechanisms for ABA synthesis: one that operates in turgid cells, and another one which becomes activated only in cells under stress conditions.

One further problem is the large overshoot in ABA production in wilted leaves. With applied ABA a doubling of the ABA content of the leaf is usually adequate for stomatal closure, while increases up to 40-fold have been reported in wilted leaves. However, extractions of whole leaves do not take into account the location of ABA within the leaf. Perhaps much of the hormone is sequestered in a compartment that has no access to the guard cells. Thus, it would be of much importance to determine the distribution of ABA at the tissue level as well as its intracellular location. Since ABA is a small water-soluble molecule, conventional fractionation techniques may not be suitable to determine its distribution in various organelles. A highly specific immunological method for detection of ABA has recently been developed (38, 39). It is conceivable that this technique could be further developed for determining the cellular localization of ABA as has already been done for the photoreceptor phytochrome (77, 78).

Acknowledgement - My research reported in this paper was supported by the U. S. Department of Energy under Contract EY-76-C-02-1338 and by grant no. PCM-7807653 from the National Science Foundation.

In Plant Growth Substances; Mandava, N.;
ACS Symposium Series; American Chemical Society: Washington, DC, 1979.

Literature Cited

1. Addicott, F. T.; Lyon, J. L. Ann. Rev. Plant Physiol. 1969, 20, 139-164.
2. Milborrow, B. V. Ann. Rev. Plant Physiol. 1974, 25, 259-307.
3. Milborrow, B. V. In: Phytohormones and Related Compounds: A Comprehensive Treatise. Vol. I. Eds. Letham, D. S.; Goodwin, P. B.; Higgins, T. J. V. Elsevier, Amsterdam, 1978. pp. 295-347.
4. Wareing, P. F.; Ryback, G. Endeavour 1970, 29, 84-88.
5. Addicott, F. T.; Carns, H. R.; Lyon, J. L.; Smith, O. E.; McMeans, J. L. Colloq. Intern. Centre Nat. Rech. Sci. Paris 1964, 123, 687-703.
6. Ohkuma, K.; Lyon, J. L.; Addicott, F. T.; Smith, O. E. Science 1963, 142, 1592-1593.
7. Ohkuma, K.; Addicott, F. T.; Smith, O. E.; Thiessen, W. E. Tetrahedron Lett. 1965, 29, 2529-2535.
8. Addicott, F. T.; Lyon, J. L.; Ohkuma, K.; Thiessen, W. E.; Carns, H. R.; Smith, O. E.; Cornforth, J. W.; Milborrow, B. V.; Ryback, G.; Wareing, P. F. Science 1968, 159, 149.
9. Wareing, P. F.; Eagles, C. F.; Robinson, P. M. Colloq. Intern. Centre Nat. Rech. Sci. Paris 1964, 123, 377-386.
10. Cornforth, J. W.; Milborrow, B. V.; Ryback, G.; Wareing, P. F. Nature 1965, 205, 1269-1270.
11. Rothwell, K.; Wain, R. L. Colloq. Intern. Centre Nat. Rech. Sci. Paris 1964, 123, 363-375.
12. Cornforth, J. W.; Milborrow, B. V.; Ryback, G.; Rothwell, K.; Wain, R. L. Nature 1966, 211, 742-743.
13. Koshimizu, K.; Fukui, H.; Mitsui, T.; Ogawa, Y. Agr. Biol. Chem. 1966, 30, 941-943.
14. Porter, N. G.; Van Steveninck, R. F. M. Life Sci. 1966, 5, 2301-2308.
15. Milborrow, B. V. Planta 1967, 76, 93-113.
16. Wareing, P. F. Phil. Trans. R. Soc. Lond. B 1978, 284, 483-498.
17. Jones, H. G. Nature 1978, 271, 610.
18. Wright, S. T. C. In: Phytohormones and Related Compounds: A Comprehensive Treatise. Vol. II. Eds. Letham, D. S.; Goodwin, P. B.; Higgins, T. J. V. Elsevier, Amsterdam, 1978. pp. 495-536.
19. Davis, L. A.; Lyon, J. L.; Addicott, F. T. Planta 1972, 102, 294-301.
20. Most, B. H.; Gaskin, P.; MacMillan, J. Planta 1970, 92, 41-49.
21. Wright, S. T. C. Planta 1969, 86, 10-20.
22. Zeevaart, J. A. D. Plant Physiol. 1971, 48, 86-90.
23. Koshimizu, K.; Fukui, H.; Mitsui, T.; Ogawa, Y. Agr. Biol.

In Plant Growth Substances; Mandava, N.;
ACS Symposium Series; American Chemical Society: Washington, DC, 1979.

Chem. 1966, 30, 941-948.
24. Tillberg, D. Physiol. Plant. 1975, 34, 192-195.
25. Ogunkanmi, A. B.; Tucker, D. J.; Mansfield, T. S. New Phytol. 1973, 72, 277-282.
26. Milborrow, B. V. J. Expt. Bot. 1978, 29, 1059-1066.
27. Andersson, B.; Häggström, N.; Andersson, K. J. Chromat. 1978, 157, 303-310.
28. Davis, L. A.; Heinz, D. E.; Addicott, F. T. Plant Physiol. 1968, 43, 1389-1394.
29. Gaskin, P.; MacMillan, J. Phytochemistry 1968, 7, 1699-1701.
30. Lenton, J. R.; Perry, V. M.; Saunders, P. F. Planta 1971, 96, 271-280.
31. Ciha, A. J.; Brenner, M. L.; Brun, W. A. Plant Physiol. 1977, 59, 821-826.
32. Seeley, S. D.; Powell, L. E. Anal. Biochem. 1970, 35, 530-533.
33. Zeevaart, J. A. D.; Milborrow, B. V. Phytochemistry 1976, 15, 493-500.
34. Little, C. H. A.; Heald, J. K.; Browning, G. Planta 1978, 139, 133-138.
35. Düring, H. Experientia 1977, 33, 1666-1667.
36. Durley, R. C.; Kannangara, T.; Simpson, G. M. Can. J. Bot. 1978, 56, 157-161.
37. Sweetser, P. B.; Vatvars, A. Anal. Biochem. 1976, 71, 68-78.
38. Walton, D.; Galson, E. Plant Physiol. (Suppl.) 1977, 59, 77.
39. Weiler, E. W. Planta 1979, 144, 255-263.
40. Assante, G.; Merlini, L.; Nasini, G. Experientia 1977, 33, 1556-1557.
41. Milborrow, B. V.; Robinson, D. R. J. Expt. Bot. 1973, 24, 537-548.
42. Taylor, H. F.; Burden, R. S. Proc. Roy. Soc. Lond. B 1972, 180, 317-346.
43. Taylor, H. F.; Burden, R. S. J. Expt. Bot. 1973, 24, 873-880.
44. Milborrow, B. V. Phytochemistry 1974, 13, 131-136.
45. Hirai, N.; Fukui, H.; Koshimizu, K. Phytochemistry 1978, 17, 1625-1627.
46. Harrison, M. A.; Walton, D. C. Plant Physiol. 1975, 56, 250-254.
47. Tietz, D.; Dörffling, K. Mitt. Inst. Allg. Bot. Hamburg 1978, 16, 101-114.
48. Sondheimer, E.; Galson, E. C.; Tinelli, E.; Walton, D. C. Plant Physiol. 1974, 54, 803-808.
49. Zeevaart, J. A. D. Plant Physiol. 1977, 57, 788-791.
50. Gillard, D. F.; Walton, D. C. Plant Physiol. 1976, 58, 790-795.
51. Raschke, K.; Zeevaart, J. A. D. Plant Physiol. 1976, 58,

In Plant Growth Substances; Mandava, N.;
ACS Symposium Series; American Chemical Society: Washington, DC, 1979.

169-174.
52. Koshimizu, K.; Inui, M.; Fukui, H.; Mitsui, T. Agr. Biol. Chem. 1968, 32, 788-791.
53. Powell, L. E.; Seeley, S. D. J. Amer. Soc. Hort. Sci. 1974, 99, 439-441.
54. Alvim, R.; Saunders, P. F.; Barros, R. S. Plant Physiol. 1979, 63, 774-777.
55. Porter, N. G. Physiol. Plant. 1977, 40, 50-54.
56. Cummins, W. R.; Kende, H.; Raschke, K. Planta 1971, 99, 347-351.
57. Kriedemann, P. E.; Loveys, B. R.; Fuller, G. L.; Leopold, A. C. Plant Physiol. 1972, 49, 842-847.
58. Raschke, K. Planta 1975, 125, 243-259.
59. Wilson, J. A.; Ogunkanmi, A. B.; Mansfield, T. A. Plant, Cell and Environment 1978, 1, 199-201.
60. Walton, D. C.; Harrison, M. A.; Cotê, P. Planta 1976, 131, 141-144.
61. Hiron, R. W. P.; Wright, S. T. C. J. Expt. Bot. 1973, 24, 769-781.
62. Walton, D. C.; Galson, E.; Harrison, M. A. Planta 1977, 133, 145-148.
63. Beardsell, M. F.; Cohen, D. Plant Physiol. 1975, 56, 207-212.
64. Wright, S. T. C. Planta 1977, 134, 183-189.
65. Zabadal, T. J. Plant Physiol. 1974, 53, 125-127.
66. Davies, F. S.; Lakso, A. N. J. Amer. Soc. Hort. Sci. 1978, 103, 310-313.
67. Pierce, M.; Raschke, K. Plant Physiol. (Suppl.) 1978, 61, 25.
68. Loveys, B. R.; Kriedemann, P. E. Physiol. Plant. 1973, 28, 476-479.
69. Kriedemann, P. E.; Loveys, B. R.; Downton, W. J. S. Aust. J. Plant Physiol. 1975, 2, 553-567.
70. Mansfield, T. A.; Wellburn, A. R.; Moreira, T. J. S. Phil. Trans. R. Soc. Lond. B 1978, 284, 471-482.
71. Jones, R. J.; Mansfield, R. A. Physiol. Plant. 1972, 26, 321-327.
72. Mizrahi, Y.; Scherings, S. G.; Malis Arad, S.; Richmond, A. E. Physiol. Plant. 1974, 31, 44-50.
73. Larqué-Saavedra, A.; Wain, R. L. Nature 1974, 251, 716-717.
74. Dashek, W. V.; Singh, B. N.; Walton, D. C. Plant Physiol. (Suppl.) 1977, 59, 76.
75. Walton, D. C.; Sondheimer, E. Plant Physiol. 1972, 49, 285-289.
76. Sharkey, T.; Raschke, K. Plant Physiol. 1979, in press.
77. Pratt, L. H.; Coleman, R. A. Proc. Nat. Acad. Sci. USA 1971, 68, 2431-2435.
78. Coleman, R. A.; Pratt, L. H. Planta 1974, 121, 119-131.

RECEIVED June 19, 1979.

6

Role of Ethylene in Plant Growth, Development, and Senescence

MORRIS LIEBERMAN

Post Harvest Plant Physiology Laboratory, Beltsville Agricultural Research Center, USDA, Beltsville, MD 20705

The simplest unsaturated carbon compound, ethylene, exerts a major influence on many if not all aspects of plant growth and development. Although ethylene is a gas at physiological temperatures and pressures, it is now recognized as a plant hormone because it is a natural product of metabolism, acts in trace amounts and is neither a substrate nor cofactor in reactions which are associated with major developmental plant processes. Whether or not ethylene meets all the standard criteria established for hormones, there is no question that this gas is a powerful natural regulating substance in plant metabolism, and that it acts and interacts with other recognized plant hormones. With the advent of gas chromatography, ethylene has become the simplest plant hormone to assay since it is evolved from the tissues and requires no extraction or purification prior to analysis.

An important advance in understanding ethylene action was realized with the rediscovery that auxin influences ethylene biosynthesis in juvenile tissues (1,2,3). These studies have led to an appreciation of the general nature of the hormonal action of ethylene, an action which extends beyond fruit ripening and senescence (its classical role) to seed germination (4), seedling growth (5), root growth (6), stress phenomena (7) and other physiological processes (8) that may be considered to be under hormonal control. Ethylene is therefore an important component in the mix of hormones that control plant metabolism.

Ethylene Biosynthesis

Methionine is the major precursor in the biochemical pathway to ethylene (9). Ethylene is formed from carbons 3 and 4 of methionine which is degraded in reactions possibly involving free radicals and oxygen (9). Recently Adams and Yang (10,11) identified S-adenosylmethionine (SAM) and 1-aminocyclopropane-1-carboxylic acid (ACC) as intermediates in the pathway from methionine to ethylene. The sequence of reactions in the pathway

In Plant Growth Substances; Mandava, N.;
ACS Symposium Series; American Chemical Society: Washington, DC, 1979.

from methionine to ethylene, including intermediates, is shown in Fig. 1.

Recognition of SAM as an intermediate indicates that ATP is involved in ethylene biosynthesis. ATP may thus provide a locus for regulatory control. Inhibitors are known for every step in the pathway from methionine to ethylene. The reaction from methionine to SAM is inhibited by L-2-amino-4-hexynoic acid (AHA) or L-2-amino-4-trans-hexenoic acid and related methionine analogues (13), and the step from SAM to ACC is inhibited by aminoethoxy vinylglycine (AVG) and canaline (12,14). The final step from ACC to ethylene probably involves oxygen, perhaps in a free-radical chain reaction, because it is inhibited by anaerobiosis as well as a number of antioxidant radical-quenching agents, such as n-propyl gallate and 3,4,5-trichlorophenol.

Some of the enzymes involved in this reaction pathway have been identified. The enzyme of the first step in the reaction pathway, SAM synthetase, is a known enzyme in plant tissues (15). The enzyme converting SAM to ACC has been isolated from tomato tissues (14) and appears to be a pyridoxal phosphate-mediated enzyme. However, the enzyme converting ACC to ethylene has not been isolated as yet, although indications are that it reacts with oxygen by a complex mechanism, perhaps to form free-radical intermediates.

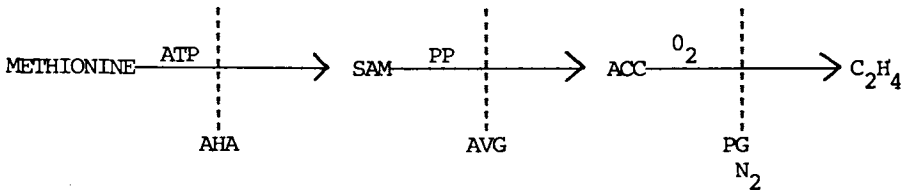
Adams and Yang (10) have suggested that the S atom of methionine is recycled in the ethylene reaction pathway, as shown in Fig. 2. In this scheme, 5'-methylthioadenosine, the residual molecule which derives from the reaction converting SAM to ACC, is further metabolized to 5'-methylthioribose, which then transfers the S-methyl group to homoserine to form methionine. This scheme is hypothetical, and the enzymes necessary for all these reactions have not as yet been demonstrated.

Knowledge of the complete reaction pathway for ethylene production and the characteristics of the enzymes systems involved, should shed light on the control and regulation of ethylene production and perhaps also its relationship to other hormones.

Ethylene Action

Action of ethylene in ripening and senescence. Historically the action of ethylene is associated with ripening fruit. Ethylene production in mature fruit coincides with the onset of the ripening process and the climacteric rise in respiration. Exogenously applied ethylene can induce mature unripe fruit to ripen and senesce as they would naturally, but at an accelerated rate. Ethylene was therefore considered a ripening or aging hormone associated especially with senescent fruit metabolism. However other plant hormones also play a role in fruit ripening and senescence. For example cytokinins can suppress ethylene production in slices of ripe avocado fruit suggesting an interaction

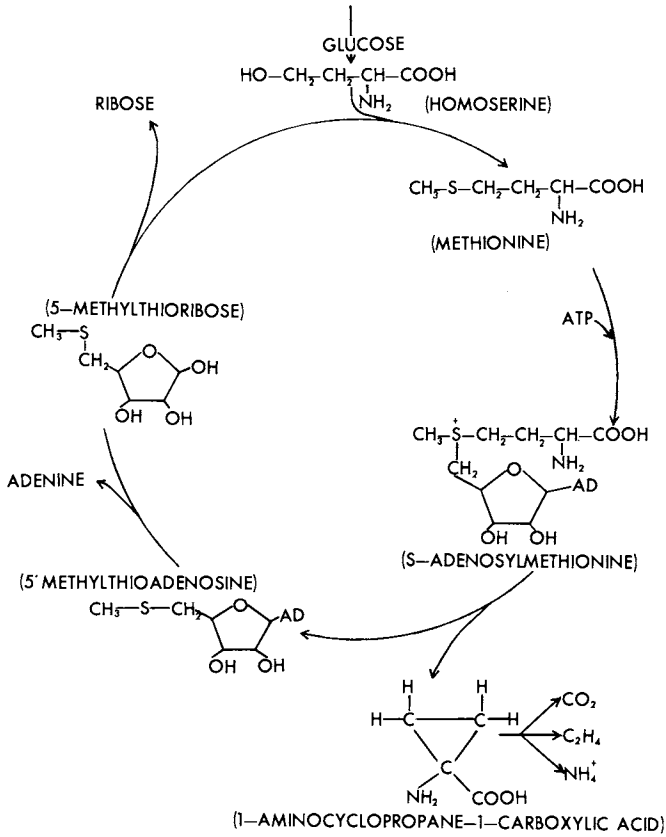
In Plant Growth Substances; Mandava, N.;
ACS Symposium Series; American Chemical Society: Washington, DC, 1979.



AHA= L-2-Amino-4-hexynoic acid
PP= Pyridoxal phosphate
AVG= Aminoethoxy vinylglycine
PG= Propyl gallate

Figure 1. Reactions from methionine to ethylene showing intermediates and inhibitors of each step in the pathway and the possible direct conversion of methionine to ethylene

In Plant Growth Substances; Mandava, N.;
ACS Symposium Series; American Chemical Society: Washington, DC, 1979.



Plant Physiology

Figure 2. Proposed pathway from methionine to ethylene indicating recycling of the S atom according to Adams and Yang (10)

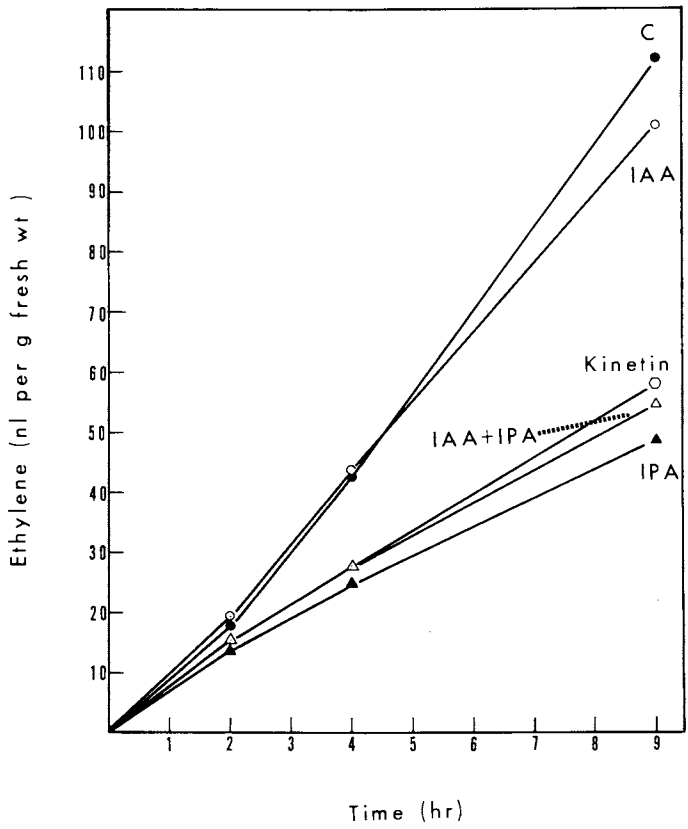
of cytokinins with the ethylene-forming system (Fig. 3) (16).

The production and action of ethylene are, however, not confined to mature fruit and senescent metabolism. Ethylene also influences many facets of plant growth and development. The influence of ethylene on growth of young tissues is observed vividly in its effect on etiolated pea seedlings to cause the well-known triple response--stunting, subapical swelling and diageotropism--which involves all aspects of growth. Evidence of the triple response to exogenously applied ethylene suggest interactions of the gas with the total spectrum of plant hormones, and it is these interactions that require elucidation.

Ethylene and auxin. The inter-relationship between auxin and ethylene was suggested by the discovery that supraoptimal levels of auxin (10^{-5}-10^{-3}M) stimulated ethylene production in a number of plant tissues (17,18,19). Auxin-induced ethylene production in subapical stem sections of pea seedlings occurs after a lag period of about 1 to 3 hours, requires continuous presence of auxin and is inhibited by inhibitors of RNA and protein synthesis (Fig. 4) (20,21). These data suggest that the ethylene-forming system is induced by high levels of auxin and may involve RNA to protein synthesis. In some tissues auxin can activate ethylene production in 15 minutes or less (22), which is too short a time span for protein synthesis. Pea root tips also appear to have an ethylene-forming system which can respond to low levels of IAA (1 μM) without a lag period, and this system is not inhibited by cycloheximide (23). However, higher levels of IAA-induced ethylene production by pea root tips (10-100 μM) involves a lag period, is inhibited by cycloheximide and probably requires protein synthesis.

The induction of ethylene in plant tissues by supraoptimal concentrations of auxin is well established, but the reverse effect, that is, the influence of ethylene on auxin concentration is less well known. There are reports which indicate that levels of exogenous ethylene (10-36 ppm) cause significant reductions in endogenous levels of IAA (Table I) (24,25,26). These influences of auxin on ethylene production and ethylene on auxin levels suggest feedback relationships between these hormones which regulate the levels of auxins and ethylene to cause specific growth phenomena (27) as, for example, the regulation of cell shape and size (28). Goldwin and Wain (29) showed that auxin-induced ethylene production was related exponentially to growth, and is a consequence of cellular growth processes induced by auxin. Thirty-four compounds which were considered to be analogues of auxin were tested for their ability to induce ethylene production. Only those compounds which promoted considerable extension growth were effective inducers. This finding suggests a relation between growth rate and ethylene production. Perhaps via a feedback mechanism ethylene serves to slow down excessive growth in plants excessively stimulated by high con-

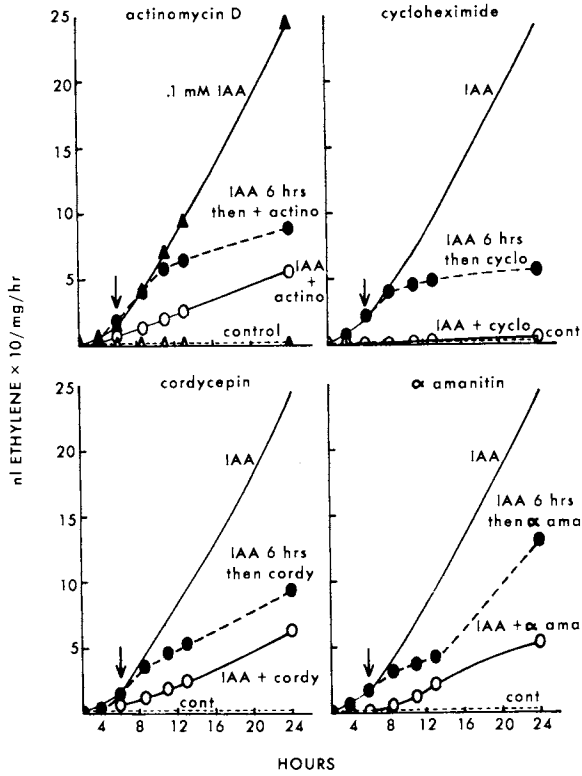
In Plant Growth Substances; Mandava, N.;
ACS Symposium Series; American Chemical Society: Washington, DC, 1979.



Plant Physiology

Figure 3. Influence of IAA and cytokinins on ethylene production by post-climacteric avocado tissue slices (16)

In Plant Growth Substances; Mandava, N.;
ACS Symposium Series; American Chemical Society: Washington, DC, 1979.



Plant Physiology

Figure 4. Effect of inhibitors of RNA and protein synthesis on IAA-induced ethylene production in subhook sections of etiolated pea seedlings. Arrows indicate time inhibitors were applied (20).

In Plant Growth Substances; Mandava, N.;
ACS Symposium Series; American Chemical Society: Washington, DC, 1979.

centrations of growth substances. The ultimate size and shape of cells, tissues and organs of a plant may represent the result of interaction of ethylene, as the modulating or braking regulator, with the mix of growth-accelerating hormones, such as auxins, gibberellins and cytokinins.

Ethylene and GA. Scott and Leopold (30) noted the opposing actions of GA_3 and ethylene in the lettuce hypocotyl elongation assay for GA_3, in the α-amylase induction assay for GA, and in induction of invertase in sugar beet tissue. The opposing actions of GA_3 and ethylene in the subhook region of etiolated pea seedlings were also observed. This region, where cell elongation occurs most rapidly, elongates abnormally when treated GA_3 (10^{-5}M) but thickens and does not elongate when treated with 1 ppm ethylene. Pretreatment of seedlings with GA_3 before treatment with ethylene prevents the stunting and swelling of the sub-apical stem tissue (31). Figure 5 shows the appearance of cells in the subapical region of pea seedlings treated with GA_3, ethylene, and a combination of GA_3 and ethylene (Fig. 5). Treatment with GA_3 elongated the cells excessively, and treatment with ethylene caused the development of isodiametric swollen cells. When treated with both GA_3 and ethylene, the cells were very similar to control cells in size and shape. These data illustrate the interactions between GA_3 and ethylene in determining size and shape of cells.

Cytokinins and ethylene. Cytokinins can synergize IAA-induced ethylene production (32) in etiolated pea seedlings, probably by increasing the concentration of free IAA via both suppression of IAA conjugation and enhancement of IAA uptake (33). However, the influence of cytokinins on ethylene production cannot be solely related to preserving free IAA, because kinetin (10^{-4}M) is much more effective in stimulating ethylene production in very young pea seedlings (2-day old) than is IAA (10^{-4}M) (32). Imaseki et al. (34) also noted that cytokinin can influence ethylene production in the presence of IAA by some metabolic process unrelated to maintaining the level of free IAA. Although cytokinins appear to enhance ethylene production in seedlings and excised segments of seedling, especially in conjunction with auxin, they tend to suppress ethylene production in climacteric and postclimacteric apple and avocado fruits (16). This tendency may relate to the known action of cytokinins in suppressing loss of chlorophyll and senescence in aging leaves (35).

Ethylene and ABA. Abscisic acid (ABA), like ethylene, inhibits growth of etiolated seedlings; but the seedlings do not show the triple response characteristic of ethylene-treated seedlings (36). ABA-inhibited pea seedlings produce less ethylene than seedlings not treated with ABA in response to high

In Plant Growth Substances; Mandava, N.;
ACS Symposium Series; American Chemical Society: Washington, DC, 1979.

levels of IAA (10^{-4}M) (37). Apparently, ABA and ethylene inhibit growth and interact with auxins by different mechanisms. However, ABA stimulates ethylene production in mature fruit (16,37) and appears to act like ethylene in hastening senescence. There appears to be a close relationship between ABA and ethylene in inducing and accelerating the aging process in plants.

Ethylene as a stimulator of growth and development. The most observed actions of ethylene on growing plants involves growth inhibition, or acceleration of senescence. These actions are especially evident in the antagonism or opposition of ethylene to auxins, gibberellins and cytokinins (27), as already outlined above. Actually ethylene stimulates growth in many types of cells, especially in water plants (Table II). When ethylene acts to stimulate cell elongation, as in water plants, auxins and CO_2 enhance the ethylene effect (38,39). This interaction is the reverse of that observed on land plants wherein ethylene opposes the effects of auxin, GA_3 and cytokinins.

Considering ethylene a growth inhibitor may be inaccurate in light of the natural physiological role of this gas in growth, development and senescence. Whether or not ethylene acts to inhibit growth depends on its concentration level, the stage of growth of the tissues on which it acts, and the type of cells and tissues to which it is applied. The tomato mutant diageotropica, which is characterized by horizontal growth of shoots and roots, assumes a normal growth habit when subjected to very low concentrations of ethylene, in the order of 5 ppb, or high levels of auxin (10^{-4}M) (40,41). These studies suggest that the morphological development of this mutant may be controlled by very low levels of endogenous ethylene production resulting from an auxin-ethylene feedback mechanism.

The inhibition of growth by ethylene in subhook regions of etiolated pea seedlings is largely due to reduction in the polar auxin transport system which supplies auxin to the cells (42, 43). In the presence of ethylene the subhook region of the pea stem does not grow in length but does continue to grow in diameter, and there is little difference in increase in fresh weight between the control and ethylene-treated seedlings in the first 24 hr. During the next 2 days (72 hr after continuous treatment with ethylene), the increase in fresh weight is greater for the ethylene treatment than for the control in spite of the noticeable difference in elongation of the subhook.

Ethylene at concentrations of 1 ppm and higher inhibited root elongation in tomato, peas and rice. However, stimulation of root elongation was obtained with less than 0.02 ppm in tomatoes, less than 0.15 ppm in peas and less than 1 ppm in rice (44). Extension of roots in all three species could be increased by ethylene, but different concentrations were required for each species. These data allow speculation that dynamic low levels of ethylene may be required for normal growth and

In Plant Growth Substances; Mandava, N.;
ACS Symposium Series; American Chemical Society: Washington, DC, 1979.

Table I. IAA Content of Epicotyls from Control and Ethylene-Treated (24 hr) Etiolated Pea Seedlings

Seedlings	IAA ng/seedling	Percent Difference
'Alaska'		
Control	6.5 ± 0.7	--
Ethylene-treated (10-20 ppm)	2.7 ± 0.3	-58.5
Sweet Eminent		
Control	1.4 ± 0.2	--
Ethylene-treated (19-36 ppm)	0.7 ± 0.1	-50.0

Table II. Effect of Ethylene and Other Hormones on Growth of Land and Water Plants

Plant or Tissue	Auxin	GA	Ethylene
Land Plants			
Pea Seedling Epicotyls	+	++	--
Pea Seedling Roots	+	+	--
Water Plants			
Rice Seedlings	+	+	+
Ranunculus Acleratus	+	+	+

In Plant Growth Substances; Mandava, N.;
ACS Symposium Series; American Chemical Society: Washington, DC, 1979.

development of roots.

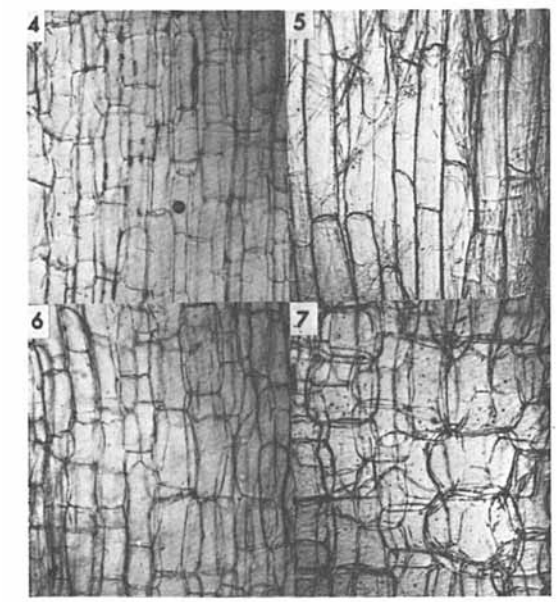
Wound or stress ethylene production. The significance of ethylene in plant metabolism may be indicated by the changes in ethylene production that occur when tissue is wounded or placed under stress. Tissue which normally evolve little or no ethylene show a surge in ethylene production 3 to 10 times the basal level upon physical wounding (45), bruising (46), freezing (47), irradiation (48), attack by microorganisms (49) and other stresses. The profile of the surge in ethylene production, after a short lag, is shown in Fig. 6 and suggests a dampened oscillation, perhaps related to a negative feedback system in the tissues (50,51).

Plants under water stress are known to produce increased amounts of ethylene, show a rise in ABA and a decline in endogenous cytokinins (52,53). Other plant hormones are also probably involved in the response to water stress and other stress and wounding actions. The surge of ethylene production upon stress may therefore represent a response to a disturbance of the hormonal balance in tissues. The dampened oscillation curve for wound ethylene production may reflect the dynamic return of the disturbed hormonal system to a proper hormonal balance under the new tissue conditions, and thus may also reflect a healing phenomenon.

Ethylene receptors and regulatory control. The mode of action of ethylene at the molecular level is unknown. Some attempts, however, have been made to determine the receptor sites for ethylene (54) as well as their characteristics (55). There appears to be very little incorporation of ethylene applied to tissues (only about 0.05%). The ^{14}C ethylene incorporated into pea seedling tissues which responded physiologically to the gas was metabolized to $^{14}CO_2$ and water-soluble ^{14}C metabolites (55). Metabolism of the incorporated ^{14}C ethylene by pea seedlings and other tissues was inhibited by high levels of CO_2 (7-10%) and Ag^+ ions (10-500 ppm) (56). Ag^+ ions prevented the incorporation of ^{14}C ethylene into water-soluble tissue metabolites and counteracted the physiological effects of ethylene in retarding epicotyl growth in pea seedlings, abscission in cotton and senescence of orchids (57). Ag^+ ions had little effect on the metabolism of ^{14}C ethylene to $^{14}CO_2$. On the other hand, high levels of CO_2 (7-10%) inhibited the oxidation of ^{14}C ethylene to $^{14}CO_2$ without affecting incorporation of $^{14}C_2H_4$ into water-soluble labeled compounds in the same tissue. These results suggest two sites at which ethylene may be metabolized. One site is blocked by Ag^+ ions and the other by CO_2. High levels of ethylene could overcome the inhibition due to Ag^+ ions and CO_2.

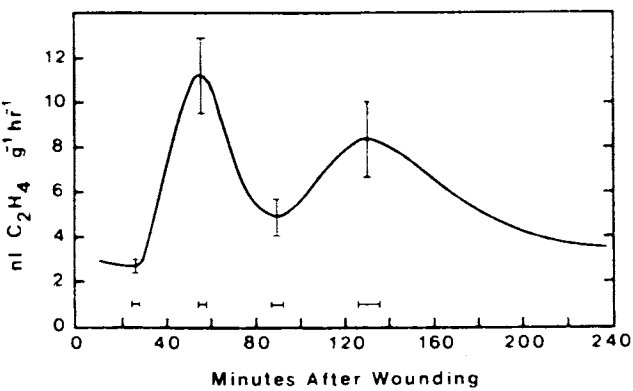
The influence of Ag^+ and CO_2 on ethylene action was observed in senescing tobacco leaf disks in which loss of chlorophyll was taken as an index of senescence (Fig. 7).

In Plant Growth Substances; Mandava, N.;
ACS Symposium Series; American Chemical Society: Washington, DC, 1979.



Plant Physiology

Figure 5. Subapical cells in cortical region of etiolated pea seedlings held for 24 hr in water exposed to air (water–air) (4), GA–air (5), GA–ethylene (6), and ethylene (7) from Stewart et al. (31)



Plant Physiology

Figure 6. Profile of ethylene production by wounded stems of etiolated pea seedlings. The profile shows kinetic changes suggesting dampened oscillation (from Saltveit and Dilley (50))

In Plant Growth Substances; Mandava, N.;
ACS Symposium Series; American Chemical Society: Washington, DC, 1979.

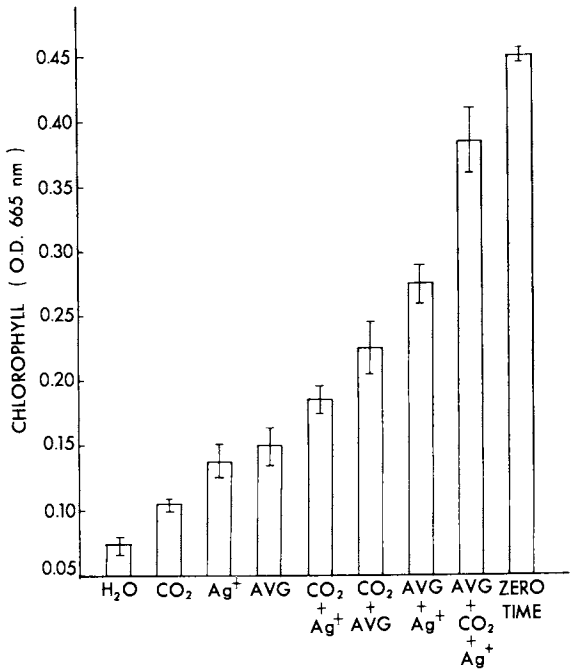


Figure 7. Influence of Ag^+ (10 ppm), CO_2 (10%), and AVG (0.1mM) on chlorophyll retention in aging tobacco leaf disks senesing in the dark for six days

In Plant Growth Substances; Mandava, N.;
ACS Symposium Series; American Chemical Society: Washington, DC, 1979.

Carbon dioxide and Ag^+ ions clearly suppressed senescence, as determined by chlorophyll loss. Aminoethoxy vinylglycine (AVG), the inhibitor of ethylene biosynthesis, also significantly suppressed senescence, as determined by preservation of chlorophyll in the leaf disks aging in the dark. Combinations of CO_2, Ag^+ ions and AVG were especially effective on preserving chlorophyll, presumably by suppressing both ethylene biosynthesis and action at the two receptor sites. After 6 days' aging at 25° in the dark, the controls contained only 7% of the chlorophyll present at the start, whereas 84% of the chlorophyll was retained by the leaf disks treated with a combination of CO_2, Ag^+ and AVG.

Leaf disks which had been treated with Ag^+ (10 ppm) and CO_2 (10%) to prevent ethylene action, actually showed considerable increase in ethylene production (Fig. 8). In the absence of auxin or cytokinins, which increase ethylene production, ethylene biosynthesis increased 2.5 times in the presence of 5-15% CO_2 and pretreatment with 10 ppm of Ag^+ ions. On the addition of IAA or IAA and kinetin to Ag^+ and CO_2 - treated leaf disks, ethylene production increased 8 to 9 times. This observation suggests the possibility that the biosynthesis of ethylene depends on its utilization, perhaps through some negative feedback signal. Presumably the binding of ethylene to its receptor or the metabolism of ethylene tends to dampen its biosynthesis. Auxin and combinations of auxin and cytokinins considerably augment ethylene production in Ag^+ and CO_2-treated leaves. Once again this points out the interaction between auxins, cytokinins and ethylene. Production of ethylene appears to be dependent on auxin and also on the degree to which ethylene is metabolized.

Localization of the ethylene forming system. The ethylene-forming system in plants has never been isolated *in vitro* because it does not survive destruction of the cell. In recent studies protoplasts prepared from apple tissue did not produce ethylene. The loss of ethylene-producing ability by tissue slices incubated in cell wall-digesting enzymes during preparation of protoplasts is shown in Fig. 9. Methionine, the precursor of ethylene, delayed the loss of ethylene production somewhat during preparation of the protoplasts. Ethylene production was restored to some extent when the protoplasts were cultured for 3 or more days (Fig. 10) (58). Restoration of ethylene producing ability by culturing was correlated with regeneration of some cell-wall material, as shown by incorporation of myoinositol in the ethanol-insoluble fraction of the protoplasts and by increased fluorescence with calcafluor-white (58). Regeneration of cell-wall material was correlated with ethylene production in response to methionine added to the cultured protoplasts. Production of ethylene by these cultured protoplasts was not only dependent on addition of

In Plant Growth Substances; Mandava, N.;
ACS Symposium Series; American Chemical Society: Washington, DC, 1979.

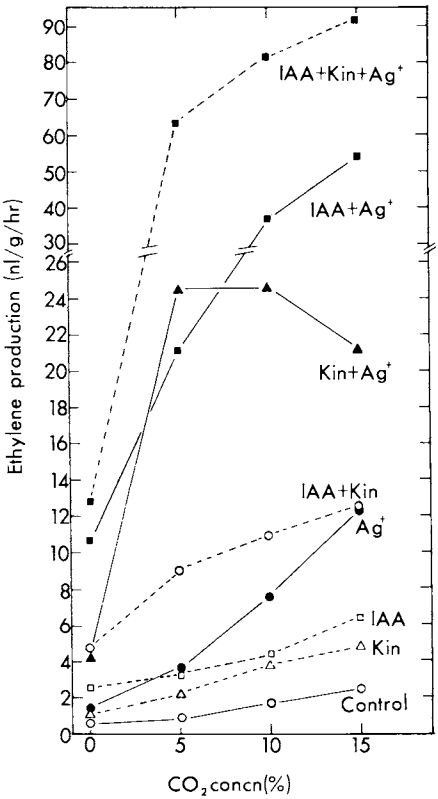
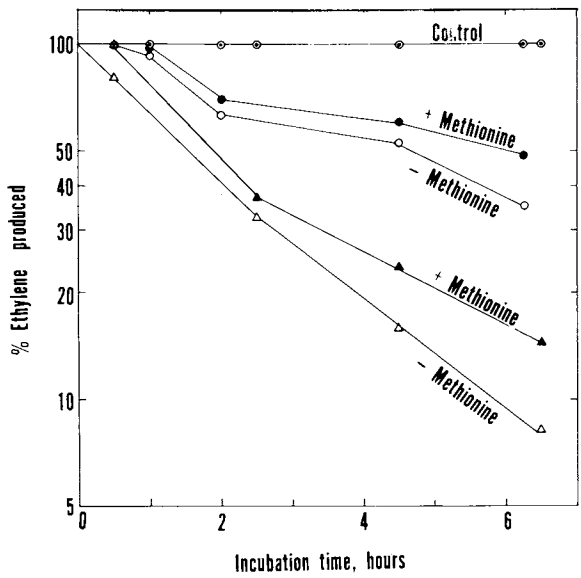


Figure 8. Ethylene production of aging tobacco disks in the presence of Ag^+, CO_2, IAA, and kinetin in various concentrations

In Plant Growth Substances; Mandava, N.;
ACS Symposium Series; American Chemical Society: Washington, DC, 1979.



Plant Physiology

Figure 9. Loss of ethylene-producing ability in apple slices treated with a mixture of cell-wall-digesting enzymes in presence and absence of methionine (58): (○, ●), preclimacteric; (△, ▲), climacteric.

In Plant Growth Substances; Mandava, N.;
ACS Symposium Series; American Chemical Society: Washington, DC, 1979.

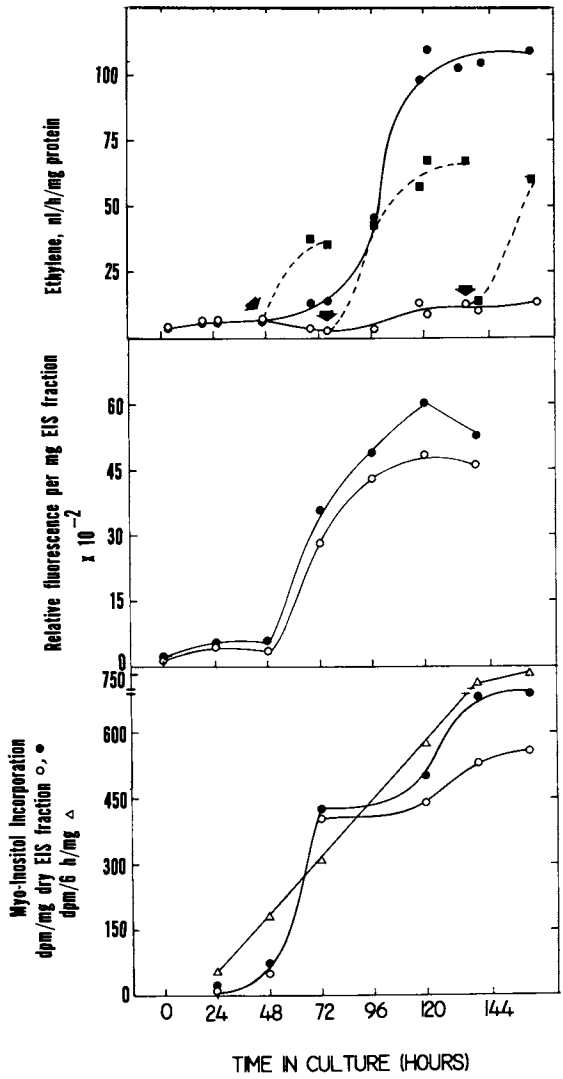


Figure 10. Evidence for restoring ethylene-producing ability in cultured protoplasts, as correlated with myoinositol incorporation and increased fluorescence of EIS components of cultured protoplasts (58)

methionine but was also inhibited by AVG and n-propyl gallate, the inhibitors of ethylene biosynthesis.

A more recently used technique of isolating protoplasts from apple fruit is less drastic and involves the use of cellulysin. The protoplasts so isolated appear to have their plasma membrane intact and produce ethylene from methionine and 1-amino-cyclopropane-1-carboxylic acid (ACC), without prior culturing. These protoplasts are very sensitive to osmotic shock and suggest the importance of intact membrane structure for ethylene biosynthesis. Once the membrane system is damaged, the ethylene-synthesizing capability is lost. It therefore appears that the system for ethylene biosynthesis, and perhaps also the receptor sites for ethylene, is localized in membranes, possibly the plasma membrane. This could account for a well-integrated highly structured system linking production to action and to other hormonal systems, whose receptor sites have also been associated with membranes (59).

Conclusions

Advances in ethylene biochemistry and physiology have preceded along a number of fronts. Firstly the biosynthetic pathway from methionine to ethylene has been further clarified and intermediates identified. Secondly some progress has been made in recognizing two possible receptor sites which are inhibited by Ag^{+} ions and CO_2, respectively. Thirdly the localization of ethylene production has been shown to be associated with membranes in studies with protoplasts.

There also have been clear indications that interactions of ethylene with auxins, cytokinins, gibberellins and ABA are involved in both ethylene production and action. Generally the effects of ethylene tend to antagonize those of auxins, cytokinins and gibberellins, and tend to reinforce those of ABA, depending, however, on tissue systems involved. Reinforcement of ethylene by ABA and vice versa occurs more frequently in senescence.

Although ethylene has been recognized mostly as an inhibitor of growth, especially of young tissues, there are examples that it acts in conjunction with auxins and gibberellins to enhance growth, especially in water plants. Some other experiments suggest that ethylene regulates morphogenesis at extremely low concentrations, in the ppb range, which may in fact be the natural physiological concentrations in many tissues. At higher concentrations, in the ppm range, ethylene appears to be inhibitory. Because ethylene can either inhibit or stimulate growth and development, depending on the tissue type (18) and concentration, I suggest that ethylene modulates the action of the so-called growth hormones--auxins, gibberellins and cytokinins. Conversely auxins, gibberellins and cytokinins appear to influence ethylene production and action. These

In Plant Growth Substances; Mandava, N.;
ACS Symposium Series; American Chemical Society: Washington, DC, 1979.

observations, which are for the most part only at the descriptive level of sophistication, indicate that an understanding of integrated interactions between all hormones at the molecular level is necessary if the growth and development of crops is to be fully controlled.

Literature Cited

1. Morgan, P.W.; Hall, W.C. Plant Physiol., 1962, 15, 420.
2. Abeles, F.B. Plant Physiol.,1966, 41, 585.
3. Burg, S.P.; Burg, E.A. Proc. Natl. Acad. Sci. USA, 1966, 55, 262.
4. Ketring, D.L.; Morgan, P.W. Plant Physiol.,1972, 50, 382.
5. Burg, S.P.; Burg, E.A. Plant Physiol.,1968, 43, 1069.
6. Chadwick, A.V.; Burg, S.P. Plant Physiol., 1970, 45, 192
7. Yang, S.F.; Pratt, H.K. In Biochemistry of Wounded Plant Storage Tissues, ed G. Kahl, 1978, de Gruyter, Berlin. In press.
8. Osborne, D.J. Sci. Prog., 1977, 64, 5[illegible].
9. Lieberman, M. Ann. Rev. Plant Physiol., 1979, 30, 533.
10. Adams, D.O.; Yang, S.F. Plant Physiol., 1977, 60, 892.
11. Adams, D.O.; Yang, S.F. Proc. Natl. Acad. Sci. U.S.A. 1979, 76, 170.
12. Rahiala, E.L.; Kekomaki, M.; Janne, J.; Raina, A.; Raiha, N.C.R. Biochim. Biophys. Acta 1971, 227, 337.
13. Lombardini, J.B.; Coulter, A.W.; Talalay, P. Molec. Pharm., 1970, 6, 481.
14. Boller, T.; Herner, R.C.; Kende, H. Planta, 1979 (in press).
15. Mudd, S.H.; Biochim. Biophys. Acta., 1960, 38, 354.
16. Lieberman, M.; Baker, J.E.; Sloger, M. Plant Physiol., 1977, 60, 214.
17. Zimmerman, P.W.; Wilcoxon, F. Contrib. Boyce Thompson Inst. 1935, 7, 209.
18. Morgan, P.W.; Hall, W.C.; Nature, 1964, 201, 91.
19. Abeles, F.B.Ann. Rev. Plant Physiol., 1972, 23, 259.
20. Lieberman, M.; Kunishi, A.T.; Plant Physiol., 1975, 55, 1074.
21. Kang, B.G.; Newcomb, W.; Burg, S.P. Plant Physiol., 1971, 47, 504.
22. Franklin, D.; Morgan, P.W.; Plant Physiol., 1978, 62, 161.
23. Steen, D.A.; Chadwick, A.V. Plant Physiol., 1973, 52, 171.
24. Lieberman, M.; Knegt, E. Plant Physiol., 1977, 60, 475
25. Michener, H.D.; Amer. J. Bot., 1938, 25, 711.
26. Valdovinos, J.G.; Ernest, L.C.; Henry, E.W. Plant Physiol., 1967, 42, 1803.
27. Lieberman, M.; Kunishi, A.T. In Plant Growth Substances, 1970, ed. D.J. Carr, 1972, Springer-Verlag, Berlin, New York, 549.
28. Osborne, D.J., in Perspectives in Experimental Biology, ed N. Sutherland, 1976, 2: 89, Pergamon, Oxford.
29. Goldwin, G.K.; Wain, R.L. Ann. Appl. Biol. 1973, 75, 71.
30. Scott, P.C.; Leopold, A.C. Plant Physiol., 1967, 42, 1021.

In Plant Growth Substances; Mandava, N.;
ACS Symposium Series; American Chemical Society: Washington, DC, 1979.

31. Stewart, R.N.; Lieberman, M.; Kunishi, A.T. Plant Physiol. 1974, 54, 1.
32. Fuchs, Y.; Lieberman, M. Plant Physiol., 1968, 43, 2029
33. Lau, O.L.; Yang, S.F. Plant Physiol., 1973, 51, 1011
34. Imaseki, H.; Kondo, K.; Watanabe, A. Plant Cell Physiol., 1975, 16, 777.
35. Richmond, A.E.; Lang, A. Science, 1957, 125, 650.
36. Lieberman, M.; Kunishi, A.T. Plant Physiol. Suppl. 1971, 47 22.
37. Gertman, E.; and Fuchs, Y. Plant Physiol. 1972, 50, 194.
38. Imaseki, H.; Pjon, C.J. Plant and Cell Physiol., 1970, 11, 827.
39. Ku, H.S.; Suge, H.; Rappaport, L; Pratt, H.K. Planta 1970, 90, 333.
40. Zobel, R.W.; Plant Physiol. 1973, 52, 385.
41. Zobel, R.W.; Roberts, L.W. Can. J. Bot., 1974, 32, 735.
42. Apelbaum, A.; Burg, S.P. Plant Physiol., 1972, 50, 125.
43. Morgan, P.W.; Beyer, E.; Gausman, H.E. In Biochemistry and Physiology of Plant Growth Substances ed. F. Wightman and G. Setterfield, 1968, Runge, Ottawa, 1255-73.
44. Konings, H.; Jackson, M.B. Ann. Rep. Agric. Res. Council, Letcomb Lab., 1974, pp 23-24.
45. Meigh, D.F.; Norris, K.H.; Craft, C.C.; Lieberman, M. Nature, 1960, 186, 902.
46. Hanson, A.D.; Kende, H. Plant Physiol., 1976, 57, 538.
47. Elstner, E.F.; Konze, J.R. Nature, 1976, 263, 351.
48. Abdel-Kader, A.S.; Morris, L.M.; Maxie, E.C.Proc. Am. Soc. Hortic. Sci., 1968, 92, 553.
49. Imaseki, H.; Uritani, I; Stahmann, M.A. Plant Cell Physiol., 1968, 9, 757.
50. Saltveit, M.E.; Dilley, D.R. Plant Physiol., 1978, 61, 447.
51. Saltveit, M.E.; Dilley, D.R. Plant Physiol., 1978, 61, 675.
52. Aharoni, N.; Richmond, A. Plant Physiol., 1978, 61, 658.
53. Hall, M.A.; Kapuya, J.A.; Sivakumaran, S.; John, A. Pestic. Sci., 1977, 8, 217.
54. Sisler, E.; Wylie, P.A.; Plant Physiol. Suppl. 1978, 61, 91.
55. Beyer, E.M. Plant Physiol., 1975, 56, 273
56. Beyer, E.M. Plant Physiol., 1979 (In press).
57. Beyer, E.M. Plant Physiol., 1976, 58, 268
58. Mattoo, A.K.; Lieberman, M. Plant Physiol.,1977, 60, 794.
59. Hertel, R.; St. Thomson, K.; Russo, V.E.A. Planta, 1972, 107,325.

RECEIVED June 19, 1979.

7

Natural Products in Plant Growth Regulation

N. BHUSHAN MANDAVA

U.S. Department of Agriculture, Science and Education Administration, Agricultural Research, Beltsville Agricultural Research Center, Plant Physiology Institute, Plant Hormone and Regulators Laboratory, Beltsville, MD 20705

The literature documents several hundred plant products that appear to exhibit growth-regulating activity. Naturally occurring plant growth substances can be broadly categorized into two types. The first type includes growth substances such as auxins, gibberellins, cytokinins, the inhibitor abscisic acid (ABA) and ethylene. These are commonly referred to as plant hormones. They appear to be present in very minute quantities in all plant species, particularly in higher plants whose growth and development are controlled by these hormones. The second group of natural products, commonly referred to as secondary plant growth substances, about which we are concerned in this article, includes compounds such as phenols, aliphatic and aromatic carboxylic acids and their derivatives, steroids, terpenoids, amino acids, and lipids. Some of these compounds are produced by plants in abundant quantities, but lack growth specificity; others are present in minute quantities, but show high specificity. Based on this information, natural products produced by animals, plants, fungi, and bacteria may broadly be categorized into: (I) those compounds that are widely distributed and are present in abundance but lack specificity. Examples in this category are phenolic compounds (including coumarins and flavonoids), lipids and amino acids (including polypeptides). (II) those compounds that have limited distribution (sometimes, species-specific) and are produced in small quantities, but with some specific activity. Examples are simple unsaturated lactones, terpenoids, steroids, and alkaloids. Although there is a clear distinction between the two categories, this conventional approach was avoided in this review. A novel approach followed here that will take into account both categories (specific and nonspecific growth substances) is based on their biosynthetic origin.

According to Robinson (1), Whittaker and Feany (2), and Rice (3), a great majority of secondary plant products are biosynthesized from acetate and shikimic acid as shown in Figure 1, which describes the formation of 15 groups of natural products.

In Plant Growth Substances; Mandava, N.;
ACS Symposium Series; American Chemical Society: Washington, DC, 1979.

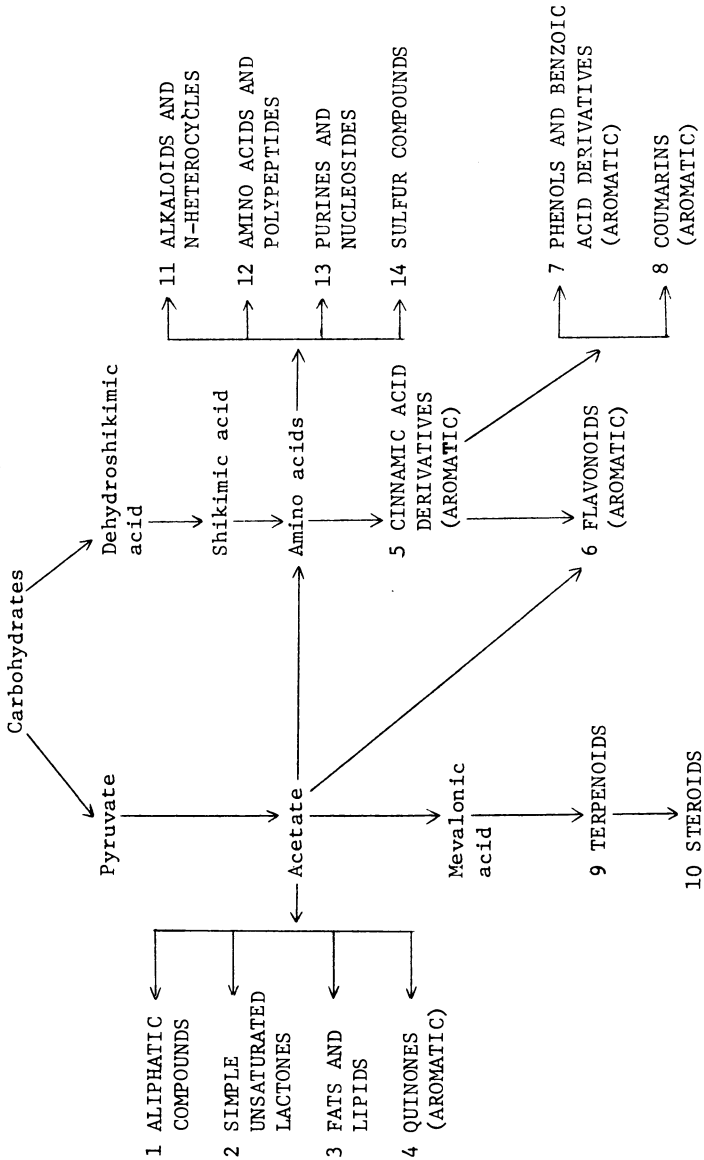


Figure 1. Biosynthetic pathways for secondary plant growth substances

In Plant Growth Substances; Mandava, N.;
ACS Symposium Series; American Chemical Society: Washington, DC, 1979.

Tannins and lignins are also derived from these pathways but are not included in Table 1. To make the list as simple as possible, all compounds of aromatic nature, viz., simple phenols, benzoic and cinnamic acid derivates, coumarins, flavonoids and quinones are condensed into one group - aromatic compounds. Thus I will attempt to cover systematically the secondary plant growth substances that fall into 11 major groups as shown in Table 1.

Interestingly, several natural products, besides their role as antibiotics and phytotoxins, exhibit plant growth responses (mainly inhibition). In order to keep their identity as such, these were listed under miscellaneous natural products (Table 1). Furthermore, compounds which have structural features similar to those present in hormones (gibberellins and ABA) and which exhibit similar growth responses are also listed under miscellaneous natural products (Table 1).

This review also includes some work on plant growth substances from the United States Department of Agriculture. A section on the mode of action of the secondary plant growth substances is included. A closely related subject which is now receiving considerable attention is the biochemical interactions of natural compounds from plants with other plants in the natural habitat (allelopathy) and this topic is included in this review. It is not my intention to make an exhaustive survey of the literature on all compounds reported to be biologically active. Instead, limited but pertinent information on a few selected groups of compounds will be provided. In reviewing these compounds, the biological activity information is cited only to indicate their importance.

Natural Products Originating from Acetate and Shikimic Acid Pathways

Aliphatic Compounds.

There are numerous reports indicating that many volatile substances such as methanol, ethanol, n-propanol and n-butanol produced by fungi and yeast (4) inhibit the growth of rice and sugar cane at 10^{-2} - 10^{-4}M. Further, ethanol inhibits the seed germination of lettuce (*Lepidium sativum*) (4). 3-Methyl butanol-1-ol and hexa-1,3,5-tryne also produced by soil microorganisms and culture gases act as inhibitors of seed germination (4, 5). Certain simple aliphatic acids have also been reported as plant growth regulators (Table 2). For instance, formic, acetic, propionic and butyric acids produced by soil organisms showed strong inhibitory effect on rice seedling growth (6). The activity of carboxylic acids decreased in the following order: stearic > caprinic > caprylic > iso-valeric > caproic > butyric > palmitic > propionic > formic > acetic > pyruvic. Another example is γ-aminobutyric acid, found in a crown-gall tumor in wilted bean leaves, which stimulates tumor growth (7). Citric acid from orange juice induces growth promotion in tissue

In Plant Growth Substances; Mandava, N.;
ACS Symposium Series; American Chemical Society: Washington, DC, 1979.

TABLE 1.

Major Groups of Natural Products Originating From Acetate and Shikimic Acid Pathways

1. Aliphatic compounds
2. Simple unsaturated lactones
3. Fats and other lipids
4. Aromatic compounds
    a) Phenols, aldehydes, benzoic and cinnamic acid derivatives
    b) Coumarins
    c) Flavonoids
    d) Other aromatic compounds
5. Terpenoids
6. Steroids
7. Alkaloids and N-heterocycles
8. Amino acids and polypeptides
9. Purines and nucleosides
10. Sulfur compounds
11. Miscellaneous natural products
    a) Antibiotics
    b) Phytotoxins
    c) Lichens
    d) Others (Structure-activity relationship and other growth inhibitors)

In Plant Growth Substances; Mandava, N.;
ACS Symposium Series; American Chemical Society: Washington, DC, 1979.

cultures (8).

In the 1930's, Bonner and his group investigated the effects of several aliphatic compounds on plant growth. A classic example is traumatin, also referred to as the wound hormone, which was isolated from bean pods and identified as 1-decene-1, 10-dicarboxylic acid (9, 10). This substance is capable of inducing renewed cell division and cell extension activity in the parenchymatous cells of the bean pod mesocarp. It is also capable of inducing wound periderm formation in washed disks of potato tuber and therefore functions as a wound hormone of the potato. Further, it was shown to be capable of partially replacing the juice of tomato fruit for reversibly inhibiting the germination of tomato seeds (11, 12, 13).

A long-chain dihydroxy compound, isolated from Avocado mesocarp, inhibited soybean callus growth and induced elongation of wheat coleoptiles. This compound was identified as 1-acetoxy-2,4-dihydroxy-n-heptadeca-16-ene, otherwise known as avocado inhibitor (14).

Radiclonic acid and its 0-acetyl and 12-hydroxy derivatives were isolated from the culture filtrate of a Pencillium sp. fungus. These compounds promoted root elongation in many types of seedlings, the maximum activity was about 25 ppm for chinese cabbage seedlings; for rice seedlings, it was inhibitory above 100 ppm and stimulatory below this concentration (15, 16).

Unsaturated Lactones.

This group represents compounds that include some analogs of 2-pyrones which constitute an important subgroup of the acetogenins having plant growth regulating properties (Table 3). Pestalotin, found in Pestalotia cryptomeriacola, was reported to be a gibberellin synergist (17, 18). The leaves of Lindera obtusiloba contain obtusilactone which is the main component showing growth-inhibiting properties (19).

Protoanemonin, an antibiotic isolated from Ranunculacea sp., is a potent growth inhibitor (20) of seed germination and many bacteria. This aglycone can be obtained by the hydrolysis of its glycoside, ranunculin which is produced by several species of Ranunculaceae (21). Parasorbic acid which is 6-methyl-5,6-dihydro-2-oxo-2H-pyran was identified from the unripe berry fruits of Sorbus aucuparia (22) and is very inhibitory to seed germination and seedling growth, and is also antibacterial (21). Another δ-lactone, massoilactone (6-n-amyl-5,6-dihydro-2-oxo-2H-pyran), found in the bark of Massvia aromatica also inhibits the germination of seeds (23). Psilotin which is 6(4-β-D-glucopyranosyloxyphenyl)-5,6-Dihydro-2-oxo-2H-pyran and its aglycone, psilotinin were identified from Psilotum nudum L. (24) as well as from Tmesipteris tannensis (25). Psilotin showed an inhibitory effect on seedling growth of turnip, onion and lettuce. These inhibitory effects of psilotin were overcome by glutathione and other thiols, and also be gibberellin A_3 (26, 27).

In Plant Growth Substances; Mandava, N.;
ACS Symposium Series; American Chemical Society: Washington, DC, 1979.

Table 2. Aliphatic Compounds With Growth Regulating Activity

Compound	Source	Biological Activity	Reference
1. Alcohols			
Methanol, ethanol n-propanol, n-butanol, and 3-methyl-butan-1-ol	Fungus and yeast	Inhibit sugar cane, rice and corn growth at 10^{-3} - 10^{-4}M	4
2. Monocarboxylic Acids			
Formic, acetic, propionic, butyric, isovaleric and pyruvic acid	Soil microorganisms	Inhibit root growth of rice seedlings	6
3. Dicarboxylic Acids			
Oxalic, fumaric, succinic, and citric acids	Vegetables and Citrus	Inhibit the growth of several plants	8

In Plant Growth Substances; Mandava, N.;
ACS Symposium Series; American Chemical Society: Washington, DC, 1979.

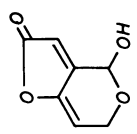
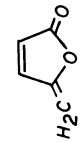
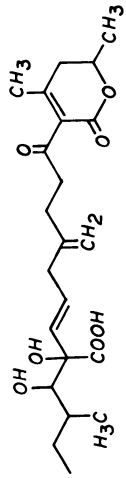
4. Other Carboxylic Acids			
a. v-aminobutyric acid	Wilted bean leaves	Stimulates tumor growth	7
b. Radiclonic acid and its derivatives	**Penicillium sp.**	Promote root elongation	15,16
c. Traumatin (1-Decene-1,2-dicarboxylic acid)	Bean pods	Induces cell division and extension	9,10
5. Hexa-1,3,5-triene	Formes annosus	Inhibits cress seed germination	5
6. Avocado inhibitor (1-Acetoxy-2,4-dihydroxy-n-heptadeca-16-ene)	Avocado mesocarp	Inhibits soybean callus growth	14

In Plant Growth Substances; Mandava, N.;
ACS Symposium Series; American Chemical Society: Washington, DC, 1979.

TABLE 3. UNSATURATED LACTONES

COMPOUND	STRUCTURE	SOURCE	BIOLOGICAL ACTIVITY	REFERENCE
1. PARASORBIC ACID		SORBUS AUCUPARIA	INHIBITS SEED GERMINATION AND SEEDLING GROWTH	21,22
2. MASSOILACTONE		MASSVIA AROMATICA	INHIBITS SEED GERMINATION	23
3. PSILOTIN (R=β-D-GLUCOSE) PSILOTININ (R=H)		PSILOTUM NUDUM L. AND TMESIPERIS TANNENSIS	INHIBITS SEED GERMINATION AND SEEDLING GROWTH	24,25,26, 27
4. PESTALOTIN		PESTALOTIA CRYPTOMERIACOLA	GIBBERELLIN SYNERGIST	17,18

In Plant Growth Substances; Mandava, N.;
ACS Symposium Series; American Chemical Society: Washington, DC, 1979.

5. PATULIN		SEVERAL *PENICILLIUM* SP.	INHIBITS SEED GERMINATION AND SEEDLING GROWTH 28,29,30 31,32
6. PROTOANEMIN		SEVERAL *RANUNCULACEA* SP.	INHIBITS SEED GERMINATION 20,21
7. OBTUSILACTONE		*LINDERA OBTUSILOBA*	INHIBITS SEED GERMINATION 19
8. ALTERNARIC ACID		*ALTERNARIA SOLANI*	INHIBITS SEEDLING GROWTH 33

The antibiotic, patulin (Table 3) shows a strong inhibitory effect on higher plants. At 10 μg/ml this compound completely inhibits the germination and growth of seedlings on certain cultivated plant species (28, 29). It is produced by a number of fungal species (Pencillium sp.) and is sometimes referred to by such names as calviformin, clavacin, clavatin and expansine. Bergel et al. (30) reported the isolation of patulin, the synthesis of which was later accomplished by Woodward and Singh (31, 32).

Alternaria solani (33) contains an unsaturated lactone moiety with a side chain (C_{15}) and this lactone, termed alternaric acid, exhibits inhibitory properties in seedling growth of test plants. Some of the unsaturated lactones described above are summarized in Table 3.

Fatty Acids and Other Lipids.

Lipids have long been known to exhibit plant growth-regulating activity and in fact, the oldest known growth substance is a lipid, namely olive oil, which was used by the ancient Egyptians in the third century B.C. to hasten the ripening of figs (34). Hirata and his coworkers in Japan (35) and Stowe's group in the U.S.A. (36), conducted a systematic study on therole of lipids in plant growth regulation and found that several classes of lipids including fatty acids and their esters, fatty alcohols and different glycerides stimulate the enlargement and ripening of figs and auxin-induced elongation of pea stems. Stowe concluded that a group of long chain (20 Å) lipids, termed oleamins (37), stimulate cell enlargement, respiration and ethylene formation, and they often act synergistically with auxins to induce these responses. Vitamins also appear to elicit plant growth regulating effects. For example, α-tocopherol (vitamin E) (38) showed differentiation of a near primordium in winter rye (Secale cereale L.) and vitamins H and K (39, 40) alone or with IAA or NAA cause growth effects in root formation in pea stem, bean and Datura plants. Although sterols belong to the lipids class, they will be described later in a separate section. Several long chain and short chain fatty acids and their esters and alcohols have been reported to exhibit such growth responses as (a) inhibiting seed germination (41) and bacterial growth (42), (b) inhibiting the tobacco auxillary bud growth (sucker control chemicals) (43, 44, 45), (c) killing meristamatic tissues in ornamentals (chemical pruning agents) (46, 47), (d) inhibiting the growth of roots and other plant organs (48), (e) stimulating auxin-type induced growth of roots and other plant organs (48), (e) stimulating auxin-type induced growth (49, 50), and (f) stimulating the overall growth and yields of some crop plants (51). Essentials of these results are summarized in Table 4.

In Plant Growth Substances; Mandava, N.;
ACS Symposium Series; American Chemical Society: Washington, DC, 1979.

Table 4: Plant-Growth Activity of Lipids

Lipid	Growth Response	Reference
1. Oils (e.g. Olive oil)	Stimulate fig enlargement and ethylene formation, and increase respiration	35
2. Oleanins (C_{10}-C_{24})	Stimulate auxin-induced growth in pea and fig bioassays	36,37
3. α-Tocopherol (Vitamin E)	Stimulate d-5 dwarf corn elongation, flower induction and primordia differentiation in winter rye	38
4. Vitamins H^1 and K	Stimulate root formation in peas, bean and Datura plants	39,40
5a. Fatty acid derivatives	Inhibit seed germination and bacterial growth	41,42
b. Fatty acid esters and alcohols (C_8-C_{12})	Inhibit tobacco axillary bud growth (Sucker control agents)	43,44,45
c. Fatty acid esters (C_8-C_{12})	Chemical pruning agents for ornamentals	46,47
d. Fatty acids (C_{12}-C_{20})	Inhibit plant growth	48
e. Fatty alcohols viz., docosonal triacontanol	Stimulate growth in auxin bioassay	49,50,51

In Plant Growth Substances; Mandava, N.;
ACS Symposium Series; American Chemical Society: Washington, DC, 1979.

Aromatic Compounds.

As mentioned earlier, this group of compounds occurs widely in nature and appears to be non-specific in eliciting activity. Although the chemistry of these compounds has been well defined, the endogenous role of these substances is not. Several of them appear to be inhibitors of seed germination. Since this subject was extensively reviewed by several workers (21, 56, 60), only a minimum of information with a few examples is presented here.

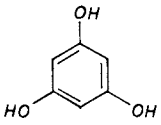
(a) Phenols, Aldehydes, Carboxylic Acids and Quinones: Many acidic growth inhibitors have been isolated from various sources and identified as substituted benzoic and cinnamic acids (e.g., furulic and caffeic acids). These aromatic acids inhibit the growth of seedlings and seed germination. The same effects are caused by certain benzaldehyde derivatives which were also isolated from plant sources (Figure 2). 3-acetyl-6-methoxy-benzaldehyde present in the leaves of Encelia farinosa is a potent inhibitor of tomato and other plants (52, 53). Some representative examples of phenols, aldehydes, carboxylic acids (particularly benzoic and cinnamic acid derivatives) and quinones are shown in Figure 2.

(b) Coumarins: Coumarin and several of its derivatives (Figure 3) were reported to inhibit seed germination and seedling growth in many test systems. Scopoletin was isolated from several plant species including 5-day-old oat (Avena sativa) seedlings (54). It inhibits the growth of roots at 10^{-5}M. Selsin was obtained from citrus roots (56) and inhibits growth in the Avena coleoptile bioassay. Psoralin was found in the seeds of Psoralea subacaulis and other members of P. corylifolia, P. drupacea and several species of Psoralea. This substance inhibits seed germination and root growth of lettuce, radish and cucumber plants (55). The biological role of coumarins has been reviewed by Evenari (21), and Mayer and Poljakoff-Mayer (56).

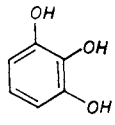
Coumarin derivatives (mostly in the form of their glycosides) are of wide occurrence in the plant kingdom and hydrolysis yields the corresponding aglycones. Published reports indicate that coumarins inhibit potato sprouting, root growth, abolition of apical dominance in sugar cane, as well as other growth effects in chicory, barley and other plants (56). Coumarin also inhibits the IAA-induced growth of coleoptiles but this effect could be reversed by 1,2-dimercaptopropane (BAL). Besides its inhibitory action (at 10^{-3}M), coumarin was shown to stimulate seedling growth and seed germination at low concentrations (10^{-7} to 10^{-10}M). Gibberellic acid A_3 reverses the inhibition of germination caused by coumarin. This reversal is a function of GA_3 and coumarin concentration (55). It may be possible that coumarin in some way affects the metabolism of the endogenous hormones (GA_3 and IAA).

Decursinol and decursin (Figure 3) were obtained from Umbelliferous plants (57). At 10 ppm, both compounds inhibited

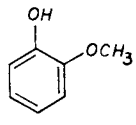
In Plant Growth Substances; Mandava, N.;
ACS Symposium Series; American Chemical Society: Washington, DC, 1979.



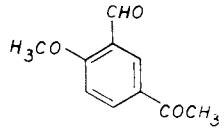
Phloroglucinol



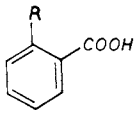
Pyrogallol



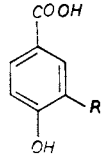
Guaicol



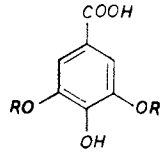
3-Acetyl-6-Methoxy-Benzaldehyde



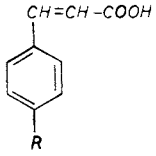
R=CH_3, o-Toluic Acid
R=OH, Salicylic Acid



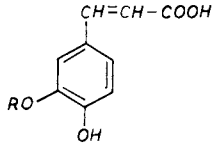
R=H, p-Hydroxybenzoic Acid
R=OCH_3, Vanillic Acid



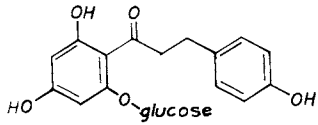
R=H, Gallic Acid
R=CH_3, Syringic Acid



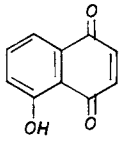
R=H, Cinnamic Acid
R=OH, p-Hydroxy-cinnamic Acid
(p-Coumaric Acid)



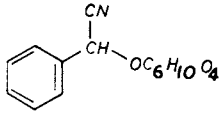
R=H, Caffeic Acid
R=CH_3, Ferulic Acid



Phlorizin



Juglone



Amygdalin

Figure 2. Phenols, aldehydes, carboxylic acids, and other aromatic compounds with growth-regulating activity

In Plant Growth Substances; Mandava, N.;
ACS Symposium Series; American Chemical Society: Washington, DC, 1979.

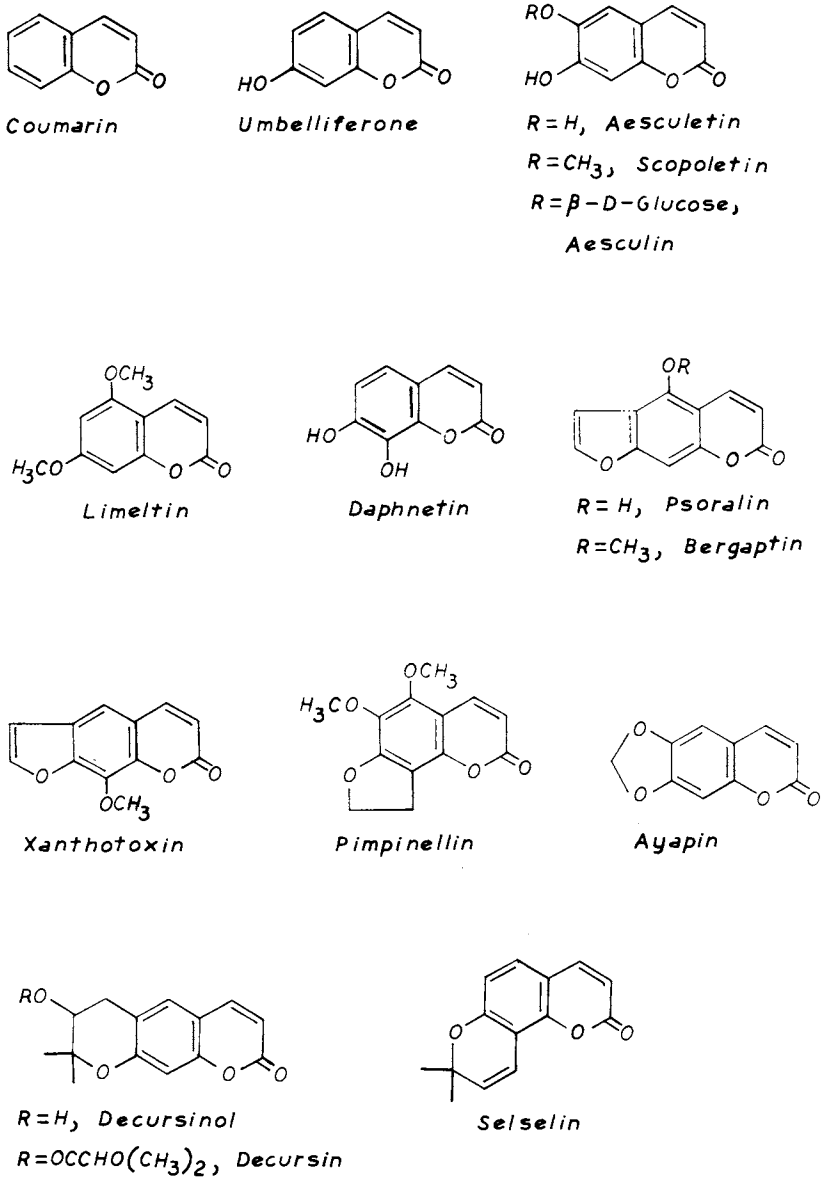


Figure 3. Coumarins in plant growth regulation

In Plant Growth Substances; Mandava, N.;
ACS Symposium Series; American Chemical Society: Washington, DC, 1979.

the growth of Avena coleoptiles sections. They also inhibited the germination of lettuce, tomato, cucumber, chinese cabbage and wheat seeds at 100 ppm. Furthermore, they promoted the adventitious root formation in bean (Phaseolus vulgaris) plants (58, 59).

(c) Flavonoids: These are another group of aromatic compounds that have widespread occurrence in both higher and lower plants. The effects of compounds such as kaempferol and quercetin (Figure 4) on plant growth have been extensively studied, especially the effect of light in leaf experiments. They have been implicated in interference with IAA oxidation at certain concentrations (60). Some of the flavonoids also play an important role in plant growth and development. For example, sayanedine exhibits a cytokinin-type activity in cultures of tobacco pith tissue (61). Another example is naringenin which is reported to be a dormancy inhibitor (62). Silybin, silydianin and silychristin, the biologically active constituents of the milk thistle (Silybum marianum L. Gaertn.) strongly stimulate the oxygen uptake of plant tissues. Since oxygen uptake (a physical effect) can affect growth, they are considered as potent growth substances. This effect manifests itself in the intensity of respiration and the time course of oxygen consumption (63).

(d) Other Aromatic Compounds: Several aromatic compounds (Figure 5) other than those described above have been reported to possess growth-regulating properties. A "lettuce cotyledon factor" identified as dihydroconiferyl alcohol was found to be a synergist of gibberellins and auxin in inducing hypocotyl elongation of lettuce and cucumber, respectively (64). This phenyl propenoid compound is now thought to be ubiquitous in nature, since the same compound is present in several plants such as pea, cucumber and Jerusalem artichoke (64). Zinniol, a toxic metabolite of Alternaria zinniae, inhibits seed germination and also has weak activity against fungi and bacteria (65).

An isocoumarin, hydrangenol from Hydrangea opulaides antagonizes gibberellin action similar to that of naringenin (62, 66). An endogenous growth inhibitor of liverwort, Lunalaria cruciata L., was identified as lunalaric acid which is a dihydrostilbene carboxylic acid (67, 68). This substance occurs in all liverworts and algae examined thus far and appears to fulfill the same growth-regulating function in lower plants that ABA does in higher plants (69, 70, 71). Further, this acid at 10-30 ppm inhibits the auxin-induced elongation of Avena coleoptile segments.

Dormancy in yam bulbs (Dioscorea batatas) was found to be induced by three inhibitors, batatasins I, II, and III (72, 73). The structures for batatasins I and III were assigned recently. Batatasin I is 6-hydroxy-2,4,7-trimethoxyphenanthrene and batatasin III is 3,3-dihydroxy-5-methoxy-bibenzyl. Biogenetically, hydrangenol, lunalaric acid and batatasin III appear to

In Plant Growth Substances; Mandava, N.;
ACS Symposium Series; American Chemical Society: Washington, DC, 1979.

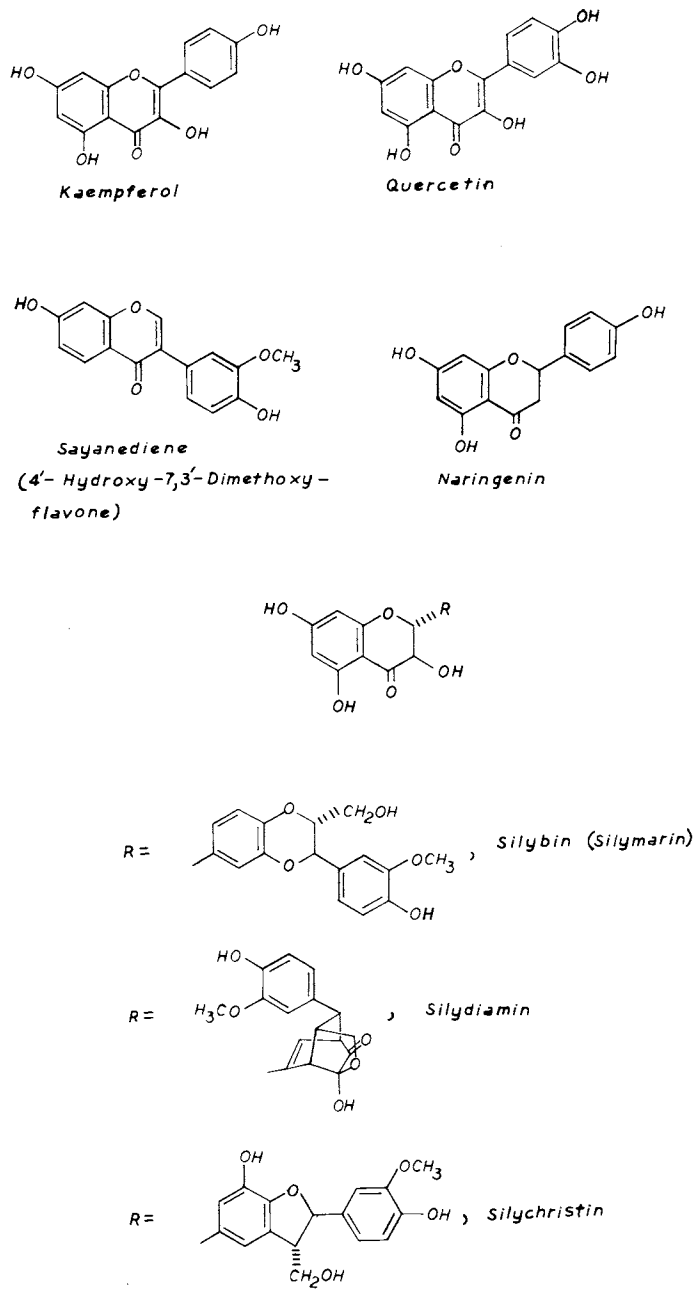


Figure 4. Flavonoids with growth-regulating properties

be derived from phenyl propanoids through an acetate pathway (70).

A group of 5 compounds, namely, sclerin, sclerolide, isosclerone and sclerotinins A and B, from Sclerotinia sp., was shown to stimulate the growth of several types of plants including rice, castor bean and mung bean seedlings. Although this group (Figure 5) has structural similarities with other phenolic compounds, they elicit only growth-promoting effects (74, 75, 76, 77). Pigments such as bikaverin and norbikaverin, also isolated from a fungal source (Fusarium sp.), affect the development of marginal hyphae, namely, the extension growth ceases and vacuolation and other morphological symptoms of aging (normally indicative of senescence). The red pigment, bikaverin, was active when tested in 30 species of fungi (78).

Some lignans whose biosynthetic origin is from cinnamic acid (by oxidative phenolic coupling) have been shown to exhibit plant growth-inhibiting activity. They include monoepoxylignolide (MEL) (79) and its methoxy derivative (80) from Aegilops ovata L. Similar epoxylignolides such as sesamin and kobusin were found in Magnolia kobus (81), but they are reported only as strong inhibitors to silkworm larvae.

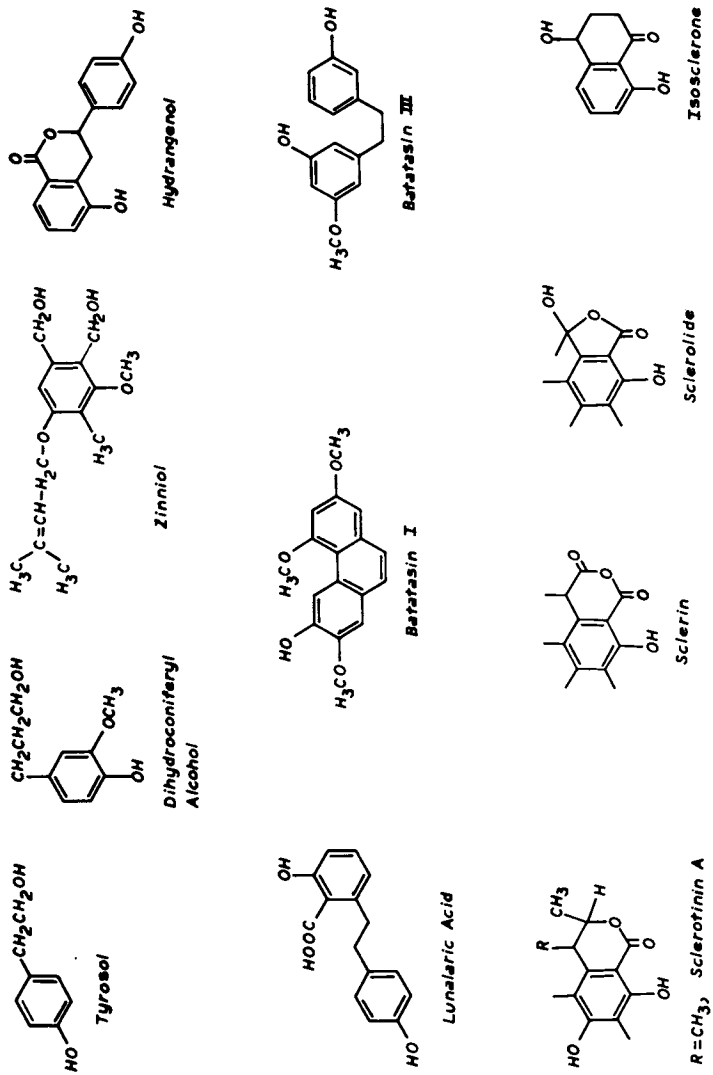
Phenylacetic acid and its hydroxy isomers (ortho, meta, and para) have been isolated from many plants and microorganisms (82) and have been reported to have plant growth activity. Other compounds such as 2-(4-hydroxybenzyl) maleic acid, a germination inhibitor from Pestalostemon galteneri (83), and L-β-phenyllactic acid, a root growth promoter and tyrosol, a lettuce seed germination inhibitor from Gleosporium lacticolor are of great interest because of their growth regulating properties (84).

Terpenoids.

Several terpenoids, particularly those with a lactone moiety, have been examined for plant growth-regulating activity. Incidentally, several of these compounds exhibit anti-tumor and insecticidal activity. They are classified into mono-(C_{10}), sesqui-(C_{15}), di-(C_{20}), tri-(C_{30}) and tetraterpenes (C_{40}). Some representative candidates among these classes possessing biological activity are as follows:

(i) Monoterpenoids or terpenes: Strictly speaking, the term "terpene" refers to a hydrocarbon of the composition $C_{10}H_{16}$. In practice, however, this term applies to all C_{10} compounds that also contain oxygen, e.g., alcohols and ketones. Monoterpenoids are the major constituents of steam-volatile essential oils of plants (1, 22, 85). Evenari (22) suggested that the inhibitory activities of essential oils on seed germination were chiefly due to the monoterpenic compounds. Volatile terpenes such as camphene, camphor, cineole, dipentene, and α- and β-pinenes (Figure 6) have been found in the shrubs, (Salvia leucophylla, S. apiana, S. mellifera and Artemisia california (86) and show growth inhibition. The most inhibitory ones are

In Plant Growth Substances; Mandava, N.;
ACS Symposium Series; American Chemical Society: Washington, DC, 1979.



In Plant Growth Substances; Mandava, N.;
ACS Symposium Series; American Chemical Society: Washington, DC, 1979.

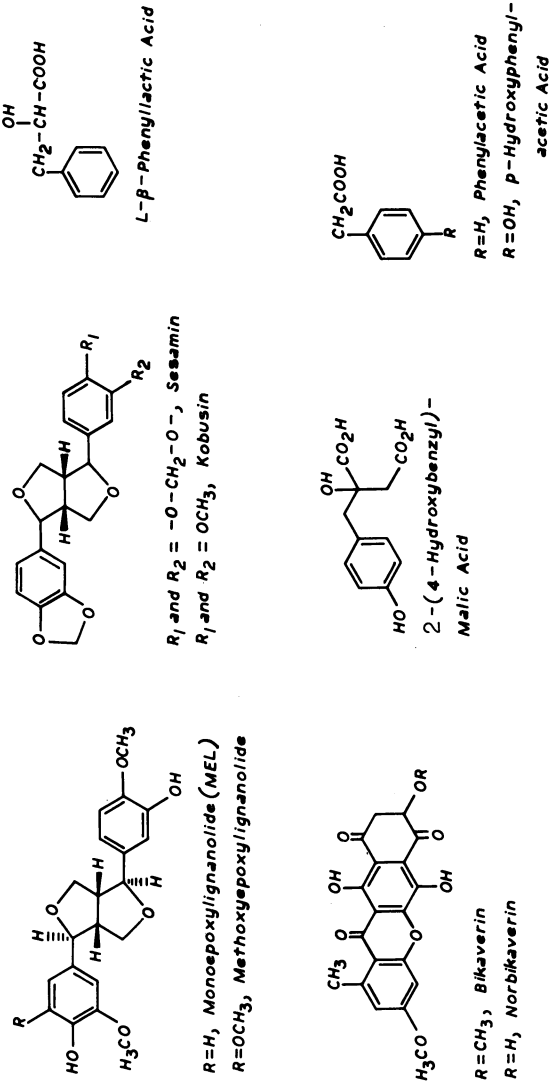


Figure 5. Other aromatic compounds with growth-regulating activity

In Plant Growth Substances; Mandava, N.;
ACS Symposium Series; American Chemical Society: Washington, DC, 1979.

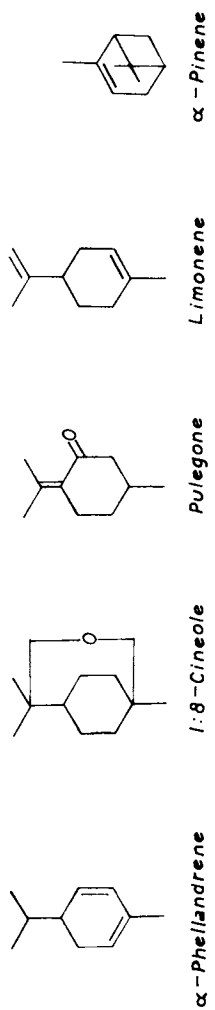


Figure 6. Monoterpenes having growth-regulating properties

camphor and cineole. Eucalyptus camaldulensis (87) and E. microtheca (88) produce cineole, α- and β-pinenes. In a study related to the structure/activity, Asplund (89) found that the compounds with functional (keto) groups [pulegone and (+)- and (-)-camphors] showed more inhibition of radish seed germination than other monoterpenes (Figure 6). The least inhibitory was β-pinene followed by 1,8-cineole, and others showed similar activity.

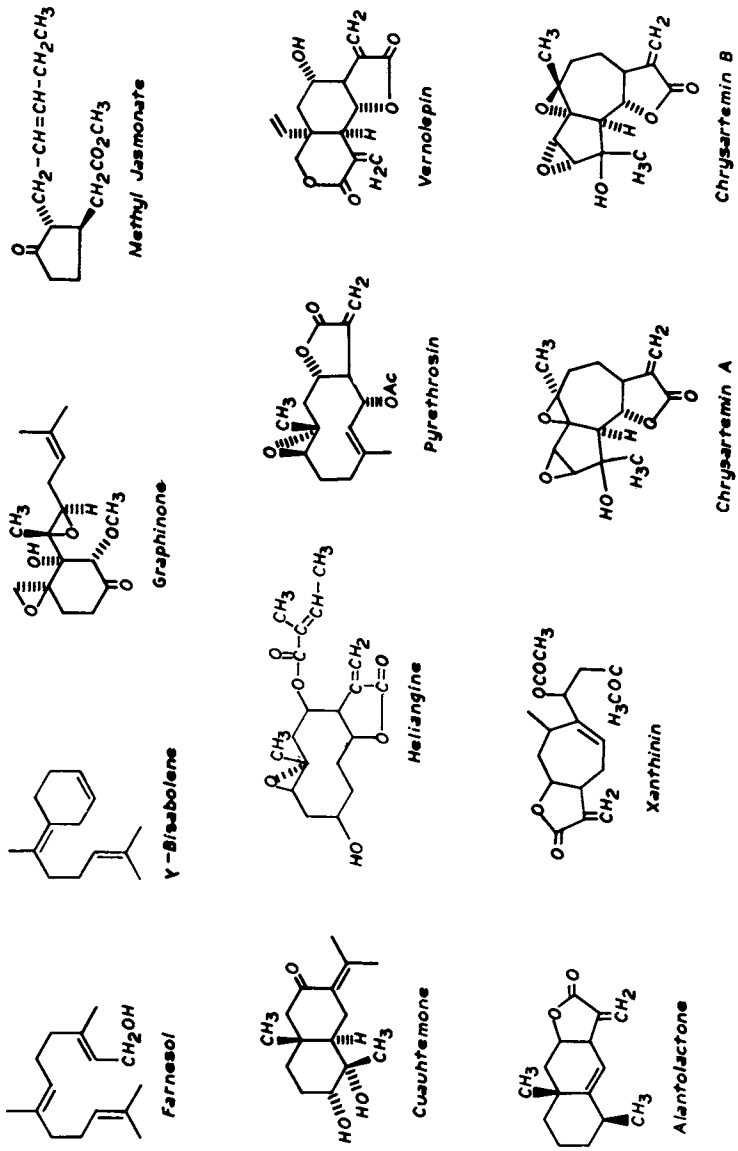
(ii) Sesquiterpenes: Biogenetically, these compounds are originated by uniting 3 isoprene units. They were found in several plants as well as in many essential oils. The simplest member of this group that follows the isoprene rule is farnesol (Figure 7) which was isolated from Sorghum sudanese (Piper) Stapf and was shown to induce stomatal closure in Commelina communis. This endogenous antitranspirant is more active than ABA in stomatal closure (90, 91).

Sesquiterpenes such as β-caryophyllene, bisabolene and chamazulene found in Artemisia absenturium were shown to possess growth-inhibiting activity (92). Other artemisia species (A. psilastachya and A. acanticarpa) produce several sesquiterpenes, some of which may also be growth inhibitors (93, 94).

Methyl jasmonate was found in the essential oils of Jasminium grandiflorum (95) and Rosmarinus officinalis (96), while the free acid was isolated from culture filtrates of Lasiodiplodia theobromae (97). The acid inhibited the growth of green plants. Cuautemone (Figure 7) was isolated from the nonsaponifiable portion from the aerial part of Pluchea odorato (Compositae, the Mexican shrub, "Cuauhtenati") and exhibits growth inhibition when tested in corn and bean plants (98). A sesquiterpene-like compound, ascochitine, was found in culture filtrate of Ascochyta fabae and this toxin causes brown necrotic spots when applied to cotyledons of broad bean (99). A plant growth inhibitor belonging to a germacrane-type sesquiterpene, heliangine, was isolated from Helianthus tuberosus L. This compound exhibited an inhibitory effect on the Avena coleoptile curvature and straight growth (100, 101, 102).

Several of the sesquiterpenes examined, particularly those with an α-methylene-γ-butyrolactone moiety, were found to be active in eliciting growth-regulating activity. A few examples are illustrated here. Vernolepin (a sesquiterpene dilactone from Veronia hymenolepis) inhibits extension growth of wheat coleoptile sections (103, 104). Pyrethrosin, from Chrysanthemum sinerariaefolium, exhibits a similar growth inhibition (105, 106). On the other hand, Chrysartemins A and B belonging to the guainolide family were isolated from the leaves of C. morifolium and C. parthenium and promoted root initiation in string bean cuttings. They also showed a synergistic effect with IAA (107) in root initiation test. When compared to heliangine and pyrethrosin, which also contain α-methylene-γ-butyrolactone, the two chrysartemins A were reported to be more active in promoting root initiation and less active as auxin inhibitors in the Avena

In Plant Growth Substances; Mandava, N.;
ACS Symposium Series; American Chemical Society: Washington, DC, 1979.



In Plant Growth Substances; Mandava, N.;
ACS Symposium Series; American Chemical Society: Washington, DC, 1979.

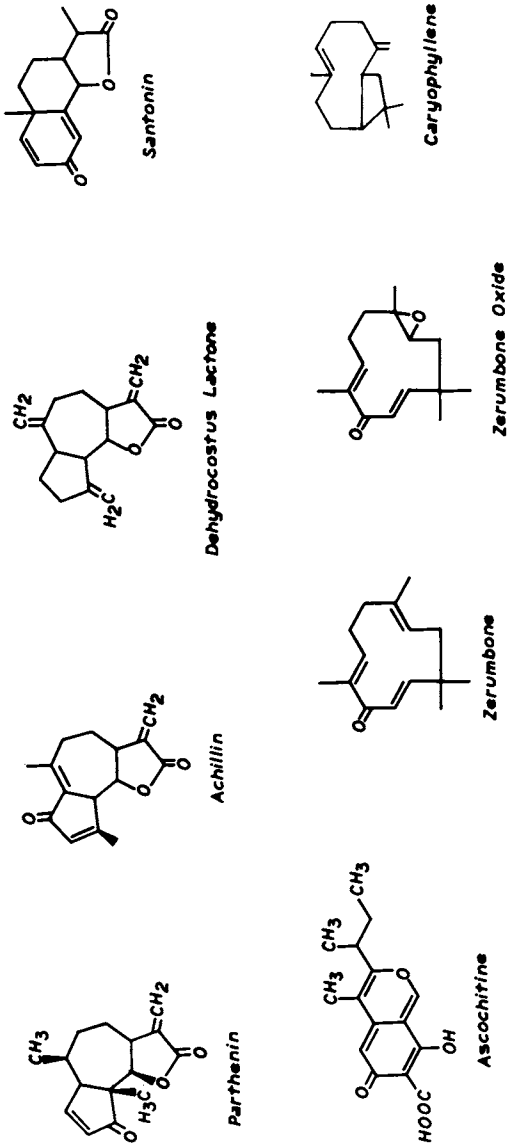


Figure 7. Sesquiterpenes with growth inhibitors

growth test (106, 107). Chrysartemin was also found in Artemisia mexicana and A. klotschiana (108). Alantolactone, found in several species of Compositae, is a potent inhibitor of seed germination and seedling growth of several species of bean seeds (109, 110, 111). Xanthinin, isolated from the leaves of Xanthium pennsylvanicum (cocklebur), acts as an auxin antagonist in the Avena coleoptile bioassay (112, 113, 114). Parthenin was isolated from bitterweed, Parthenium hysterophorus, and exhibited growth-inhibiting activity when tested at 50 ppm on radical growth, hypocotyl elongation and adventitious root formation (115, 116); achillin isolated from Achillea lanulosa (117, 118) also exhibited similar growth-inhibiting activity. Parthenine and achylline also inhibited radical development in germinating seeds and embryos of several plants cultured in vitro. Growth was also inhibited when plants were grown in Hoagland's solution and respiration was depressed. The action seems to be on cell division and not on cell elongation. Dehydrocostus lactone, found in costus (saussurea lappa) roots, showed growth promotion in the mung bean hypocotyl bioassay (119, 120). Among the cross-conjugated terpenoid ketones examined, zerumbone and its oxide, and santonmin induced root formation in the mung bean bioassay and were claimed to be more active than IAA at 10-30 ppm in this test (121).

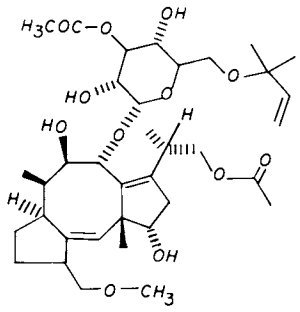
(iii) Diterpenes: Several compounds possessing plant growth-regulating activity are reported in this classification of terpenoids which are biogenetically derived from 4 isoprene units. They are produced by higher plants as well as by microorganisms.

A diterpene glucoside with an opiobolane skeleton from Fusicoccum amygdali, termed fusicoccin (Figure 8), was shown to elicit a remarkable stimulation of growth of cell enlargement in isolated plant segments. This substance induces a magnified response, an auxin-like growth in pea stem sections, cytokinin-like activity in squash and radish cotyledons, cell enlargement in leaf fragments and germination in dormant wheat, lettuce and radish seeds (122, 123, 124).

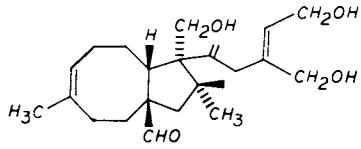
Portulal (Figure 8) is a novel diterpene containing a perhydroazulene nucleus and was isolated from Portulaca grandiflora Hook. It inhibits the elongation of Avena coleoptile sections induced by IAA. It also accelerates adventitious root formation of Azukia epicotyl cuttings.

A group of 8 diterpenes containing a common but novel ophiobolane skeleton (Figure 8) was isolated from the culture filtrate of a fungal strain, Cladosporium sp. They were termed cotylenins and are leaf growth-regulating substances. Cotylenin A is a growth promoter and showed the same biological activities as that of fusicoccin in a series of assays, except the wilting activity of tomato cuttings. Cotylenins A and C are rare fungal glycosides containing unusual sugars. From a biosynthetic point of view, cotylenins E and C could be the possible precursors for the principal metabolite, cotylenin A. Furthermore, cotylenins B

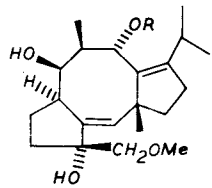
In Plant Growth Substances; Mandava, N.;
ACS Symposium Series; American Chemical Society: Washington, DC, 1979.



Fusicoccin

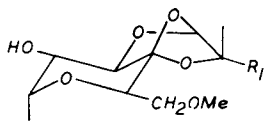


Portulal

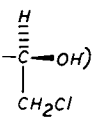


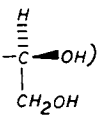
a) R = H, Cotylenol

b) R =

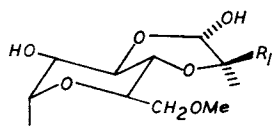


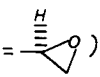
Cotylenin A (R_1 = )

Cotylenin B (R_1 = )

Cotylenin D (R_1 = )

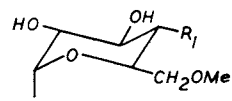
c) R =



Cotylenin C (R_1 = )

Cotylenin G ($R_1 = CH_2OH$)

d) R =



Cotylenin E (R_1 = OH)

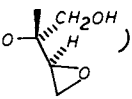
Cotylenin F (R_1 = )

Figure 8. Diterpenoids with growth-regulating activity

and D might have been formed from cotylenin A in the culture conditions. Cotylenins C, E, F and G showed about the same activity as that of the principal metabolite, cotylenin A, in a cucumber cotyledon assay (125, 126, 127, 128, 129).

Higher plants and some microorganisms produce a number of diterpene lactones such as podolactones A-E, ponolactones A and its glucoside, inumakilactones A, its glucoside, B and E, nagilatone C, momilactones A-C, and sellowin A, many of which are inhibitory to the growth of plants (Table 5).

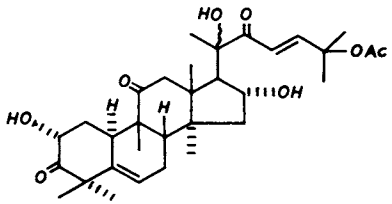
(iv) Triterpenes: A few compounds in this category have been reported to possess growth-regulating activity. A group of triterpenoids termed "cucurbitacins" (Figure 9) has been found mainly in the Cucurbitacea family (cucumbers, gourds, melons, pumpkins and squashes are colloquially known as cucurbits). To date, 17 cucurbitacins have been isolated from 6 plant sources and some of them were reported to have tumor-inhibiting properties (146). Guha and Sen (147) found that cucurbitacins B, E, I, J and K play a role as antigibberellins. For example, cucurbitacin B reduced the growth effect of GA_3 when applied to 7-day-old rice seedlings (9 µg of the compound reduced the growth effect of 5 µg GA_3 per plant by 50%). Similar growth effects were observed with other cucurbitacins. We have evaluated several of them in the bean second internode bioassay and found that they inhibit cell elongation (148). Three tetracyclic triterpenes, fasciculols (Figure 9), containing a lanostane skeleton have growth-regulating activity and were recently isolated from the fruit of Nematoloma fasiculare. Also found were the corresponding depsipeptides. All of these compounds, namely fasciculols A-F, inhibited elongation of chinese cabbage seedlings (149, 150).

(v) Tetraterpenes: Carotenes and other pigments belong to this group. Although there is no evidence concerning the growth-regulating activity of carotenoids, the photodegradation of these compounds appears to exhibit biological properties, e.g., violaxanthin. Abscisic acid and other inhibitors have been implicated to have arisen from the breakdown of these pigments.

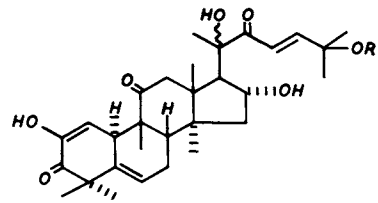
Steroids.

Several steroids including sterols, their glycosides, sex hormones and saponins have been implicated in plant growth regulation. Many of these compounds, particularly sterols and sex hormones (Table 6), show several growth-regulating properties (mainly stimulation) of several plant organs (151). For example, sitosterol stimulates the growth of 6-day-old dwarf pea (152). Moreover, sitosterol and lanosterol initiate flower buds in several species of Chrysanthemum (153). Ergosterol and cholesterol, on the other hand, inhibit root growth in excised pea embryos (154). The naturally occurring sterylglycoses derived from stigmasterol, sitosterol, lanosterol and cholesterol exhibit auxin-like activity in the Avena coleoptile bioassay (155).

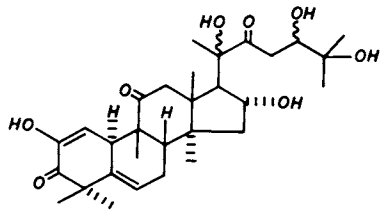
In Plant Growth Substances; Mandava, N.;
ACS Symposium Series; American Chemical Society: Washington, DC, 1979.



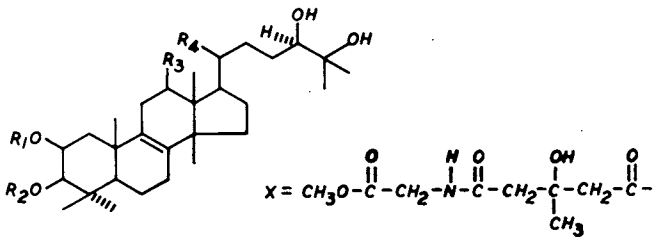
Cucurbitacin B



R=Ac, Cucurbitacin E
R=H, Cucurbitacin I



Cucurbitacin J and K
(Epimeric at C-24)



R_1=R_2=R_3=H; R_4=CH_3; FASCICULOL A (F-A)

R_1=R_2=H; R_3=OH; R_4=CH_3; FASCICULOL B (F-B)

R_1=R_2=H; R_3=OH; R_4=CH_2OH; FASCICULOL C (F-C)

R_1=X, R_2=H, R_3=OH, R_4=CH_3; DEPSIPEPTIDE OF F-B (F-D)

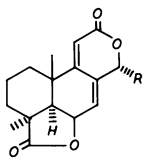
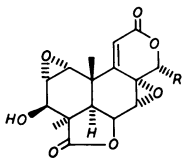
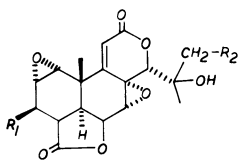
R_1=H, R_2=X, R_3=OH, R_4=CH_2OH; DEPSIPEPTIDE OF F-C (F-E)

R_1=X, R_2=H, R_3=OH, R_4=OH, R_4=CH_2OH; DEPSIPEPTIDE OF F-C (F-F)

Figure 9. Triterpenoids in plant growth regulation

In Plant Growth Substances; Mandava, N.;
ACS Symposium Series; American Chemical Society: Washington, DC, 1979.

Table 5. Diterpene Lactones

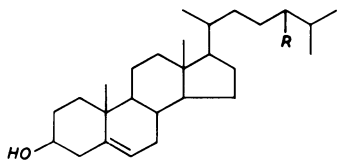
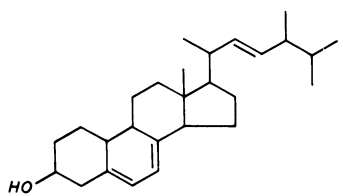
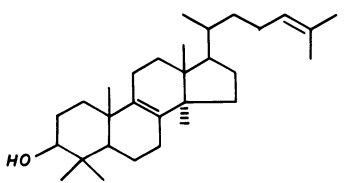
Compound	Structure	Source	Biological Activity	Reference
1. C_{16}-Terpene lactone ($R=CH_3$)		Acrostalagmus sp. NRRL-3481	Inhibits Avena coleoptile segments	132, 133
2. Sellowin-A		Podocorpus hellii	Inhibits growth of pea stem hook segments	134, 135
3. Inumakilactone A glucoside ($R=-CH(CH_3)-O-\beta$-glucose)		P. macrophyllus	Inhibits cell expansion and division	136
4. Inumakilactone B ($R=-CH=CH_2$)		P. neriifolius	Inhibits cell expansion in pea stem segments	135, 137, 138, 139, 140
5. Podolactone A ($R_1=H$, $R_2=OH$)		P. neriifolius	Inhibits cell expansion and mitosis of plant cells	137, 138, 144
6. Podolactone B ($R_1=R_2=OH$)		P. neriifolius	Inhibits cell expansion and mitosis of plant cells	137, 138, 144

In Plant Growth Substances; Mandava, N.;
ACS Symposium Series; American Chemical Society: Washington, DC, 1979.

7. *Podolactone E*		*P. nerriifolius*	*Inhibits cell expansion in pea stem segments*	135, 137, 138, 139, 140
8. *Nagilactone C*		*P. nagi*	*Inhibits cell expansion and mitosis of plant cells*	141, 142, 143
9. *Ponolactone A (R = H)*		*Pseudomonas nakii*	*Inhibits cell expansion and mitosis of plant cells*	145
10. *Ponolactone A glucoside (R = glucose)*		*P. nakii*	*Inhibits cell expansion and mitosis of plant cells*	145
11. *Momilactone A (R = H)*		*Oryza sativa L.*	*Inhibits the growth of rice roots*	130
12. *Momilactone C (R = OH)*		*O. sativa L.*	*Inhibits the growth of rice roots*	131
13. *Momilactone B*		*O. sativa L.*	*Inhibits the growth of rice roots*	130

In Plant Growth Substances; Mandava, N.;
ACS Symposium Series; American Chemical Society: Washington, DC, 1979.

Table 6: Effect of Steroids On Plant Growth

Compound	Structure	Growth Response	Reference
1. Sitosterol ($R=C_2H_5$)		a) Stimulates growth of 6-day-old dwarf P. sativum b) Initiates flower buds in Chrysanthemum	152, 153
2. Cholesterol ($R=H$)		a) Inhibits root growth in excised P. sativum embryo b) Promotes flowering and flower peduncle length in broccoli curd cuttings	154, 157
3. Ergosterol		Inhibits root growth in excised P. sativum embryo	157
4. Lanosterol		Stimulates flowering in Chrysanthemum sp.	155

In Plant Growth Substances; Mandava, N.;
ACS Symposium Series; American Chemical Society: Washington, DC, 1979.

5. Estrone ($R_1 = R_2 = O$, $R_3 = H$) 6. Estradiol-17β ($R_1 = OH$, $R_2 = R_3 = H$) 7. Estratriol ($R_1 = R_3 = OH$, $R_2 = H$)		Increase in the number of flowers of Ecballium elaterum and vegetative growth. Also influence sex expression	156
8. Testosterone ($R = OH$)		Decreases stem growth of excised P. sativum	154
9. Progesterone ($R = COCH_3$)		Increases vegetative growth	157
10. Cortisone ($R_1 = R_2 = O$) 11. Cortisol (Hydrocortisone) ($R_1 = H$, $R_2 = OH$)		a) Inhibit shoot growth of isolated pea embryo and increase number of flowers b) Stimulate Ervum lens seedling growth and also several plant species	157

In Plant Growth Substances; Mandava, N.;
ACS Symposium Series; American Chemical Society: Washington, DC, 1979.

The least understood of plant growth developmental processes is the flowering phenomenon and sex expression as influenced by plant growth substances. From a limited study, some steroids appear to exert influence on flowering. The sex hormones, estrone, estradiol and estriol, found in the seeds and pollen of date palm (Phoenix dactylifera) and pomegranate seeds, increase in the number of flowers of Ecballium elaterium and influence vegetative growth. They also appear to influence the sex expression in several plants (156). Testosterone, detected in the pollen of Pinus sylvestris, decreases the stem growth of excised Pisum sativum sections (154). Several corticosteroids, including cortisone and cortisol (11-hydroxycortisone), have been implicated in this growth phenomenon. For example, cortisone increases the number of flowers of E. elatum (157) while the female animal sex hormone, progesterone, isolated from Holarrihena florebunda, influences the vegetative growth of (E. elatum) (157). Androsterone and androstanedione, belonging to the androgens, cause a slight increase in the number of flower buds and change the sex ratio toward maleness (157).

Several saponins, such as digitogenin,diosgenin, digitoxigenin, tigogenin and hecogenin, also have been reported to influence in many ways the growth process of several types of plants (160, 161, 162, 163) as shown in Table 7. From the foregoing discussion, it is apparent that the steroids produced by plants are involved in eliciting many growth responses, a situation which warrants a more detailed study in order to fully understand their role in plant growth regulation.

Alkaloids and N-Heterocycles.

According to Swain (164), alkaloids (4500) constitute about one-half of the low molecular weight secondary metabolites (10,000) in higher plants and fungi. Very few of them have been tested for plant growth-regulating activity (Table 8). Tomatine, a triterpene alkaloid, has been shown to be a potent growth inhibitor (165). Tomatine and its aglycone, tomatidine, were isolated from several Lycopersicum species (166, 167). Narciclassine and narciprimene, found in several species of daffodils, possessed antimitotic activity (168, 169), while lycoricidinol and lycoricidine, isolated from the bulbs of Lycoris radiata Herb., showed growth-inhibiting activity on Avena coleoptile sections and rice seedling tests (170). These two compounds exhibit marked inhibitory action on cell division in tobacco tissue culture and have been shown to have carcinostatic activity. All four of these compounds are closely related to many Amaryllidaceae alkaloids, the biosynthetic scheme of which is similar to that of these compounds. Interestingly enough, narciclassine and lycoricidinol differ only in the location of a double bond and hydroxyl groups in ring C, and as Okamoto et al. (170) have pointed out, these two compounds are likely to have the same structure.

In Plant Growth Substances; Mandava, N.;
ACS Symposium Series; American Chemical Society: Washington, DC, 1979.

Delcosine and ajaconine are diterpene alkaloids that belong to the lycoctonine and atisine types, respectively, and were isolated from the seeds of common larkspur, Delphinium ajacis (171). In a pea cambium growth bioassay on phloem and xylem tissue, delcosine showed strong growth inhibition (GA_3 promotes growth response at 10^{-2} - 10^{-3}M in this test system), whereas ajaconine was devoid of activity at any concentration.

Several nitrogen-heterocyclic compounds, which can also be classified as alkaloids, have been shown to possess growth-regulating properties. Some representative compounds in this group are shown in Table 9.

Amino Acids and Peptides.

The effects of various plant amino acids on lettuce seed germination and seedling (hypocotyl and radicle) growth were reported recently (176). Seedling growth was inhibited more markedly than seed germination by these compounds (Table 10). Further, it was shown that the non-protein amino acids were more effective inhibitors of germination and seedling growth than were the protein amino acids.

Hoffinger et al. (177) showed that the naturally occurring betaines such as hypaphorine and oxyneurine induce growth promotion at low concentrations in the lentil root tip bioassay which is an auxin test system. The same compounds turned out to be inhibitors at higher concentrations.

A group of compounds that cause growth abnormalities was isolated from Aspergillus niger and termed "malformins." Malformin A was identified by the Curtis group (178) and was later synthesized by Bondansky and Stahl (179). This substance caused curvature on corn roots, showed inhibition of adventitious root formation in Phaseolus vulgaris, and also stimulated the growth of etiolated bean cuttings. Malformin C, also isolated from an A. niger source, was synthesized by Anderegg et al. (180) to confirm the structure. Biologically, it is similar to Malformin A (Figure 10).

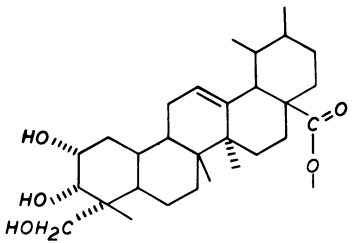
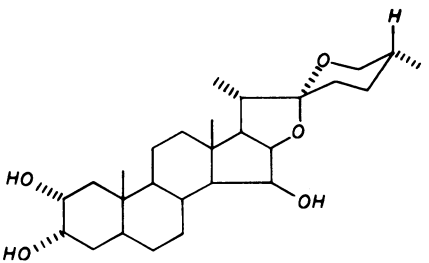
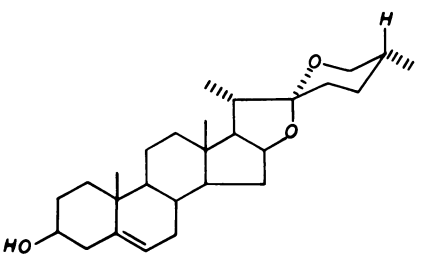
Three metabolites, Cyl-1, Cyl-2, Cyl-3, were found in the culture filtrate of Cylindrocladium scoparium (181, 182). Cyl-1 and Cyl-2 showed inhibition of root growth, whereas Cyl-3 promoted the growth of lettuce seedlings. The structure (cyclic peptide) was eluciated only for Cyl-2 (182) as shown in Figure 10.

Purines and Nucleosides.

Purines are best known, of course, as constituents of nucleic acids such as RNA and DNA in which purine and guanine are involved. If a monosaccharide sugar such as glucose is attached to purines, the resulting compounds are called nucleosides. An important class of compounds that belong to this group are cytokinins, all of which are derived from adenine (6-aminopurine) or adenosine (adenine glucoside). Cytokinins, belonging to a group of primary growth substances, are outside the scope of this

In Plant Growth Substances; Mandava, N.;
ACS Symposium Series; American Chemical Society: Washington, DC, 1979.

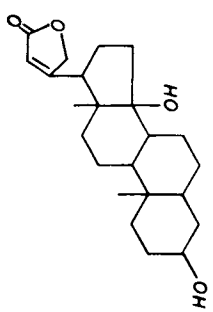
TABLE 7. SAPONINS AND RELATED COMPOUNDS ON PLANT GROWTH

COMPOUND	STRUCTURE	BIOLOGICAL ACTIVITY	REFERENCE
1. OXYASIATICOSIDE (A PENTACYCLIC TRITERPENE)		AT LOW CONCENTRATIONS, STIMULATE GROWTH AND CHLOROPHYLL SYNTHESIS IN RADISH, PEA AND LUPINUS X POLYPHYLLUS	160
2. DIGITOGENIN		INHIBITS THE ROOT GROWTH OF LEPIDIUM SATIVUM AND HORDEUM VULGARE	161
3. DIOSGENIN		STIMULATES PEA STEM AND ROOT GROWTH	162

In Plant Growth Substances; Mandava, N.;
ACS Symposium Series; American Chemical Society: Washington, DC, 1979.

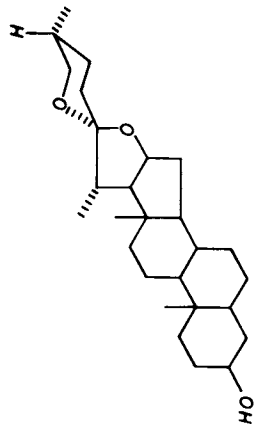
4. DIGITOXIGENIN

STIMULATES THE ADVENTITIOUS ROOT FORMATION ON TOMATO CUTTINGS 162



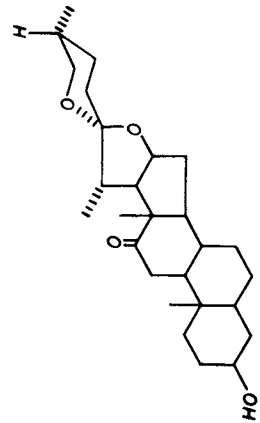
5. TIGOGENIN

STIMULATIVE EFFECT IN THE AVENA SECTION TEST 163



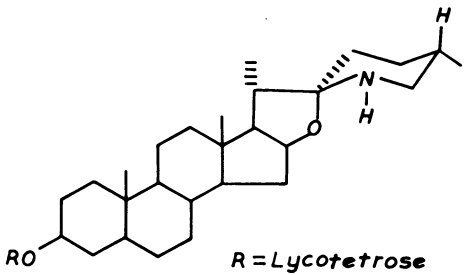
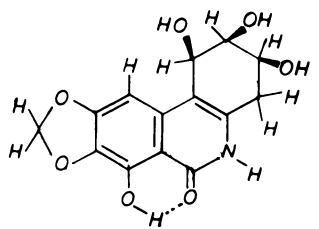
6. HECOGENIN

STIMULATIVE EFFECT IN THE AVENA SECTION TEST 163



In Plant Growth Substances; Mandava, N.;
ACS Symposium Series; American Chemical Society: Washington, DC, 1979.

TABLE 8. ALKALOIDS

COMPOUND	STRUCTURE	SOURCE	BIOLOGICAL ACTIVITY	REFERENCE
1. TOMATINE		LYCOPERSICUM PERUVIANUM, L. ESCULENTUM L. HIRSUTUM	INHIBITS SEEDLING GROWTH	165,166 167
2. NARCICLASINE		DAFFODILS (NARCISSUS TAZELTA L., N. INCOMPARITILIS MILL, N. PSEUDONARCISSUS L. AND N. TRIANDRUS L.)	ANTIMITOTIC ACTIVITY	168,169

In Plant Growth Substances; Mandava, N.;
ACS Symposium Series; American Chemical Society: Washington, DC, 1979.

3. NARCIPRIMINE		DAFFODILS (*NARCISSUS* SP.)	DEVOID OF ANTI-MITOTIC ACTIVITY 168,169
4. LYCORICIDINOL (R=OH)		*LYCORIS RADIATA* BULBS	INHIBIT *AVENA* COLEOPTILE GROWTH 170
5. LYCORICIDINE (R=H)		*L. RADIATA* BULBS	INHIBITS *AVENA* COLEOPTILE GROWTH 170
6. DELCOSINE		LARKSPUR (*DELPHINIUM AJACIS*) SEEDS	INHIBITS PHLOEM XYLEM TISSUE GROWTH IN PEA CAMBIUM BIOASSAY 171

In Plant Growth Substances; Mandava, N.;
ACS Symposium Series; American Chemical Society: Washington, DC, 1979.

TABLE 9. NITROGEN HETEROCYCLES WITH GROWTH REGULATING ACTIVITY

COMPOUND	STRUCTURE	BIOLOGICAL ACTIVITY	REFERENCE
1. FUSARIC ACID (5-N-BUTYL PICOLINIC ACID)		INHIBITS GROWTH OF SEVERAL PLANTS	173
2. α-PICOLINIC ACID		INHIBITS TOMATO SEED GERMINATION	99
3. ZEANIC ACID		PROMOTES RADISH COTYLEDON GROWTH AND RICE SEEDLING GROWTH	174

In Plant Growth Substances; Mandava, N.;
ACS Symposium Series; American Chemical Society: Washington, DC, 1979.

4. 2,6-DIHYDROXY-CINCHONIC ACID		GROWTH PROMOTION IN RICE SEEDLINGS	21
5. PAPAVARINE		INHIBITS SEED GERMINATION	21

In Plant Growth Substances; Mandava, N.;
ACS Symposium Series; American Chemical Society: Washington, DC, 1979.

In Plant Growth Substances; Mandava, N.;
ACS Symposium Series; American Chemical Society: Washington, DC, 1979.

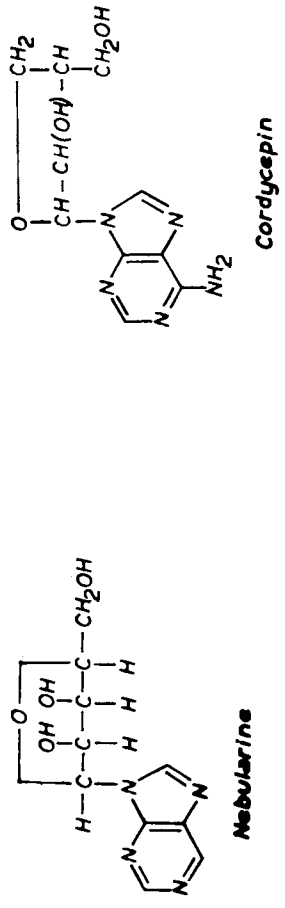


Figure 10. Polypeptides and purines involved in plant growth regulation

In Plant Growth Substances; Mandava, N.;
ACS Symposium Series; American Chemical Society: Washington, DC, 1979.

Table 10: Amino Acids and Peptides

Compound	Source	Biological Activity	Reference
1. Nonprotein and protein amino acids	Several plants	Inhibit seed germination and seedling growth lettuce	176
2. Betaines (hypaphorine and oxyneurine)	Erythrina sp. and Lycium barbarum	Stimulate at low concentration and inhibit at high concentration of lentil root tip growth	177
3. Peptides a) Malformin A	Aspergillus niger	Causes curvature of corn roots and inhibits adventitious root formation in Phaseolus vulgaris	178
b) Malformin C	A. niger	Causes curvature of corn roots and inhibits adventitious root formation in Phaseolus vulgaris	180
c) Cyclopeptides			
Cyl-1	Cylindrocladium scoparium	Inhibits lettuce root growth	181
Cyl-2	C. scoparium	Inhibits lettuce root growth	182
Cyl-3	C. scoparium	Promotes growth of lettuce seedlings	181

In Plant Growth Substances; Mandava, N.;
ACS Symposium Series; American Chemical Society: Washington, DC, 1979.

review with the exception of one example which is discadenin, isolated from a cellular slime mould, Dictyostalium discaideum (183, 184). Besides its potent spore germination inhibiting activity, discadenine exhibits cytokinin activity (about 2/3 the level of kinetin at 2.3 x 10^{-7}M) in the tobacco pith test (185).

Caffeine is one more example of compounds belonging to the purines. Evenari (21) has reported that it is one of the potent inhibitors of seed germination. Several substituted purines and nucleosides produced by microorganisms are effective antibiotics (see the Section 11). They include nebularine, cordycepin and nucleocidine which may also possess growth-inhibiting properties, as is the case with other antibiotics (Figure 10).

Sulfur Compounds.

Recent reports indicate that some sulfur compounds exhibit growth-regulating properties. Etiolated asparagus shoots contain some substances which stimulate pyruvate oxidation in asparagus itself as well as in Streptococcus faecalis 10C1. These substances (Table 11) were identified as asparagusic, dihydroasparagusic and S-acetyldihydroasparagusic acids (186, 187). Asparagusic acid S-oxide, which exists in syn and anti forms, was also found in asparagus shoots. These compounds showed inhibitory action with various kinds of plants, including seed germination. With the exception of the S-oxides of asparagusic acid, these compounds showed complete inhibition of growth of root and hypocotyl of lettuce seedlings at 6.09 x 10^{-4}M (186, 187, 188, 189).

Brugierol, isobrugierol, brugine and its hydroxy derivative were isolated from the stem and bark of Brugiera cylindrica and they are structurally related to asparagusic acids (190, 191, 192, 193).

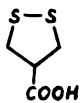
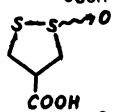
Papaya seeds contain a growth inhibitor, caricacin, which was shown to be N-benzyl-thiono-carbamic acid methyl ester (194). This compound (Table 11) inhibits the elongation of mung bean seedlings. Allicin, an essential ingredient of garlic, completely arrests bacterial growth (195).

Miscellaneous Natural Products.

Apart from the foregoing 10 major groups of organic compounds that appear to have originated from the two metabolic pathways (Figure 1), several other natural products could also be classified into those groups. Compounds such as antibiotics and phytotoxins were deliberately eliminated from the discussion because of their established identity and specific function in animals, plants and microorganisms. The plant growth regulatory role of these two classes will be covered here. Other compounds that are included in this section are some substances from lichens which have been examined recently for growth-regulating effects. A section on structural analogs of hormones is

In Plant Growth Substances; Mandava, N.;
ACS Symposium Series; American Chemical Society: Washington, DC, 1979.

Table 11. Plant Growth Regulating Activity of Sulfur Compounds

	Compound	Structure	Source	Biological Activity	Reference
1.	Asparagusic acid		Etiolated asparagus shoots	Inhibits lettuce root and hypocotyl growth at 10^{-4}M	186,187 188,189
2.	Dihydroasparagusic acid	$HS-CH_2-\underset{\underset{COOH}{|}}{CH}-CH_2-SH$	Etiolated asparagus shoots	Inhibits lettuce root and hypocotyl growth at 10^{-4}M	186,187 188,189
3.	S-Acetyldihydro-asparagusic acid	$Ac-S-CH_2-\underset{\underset{COOH}{|}}{CH}-CH_2-SH$	Etiolated asparagus shoots	Inhibits lettuce root and hypocotyl growth at 10^{-4}M	186,187 188,189
4.	Syn-and anti-asparagusic acid S-oxides		Etiolated asparagus shoots	Inhibits lettuce root and hypocotyl growth at 10^{-4}M	186,187 188,189
5.	Caricacin (N-Benzyl-Thiono-carbamic acid methyl ester)	$C_6H_5-CH_2-NH-\overset{\overset{S}{\|}}{C}-OCH_3$	Papaya seeds	Inhibits mung bean seedling elongation	194
6.	Allicin	$H_2C=CH-CH_2-\underset{\underset{H_2C=CH-CH_2-S}{|}}{S}=O$	Garlic	Inhibits bacterial growth	195

In Plant Growth Substances; Mandava, N.;
ACS Symposium Series; American Chemical Society: Washington, DC, 1979.

included here, even though many of them belong to terpenoids. Such a clear distinction seems necessary to bring it to the attention of chemists who might plan on synthesizing simple analogs or modified compounds; this could prove to be a practical and fruitful area of research, as was found to be the case with steroid hormone research (particularly for contraceptives).

(a) Antibiotics: These are mainly a group of natural products produced by microorganisms and have strong effects on other microorganisms. Several of them are potent inhibitors of plant growth, and some have growth-stimulating properties (196) as shown in Table 12. Wright (197) has compared the activity of antibiotics in seed germination and subsequent root growth with the activities of coumarin and IAA. He concluded that antibiotics had little effect on percentage germination; most were less toxic than IAA and more toxic than coumarin to root extension. The most toxic antibiotics were alternaric acid, gliotoxin, glutinosin and mycophenolic acid, and the least toxic were griseofulvin, pencillin and streptomycin.

(b) Phytotoxins: These are a group of natural products produced by microorganisms and have deleterious effects on plants. They exhibit growth inhibition at very low concentrations. As Lynch (198) has pointed out, phytotoxicity can be expressed as the inhibition of seed germination as well as the inhibition of subsequent root or shoot growth. Strobel (199) has classified phytototoxins into 4 categories, namely (i) glycoside phytotoxins, (ii) terpenoid phytotoxins, (iii) amino acid-derived phytotoxins, and (iv) miscellaneous toxins (shown in Table 13). Several of the toxins tested showed growth inhibition in several bioassays (200).

(c) Lichen Substances: Symbiotic organisms consisting of fungi and algae (Lichens) produce characteristic metabolites of different chemical structures, called "Lichen Substances." A few compounds of this group, e.g. (-)-usnic acid, have been reported to possess growth-regulating properties in lettuce seed germination (201) and dwarf maize growth (202). In a recent survey of lichen substances, Huneck (203) claimed that among 14 compounds tested in cress root growth, oat and pea seedling growth, and wheat coleoptile bioassays, mesoerythritol, planaic acid, usnic acid and lepranic acid showed growth promotion at 10^{-3} - 10^{-4}M; other substances such as caperatic acid, evernic acid, fumarprotocetraric acid, psoromic acid and leprapinic acid were strong inhibitors (10^{-3} and 10^{-7}M). Since usnic acid, widely distributed in lichen genera, is a potent inhibitor of algal growth, this compound can be regarded as a special growth regulator of lichens (203).

(d) Structure-Activity Relationship:

I. Natural products Eliciting GA_3-Type Activity: The term "GA-like activity" was first adopted by Phinney and West (204) for defining the biological activity of unknown compounds

In Plant Growth Substances; Mandava, N.;
ACS Symposium Series; American Chemical Society: Washington, DC, 1979.

Table 12. Effect of Some Antibiotics On Plant Growth (196)

ANTIBIOTIC	EFFECT ON PLANT GROWTH
1. Actidione, (Cycloheximide)	Inhibits roots, seed germination and foilage toxicity
2. Albidin	Inhibits seed germination and root growth
3. Bacillomycin A (=fungocin)	Inhibits seed germination and stimulate Ronuiex Cissae growth
4. Betracin	Inhibits root growth
5. Chloramphenical (=chloromycetin)	Inhibits root and shoot growth
6. Chlorotetracycline (=Aureomycin)	Inhibits root and shoot growth
7. Citrinin	Inhibits seed germination and root growth
8. Gladiolic acid	Inhibits seed germination and root growth
9. Gliotoxin	Inhibits seed germination and root growth
10. Glutinosin	Inhibits seed germination and root growth
11. Gramicidin	Inhibits seed germination and shoot growth
12. Griseofulvin	Inhibits seed germination, root and shoot growth, and foliage toxicity
13. Mycophenolic acid	Inhibits seed germination and root growth
14. Neomycin	Inhibits root and shoot growth
15. Oxytetracycline (=Terramycin)	Inhibits root and shoot growth and also stimulates growth in Reuiexiin
16. Penicillin	Inhibits root and shoot growth and stimulator in Renuiex taamie
17. Polymyxin	Inhibits root growth
18. Stemphylone	Inhibits seed germination and root growth and stimulate Renieux time growth
19. Streptomycin	Inhibits seed germination, root growth, shoot growth and foliage toxicity
20. Thiolutin	Inhibits root growth and stimulates Ruaexacelosa
21. Viridin	Inhibits seed germination and root growth

In Plant Growth Substances; Mandava, N.;
ACS Symposium Series; American Chemical Society: Washington, DC, 1979.

Table 13. Various Types of Phytotoxins

Glycoside Phytotoxins	Terpenoid Phytotoxins	Amino acid derived phytotoxins	Miscellaneous toxins
1. Helminthosporides (e.g. Helminthosporide)	1. Helminthosporal	1. Lycomarasmine and related compounds	1. Alternaria toxins
2. Oligosaccharides	2. Diacetoxyscirpenol	2. Tabtoxins	2. Naphthazarine toxins
3. Glycopeptides	3. Fusicoccin	3. Rhizobitoxine	
4. Lipomucopolysaccharides	4. Ophiobolins (e.g. Ophiobolin A)	4. Tentoxin	
5. Polysaccharides		5. Periconia toxins	
		6. Pseudomonas phaseolicola toxins	
		7. Helminthosporium carborum toxins	
		8. Syringomycin	

In Plant Growth Substances; Mandava, N.;
ACS Symposium Series; American Chemical Society: Washington, DC, 1979.

obtained from plant extracts. The same approach was followed to define the activity of other hormonal responses (e.g., ABA-like and IAA-like compounds). Consequently, the literature was flooded with numerous reports that resulted in a complete uncertainty as to whether such hormone-like compounds are really plant hormones, particularly when attributing them to the mode of action of hormones. The present discussion is not confined to this subject area.

Some natural products, because of either a close structural similarity or a skeletal resemblance (e.g., a portion of the molecule with the required sterochemistry or functional groups) essential for activity, appear to produce the same or similar activity as that elicited by a plant hormone, such as GA_3. In other words, to exhibit biological activity, the entire molecule (such as the tetracyclic ent-gibberellane nucleus) may not be required and only a portion of the molecule with either the proper configuration or sterochemistry and/or functional groups would produce the same or enhanced GA-like activity. A few examples to illustrate such a situation are the metabolites, helminthosporol, helminthosporal, the corresponding glycol, helminthosporic acid and dihydrohelminthosporic acid (Figure 11), all of which were obtained from the fungus, Helminthosporium sativum (204). All of these products showed stimulation similar to that of GA_3 when tested in the lettuce hypocotyl and other bioassays (205, 206, 207, 208, 209). Because of close structural similarities in the C/D ring system of both types of compounds, it has been assumed that such a ring system (C/D) is essential for exhibiting biological activity (210). Another related compound with a fused bicyclic ring system, such as the one present in the above examples, is cis-sativenediol, which was isolated from a pathogenic fungus, Colchiobolus setarial and also from H. sativum. This substance (Figure 11) promoted the elongation of rice seedlings and its activity is very similar to that of GA_3 (210). Steviol, an aglycone obtained by enzymatic hydrolysis of a sweetner, stevioside, from the leaves of Stevia rebaudiana (211), showed GA_3-type activity in lettuce and cucumber hypocotyl elongation tests and also in the growth of bean plants (212, 213). The foregoing examples suggest that some such structural features common to GA_3 as to ring size, ring junction sterochemistry, absolute configuration and functional groups such as hydroxyl and exomethylene groups must be required to exhibit the desired biological activity.

Gibbane (ent-gibberellane) type compounds were previously thought to occur only in the fungus Gibberellan fujikuroi and in higher plants. Recent work indicates that such compounds may also be produced by other organisms such as ferns. Antheridiogen (A_{An}), from antheridium-inducing fern, Avenia phyllitidis, is an example of a compound which has GA_3-type activity, but to a lesser degree (214, 215).

In Plant Growth Substances; Mandava, N.;
ACS Symposium Series; American Chemical Society: Washington, DC, 1979.

II. ABA-Type Compounds: Several metabolites of ABA and many analogous compounds bearing some structural features (β-ionone moiety) were recently found in different plant sources. For example, cis, trans-xanthoxin, a photooxidation product of a carotenoid, violaxanthin, is a potent inhibitor of germination and its activity is comparable to that of ABA (216). It has been postulated that xanthoxin must have been converted into ABA in the tested plant tissues to exhibit ABA-like activity (216). Vomifoliol (Blumenol A) was also as active as ABA in stomatal closure, but was inactive in senescence and abscission tests (217). Queisone (5-isobutyroxy-β-ionone (Figure 12) was isolated from infected tobacco (Nicotiana tabacum) plants (218, 219) and inhibited the germination of Peronospora tabacina at 0.001 ppm (220, 221). Although the biological data on several other compounds such as theaspirone, blumenols B and C, damascone and damascanone were not readily available, it is assumed that at least some of them possess ABA-like activity because of the close structural similarities (common β-ionone moiety) to ABA.

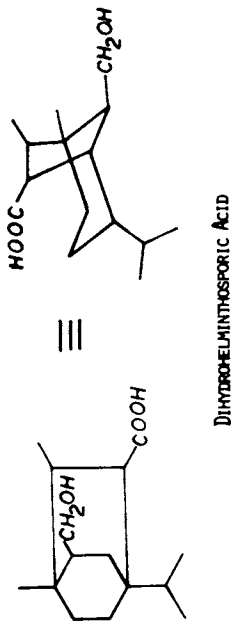
(e) Other Inhibitors: Sugar beet fruits contain cis-4-cyclohexene-1,2-dicarboxyimide which inhibits lettuce seed germination (222). Crow et al. (223) isolated from Eucalyptus grandis, three inhibitors (G_1, G_2 and G_3) the structures of which were determined by chemical and X-ray crystallographic methods. A few of the compounds containing a 5-membered ring system such as jasmonic acid (224) and cucurbic acid (225) were reported to possess growth-regulating properties. These acids are structurally related to prostaglandins which are yet to be detected in the plant kingdom (Figure 12).

New Plant Growth Regulators From USDA Laboratories

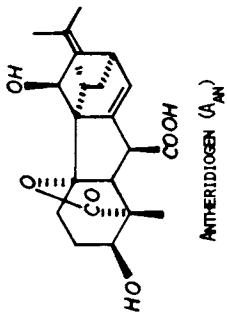
To understand the role of secondary plant products in plant growth and development and also to explore their potential use in crop improvement and protection, several USDA laboratories have been carrying out investigations to isolate and identify new growth substances, some of which are described here. A germination stimulant for the root parasite, witchweed (Striga lutea Lour.) was identified as strigol (226) (Figure 13). This compound is active at hormonal levels, causing germination at concentrations less than 10^{-8}M (226, 227). The structure for strigol was confirmed recently by synthesis independently by American (228) and British groups (229, 230). Strigyl acetate, also isolated from the same source, was found to be as active as strigol (226, 227).

A phytotoxin from the nodules produced by Rhizobium japonicum on the roots of soybean (Glycine max (L.) Merr.), termed rhizobitoxine, was identified by Owens et al. (231, 232, 233) as 2-amino,4-(2-amino-3-hydroxypropyl)-trans-but-3-enoic acid. Another closely related analog of rhizobitoxine,

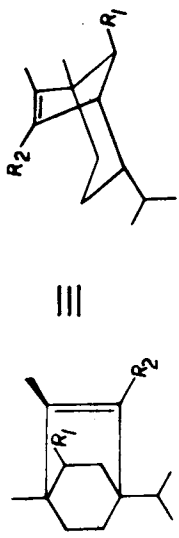
In Plant Growth Substances; Mandava, N.;
ACS Symposium Series; American Chemical Society: Washington, DC, 1979.



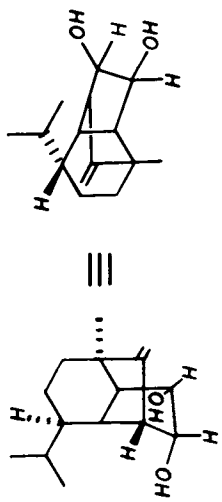
DIHYDROHELMINTHOSPORIC ACID



ANTHERIDIOGEN (A_{AN})



1) R_1=CH_2OH; R_2=CHO, HELMINTHOSPORAL
2) R_1=R_2=CHO, HELMINTHOSPORAL
3) R_1=R_1=CH_2OH, GLYCOL
4) R_1=CH_2OH, R_2=COOH, HELMINTHOSPORIC ACID



CIS-SATIVENEDIOL

In Plant Growth Substances; Mandava, N.;
ACS Symposium Series; American Chemical Society: Washington, DC, 1979.

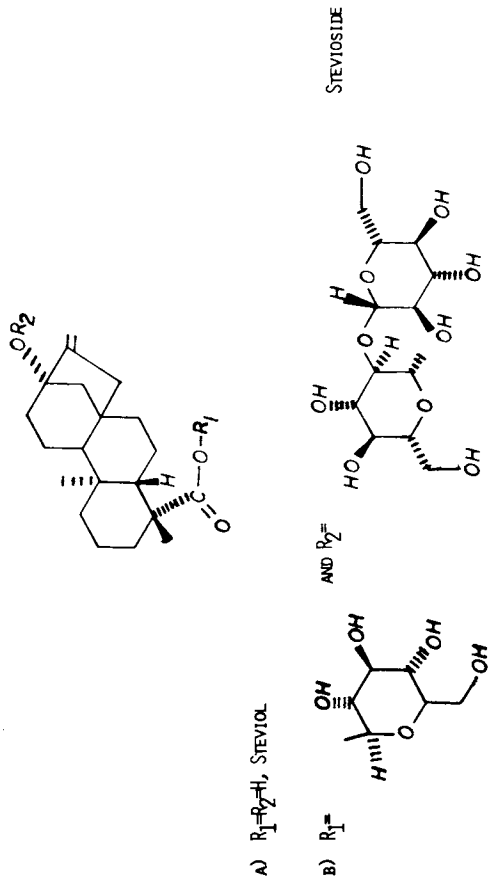
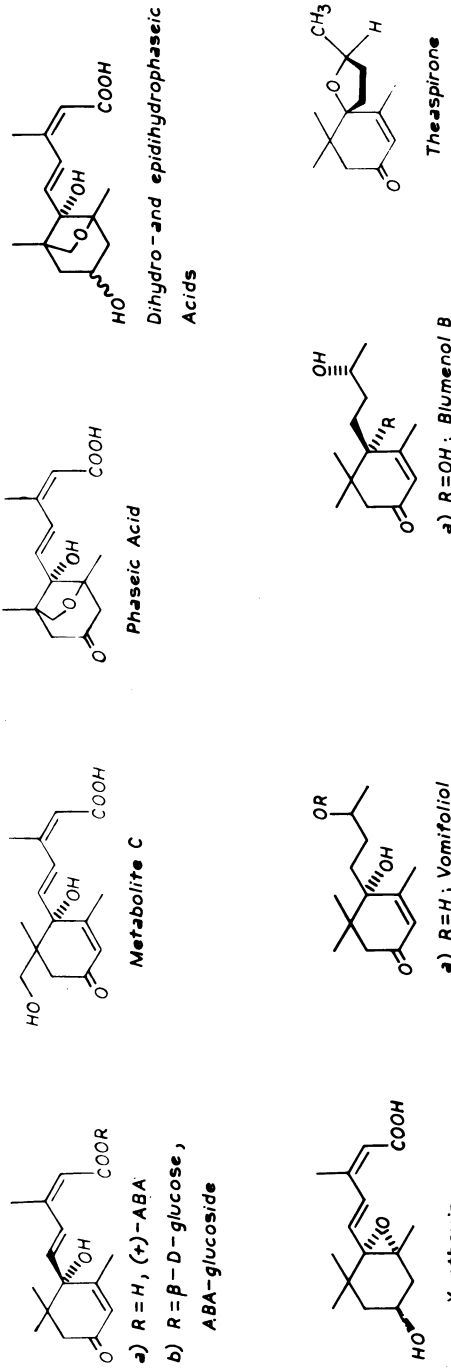


Figure 11. Compounds with GA-like activity



In Plant Growth Substances; Mandava, N.;
ACS Symposium Series; American Chemical Society: Washington, DC, 1979.

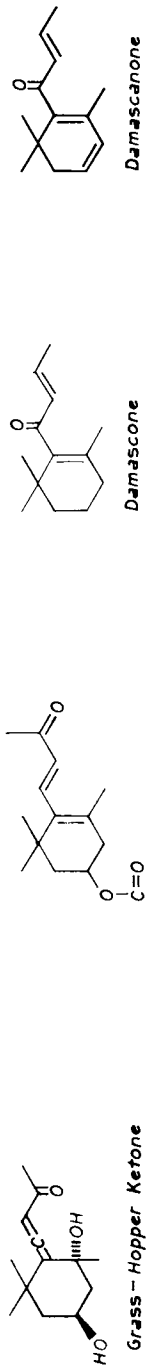


Figure 12. ABA-type compounds and other growth inhibitors

In Plant Growth Substances; Mandava, N.;
ACS Symposium Series; American Chemical Society: Washington, DC, 1979.

dihydrohizobitoxine which is O-(2-amino-3-hydroxypropyl) homoserine, was also found from the same source (234). In addition to phytotoxic properties, rhizobitoxine inhibits the production of ethylene, a process mediated through methionine (235). It also inhibits the chlorophyl synthesis and root growth in mustard (Brassica japonica L.) plants (236).

Chemical control of tobacco bud growth is of considerable economic importance. However, because of the health hazards implicated by the use of some synthetic chemicals such as maleic hydrazide presently being used to control the axillary and lateral bud growth, a search has been conducted for possible natural substitutes. Cutler and his associates (237, 238, 239) successfully isolated a growth inhibitor from Hicks variety tobacco and identified it as 4,8-13-duvatriene-1,3-diol (Figure 13) which belongs to the cambrene family. This diol exists in two forms (A and B) and is an active tobacco growth inhibitor.

Several diterpenes present in tobacco plants were evaluated by Cutler (240) for growth-regulating activity in wheat coleoptile bioassays. Except for isodihydroabienol and β-levantenolide, all of the endogenous tobacco diterpenes showed growth inhibition of the wheat coleoptiles at concentrations of 10^{-3}M or less (Table 14).

Cantharidin (Figure 13), isolated from spanish fly (Lylta vesicatoria L.), inhibits the growth of very young lupin (Lupinus albus L.) seedlings, Medico sativa L., Raphanus sativus L. and Brassica napus L. and Brassica napus L. seed germination at 20 ppm. It also inhibits the growth of wheat coleoptiles, corn, tobacco and bean plants (241, 242).

A novel fungal metabolite, hydroxyterphenyllin (242) from Aspergillus candidus found in unbleached flour was shown to be an active inhibiting ingredient when tested in wheat coleoptile bioassays at 10^{-3} - 10^{-5}M. Terphenyllin (244, 245) was also identified from the same source but was less active (at 10^{-3}M). The new compound (243) was shown to be a potent growth inhibitor by Cutler's group.

One of the metabolites of the trichothecene family of mycotoxins, termed "neosolanoil" (246) was isolated from Florunner peanuts and identified as 3α-hydroxy-4β, 8α-15-triacetoxy-12,13-epoxytrichothec-9-ene. This toxin was shown to be a potent plant growth inhibitor active to 10^{-6}M in the wheat coleoptile bioassay.

Cutler and his group (247) have also isolated cytochalasin H (or paspalin PI) from a parasite fungus, (Phomopsis sp. (Diaporthe is the perfect state of the fungus) which is often associated with leaf spot and diebark conditions of certain plants. This compound showed marked inhibition of growth and floral development of tobacco plants at concentrations of 10^{-2} and 10^{-4}M.

Other USDA research on natural products in relation to plant growth and development includes the development of tobacco

In Plant Growth Substances; Mandava, N.;
ACS Symposium Series; American Chemical Society: Washington, DC, 1979.

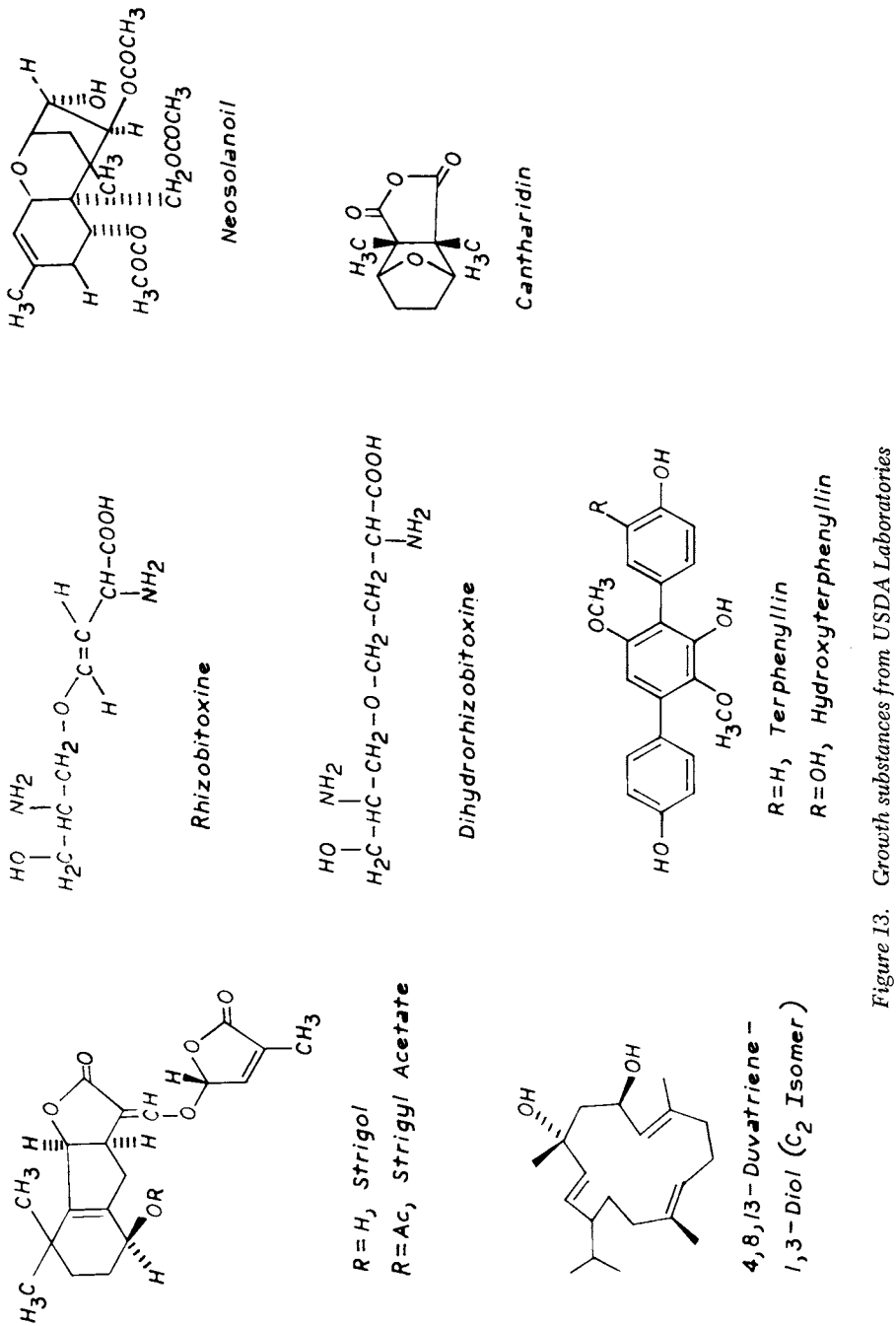


Figure 13. Growth substances from USDA Laboratories

In Plant Growth Substances; Mandava, N.;
ACS Symposium Series; American Chemical Society: Washington, DC, 1979.

sucker control agents (43, 44, 44a) and chemical pruning agents (45, 46) using short-chain fatty acids and their esters and fatty alcohols which have been described previously (Table 4).

In a search for safer biodegradable plant growth substances that may have potential uses in agriculture, particularly for crop production, our laboratory has developed some new bioassay systems to screen various plants for growth-regulating activity. Our screening efforts resulted in the discovery of both growth promoters and inhibitors.

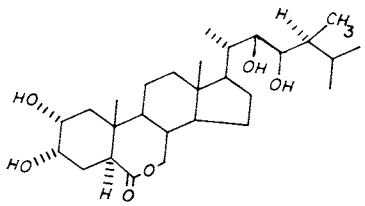
A new plant growth promoter, termed brassinolide (Figure 14) was found in rape (Brassica napus L.) pollen. The purified pollen extract, termed "brassins" (248) after the genus Brassica, is composed of a complex mixture of lipids and caused cell elongation and cell division in the treated internodes of bean plants. The active component, brassinolide, causes splitting of the bean internodes, enhances auxin-mediated response in bean hypocotyl sections (249), leaf expansion in lettuce seedlings and epicotyl elongation of mung bean seedlings (250). Similar biological responses due to brassin treatment were reported by Yopp and co-workers (251). A concentrated team effort by chemists at Beltsville Agricultural Research Center, Eastern Regional Research Center and Northern Regional Research Center led to the final identification of the active component, brassinolide, by extracting 500 lbs. of rape pollen (252). Brassinolide has now been identified as a novel steroidal lactone by a combination of several spectroscopic methods and X-ray crystallography (253). This compound, present at ppb levels in the pollen, has not yet been found in any other plant part. It appears to be unique both in chemical structure and in biological activity.

Among the compounds that have been isolated from the brassins were a new group of glucosyl esters of fatty acids, the structures of which were established by spectral methods (254, 255) as well as by chemical and biochemical synthesis (256, 257, 258). Although these esters were much less active than brassinolide in the bean second internode bioassay, their presence may be essential for seed germination since the enzymatic synthesis of these esters was correlated to germination (257, 258).

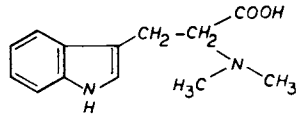
A growth inhibitor, identified as N,N-dimethyl-tryptophan (259) was found in jequirity bean (Abrus precatorius L.) seeds (260) and this substance inhibited the seedling growth of lettuce and many other plants. It also inhibited auxin-induced responses in several test systems, but not in pea stem sections (261). As shown earlier, phenylacetic acid and its derivatives exhibit plant growth-regulating activity. We have found that a pathogenic fungus, Rhizoctonia solani, contains m-hydroxy- and m-methoxyphenyl acetic acids (Figure 13) as phytotoxins that cause elongation in bean second internodes and also inhibit the hypocotyl growth of the lettuce seedlings (262).

As discussed elsewhere, several long-chain fatty alcohols show growth-regulating properties. We have isolated docosanol

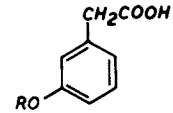
In Plant Growth Substances; Mandava, N.;
ACS Symposium Series; American Chemical Society: Washington, DC, 1979.



Brassinolide

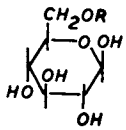


N,N-Dimethyl Tryptophane



R=H, m-Hydroxyphenylacetic Acid

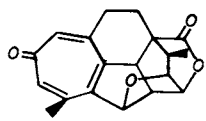
R=CH_3, m-Methoxyphenylacetic Acid



R= $\overset{O}{\overset{\|}{C}}-(CH_2)_n-CH_3$

n = 14-18

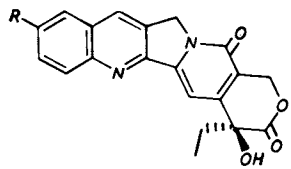
Glucosyl Esters of Fatty Acids ($C_{16}-C_{20}$)



Harringtonolide

$CH_3(CH_2)_{20}CH_2OH$

1-Docosanol

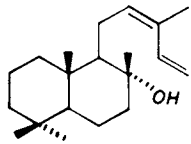
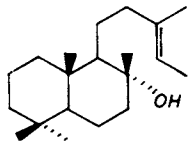
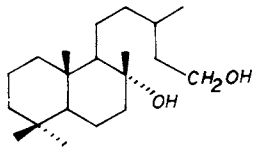
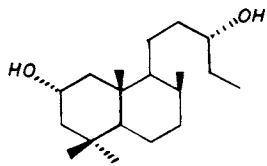
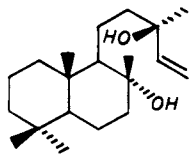
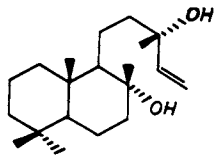


R=H, Camptothecin

R=OCH_3, 10-Methoxycamptothecin

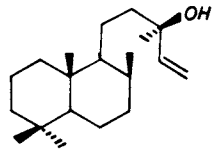
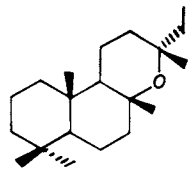
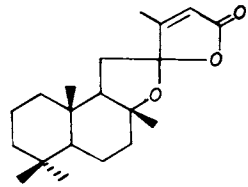
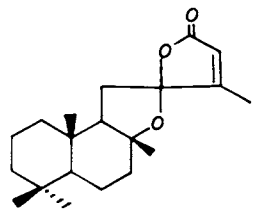
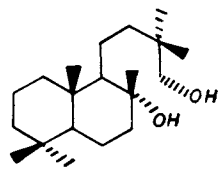
Figure 14. New growth substances from author's laboratory

TABLE 14. INHIBITORY ACTIVITIES OF SOME TOBACCO

COMPOUND	STRUCTURE	INHIBIT ACTIVITY AT CONCENTRATION
1. cis-ABIENOL		10^{-3} - 10^{-4}M
2. ISODIHYDROABIENOL		INACTIVE
3. (13-E) LABDA-1,12-ENE-8,15-DIOL (N-4)		10^{-3} - 10^{-4}M
4. 2-HYDROXYMANOOL		10^{-3} - 10^{-4}M
5. SCLAREOL		10^{-3} - 10^{-4}M
6. EPI-SCLAREOL		10^{-3} - 10^{-4}M

In Plant Growth Substances; Mandava, N.;
ACS Symposium Series; American Chemical Society: Washington, DC, 1979.

DITERPENES IN WHEAT COLEOPTILE BIOASSAY

COMPOUND	STRUCTURE	INHIBIT ACTIVITY AT CONCENTRATION
7. MANOOL		10^{-3}M
8. MANOYL OXIDE		10^{-3}M
9. α-LEVANTENOLIDE		10^{-3}M
10. β-LEVANTENOLIDE		INACTIVE
11. LABDANEDIOL		10^{-3} - 10^{-4}M

from the cotton fibers (263). 1-Docosanol was active in auxin bioassays and the same compound was also reported to be present in tobacco (264).

Our Laboratory is also involved in evaluating the plant products of medicinal interest for plant growth-regulating activity. Our main source of material is a worldwide plant collection obtained in conjunction with a search conducted by the National Cancer Institute for unique chemicals potentially useful in cancer treatment. It has been found that camptothecin, an alkaloid (267) tested in clinical trials in cancer study, is a selective growth inhibitor (265, 266). This compound inhibits tobacco axillary bud growth, root initiation in mung bean and also the growth of several vegetable plants such as tomato and potato.

A new growth inhibitor, harringtonolide (Figure 14) with tropone and lactone moieties, was also isolated from the seeds of Cephalotaxus harringtonia and its structure was assigned by spectroscopic analysis and X-ray crystallography (268). This compound inhibited the axillary bud growth in tobacco at 10^{-3}M and was also an effective growth inhibitor when tested in the bean second internode bioassay.

Growth Substances Involved in Biochemical Interactions between Plants in Natural Habitat - Allelopathy

It has long been recognized that when different species of plants grow together in a natural habitat, some plants influence (antagonize) the growth of other plants in the surrounding environment. The phenomenon (269) of such an influence under natural conditions and exerted by biochemical means other than nutritional ones, has been called "allelopathy." The original definition of allelopathy by Molisch (270) as being the beneficial as well as the detrimental biochemical interactions between all types of plants (including microorganisms) has largely been applied to higher plants by Rice (271) who uses this term as follows: "any direct or indirect harmful effect of one plant (including microorganisms) on another through the production of chemical compounds that escape into environment."

In 1907, Livingstone (272) and other workers (273) in the United States Department of Agriculture reported that the water extracts of soils, where certain crops had been grown continuously, inhibited the growth of wheat and other crop seedlings. At that time no explanation was offered for this observation. Later, several workers demonstrated the toxic effects of root exudates, leaf leachates, and soil extracts on seed germination, seedling growth, and other plant growth phenomena. We now know that several growth inhibitors are responsible for the allelopathic effects. Audus (274) divides such inhibitors into 4 groups (Table 15) based on their source of isolation as shown

In Plant Growth Substances; Mandava, N.;
ACS Symposium Series; American Chemical Society: Washington, DC, 1979.

Table 15. Growth Inhibitors in Allelopathy (274)

Inhibitors in		Volatile Inhibitors	Sick Soil Toxicants
Root Exudates	Leaf Leachates		
1. Allicin	1. 3-Acetyl-6-methoxybenzaldehyde	1. Ethylene	1. Decomposition products of amygdalin (e.g. HCN)
2. Chlorogenic acid	2. Genistic acid	2. β-Pinene	2. Phlorizin
3. Melilotic acid	3. Dihydroxy-stearic acid	3. Camphene	3. Phloretin
4. Gallic acid	4. Protocatechuic acid	4. Cineole	
5. o-Coumaric acid	5. Caffeic acid		
6. Piperic acid	6. Syringic acid		
7. 2-Furanacrylic acid	7. p-Hydroxy-benzoic acid		
8. Juglone (β-hydroxy-naphthoquinone)			
9. p-Hydroxy-benzaldehyde			
10. Phenylpropianic acid			

In Plant Growth Substances; Mandava, N.;
ACS Symposium Series; American Chemical Society: Washington, DC, 1979.

below:

(1) Root exudates: A wide variety of chemicals such as sugars, amino acids, and aromatics, is excreted by roots of plants. Very little information is available on the allelopathic interaction of root exudates with the higher plants, except for the identification of a few products in isolated cases.

(2) Leaf leachates and decomposition products: Inhibitors from leaves may be washed into the soil or compounds from leachates further decompose into toxic products which inhibit seed germination and prevent seedling establishment.

(3) Volatile toxicants: A great many volatile substances, including gaseous compounds and volatile terpenoids, are involved in allelopathy.

(4) "Sick soil" toxicants: Accumulation of toxicants from previous inhabitants evidently results in growth inhibition of roots and seedling establishment.

Although much of the published information on allelopathy deals with the toxic effects of compounds, it is also possible that some of the secondary plant products released into the environment possess growth-promoting properties, thereby stimulating the growth of plants. They may help to control weeds, plant diseases, and many pests. It is estimated that in the United States alone the losses caused by weeds, diseases, and pests are about 30% of the potential agricultural production. When translated into dollar values, the losses amount to more than $20 billion each year. Proper understanding of allelopathic processes, particularly for plant growth, could lead to several beneficial effects (for example, biochemically control of weeds) on agricultural production.

Mechanism of Action

When one observes that a certain secondary plant product exerts a growth-regulating activity, obviously the first question that concerns the physiologist is its mode of action. From the foregoing discussion, we know that several diversified organic compounds exhibit different growth phenomena in isolated or intact plant systems. But it is difficult to discuss the mechanism of action of these natural products because of:

1) Complications in separating the secondary effects from the primary causes,

2) The uncertainty in translating the observed effects in isolated enzyme and other biochemical systems to intact plant systems,

3) The lack of understanding of the effect of these substances on whole plant photosynthetic processes (e.g. changes in stomatal opening, membrane permeability, water content, or many other processes that affect the overall photosynthetic process). However, from the available information, an attempt has been

In Plant Growth Substances; Mandava, N.;
ACS Symposium Series; American Chemical Society: Washington, DC, 1979.

made here to bring some generalizations concerning the mode of action of natural products. Also, it must be pointed out that the mode of action of most secondary growth substances is implicated through the mediation of plant hormones.

1) Cell Division and Cell Elongation.

The main criteria used in determining the effectiveness of the use of natural products are the changes in weight and size of the test organisms. Appreciable increases in either of these require cell elongation and cell division. Evidence presented on the plant test organisms with the natural products indicates either an increase or a decrease in the cell elongation or cell division. For example, coumarin blocks all mitosis (prevents the entry of cells into mitosis) in onions and lily roots similar to those effects observed with colchicine (275). Similarly, parasorbic acid prevents the inception of mitosis (276). Trans-cinnamic acid and juglone showed a marked decrease in number of root cells of peas (Pisum sativum) and this decrease may be interpreted as an effect on the mitotic activity. Other examples cited earlier, namely scopoletin, and several terpenoid compounds, prevent mitosis in roots and other test organs.

Several compounds tested in our laboratory showed either marked increase or decrease in cell elongation and cell division. For example, brassinolide enhances cell enlargement and cell proliferation, whereas camptothecin completely inhibits the growth of the test organs, apparently blocking all mitosis.

2) Effect on Gibberellin or Auxin-Induced Growth.

Considerable work has been done on many natural products affecting the gibberellin- or auxin-induced growth in many test systems. Particularly, the mode of action of non-specific growth substances such as the phenolic compounds (e.g., substituted benzoic and cinnamic acids, coumarins and flavonoids) has been implicated with the IAA-oxidation system. These compounds (Tables 2-4) appear to act as substrates on the IAA-oxidase-peroxidase enzyme system, thereby increasing or decreasing the oxidizing (decarboxylating) activity of IAA. For example, p-hydroxybenzoic acid decreases the growth of segments in IAA (277) and at the same time increases the rate of decarboxylation of IAA (278, 279). Polyphenols (including methyl ethers) such as guaicol and pyrogallol, on the other hand, were reported to inhibit the activity of the IAA oxidation system (280, 281). These polyphenols also show synergistic effects with IAA (growth promotion), at least in the Avena curvature bioassay (282). In other words, monophenols are active in promoting the oxidation of IAA, whereas di- and polyphenols show the inhibitory effects on IAA oxidation system. Putting it in a different perspective, monophenols tend to destroy IAA in plant tissue, while the latter groups protect IAA (i.e., they maintain the IAA levels intact in

In Plant Growth Substances; Mandava, N.;
ACS Symposium Series; American Chemical Society: Washington, DC, 1979.

the tissue). Coumarin and its derivatives appear to fit the same explanation described for phenols. Monophenols such as umbeliferone promote the IAA oxidation system, while polyphenols (including methyl ethers) such as scopoletin inhibit it. The mode of action for other nonspecific growth substances may follow the same patterns as in phenols. p-Quinones may be considered as p-diphenols (converted by the photosynthetic chain) and certain amino acids (tyrosine and dihydroxyphenylamine, DOPA) are mono- and o-diphenols. Tannins are also phenolic in this context. The flavonoids also fall under mono-, di- and triphenols. Amines may function like phenols and act also as either promoters or inhibitors of IAA oxidation system. For example, benzidine is a potent inhibitor of IAA-oxidation in tissue culture (283).

Several of the secondary growth substances such as phenolics, antibiotics, and alkaloids (narcotics) not only affect the IAA-induced growth but also inhibit gibberellin-induced elongation (284). Many natural products that affect seed germination were specifically attributed to the GA-induced growth responses (285), since it has been elegantly demonstrated that the gibberellins (e.g. GA_3) are responsible for the germination of seeds including cereals such as barley which on GA treatment synthesize de novo a host of hydrolytic enzymes (α-amylase, proteanase, ribonuclease, and endo-β-gluconase) (285). A similar mechanism of GA-induced enzyme activity (286) was shown in several plant tissues (increased invertase activity in Avena sativum, Zea mays, Jerusalem artichoke, sugar cane, and beet root) and changes in nucleic acid metabolism were also attributed to GA interaction. Corcoran (287) has found that the growth substances from Carob (Ceratonia siliqua) inhibit only GA_3-induced growth, but not that of the IAA-induced growth. Cucurbitacins also inhibit only GA_3-induced growth in rice seedlings (288). Several tannins and a number of phenolic compounds inhibit only GA-induced growth in cucumber and dwarf pea seedlings (289).

3) Effect on Sulfhydryl Enzymes.

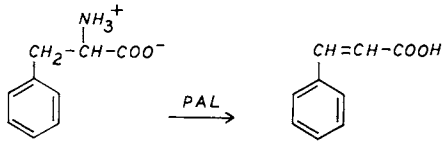
One possible explanation for the mode of unsaturated lactones such as coumarin, protoanemonin, and ν-pyrone-2,6-dicarboxylic acid is to inhibit the action of sulfhydryl enzymes essential for growth. This occurs as a result of a chemical reaction between the ν-lactone and cysteine. The growth-inhibiting effects of these lactones are prevented by 1,2-dimercaptopropane (an - SH contributor) known as BAL (290). This was proven for split pea stem curvature and also for coleoptile growth (290). The presence of auxin appears to shift the range of inhibiting concentration to higher levels, as shown with chelidonic acid or ν-pyrone-2,6-dicarboxylic acid on the growth of pea stem segments. With coumarin, the inhibition is reversed by BAL. In substituted coumarins, additional effects as shown earlier (phenolics on IAA oxidation system) were also involved.

In Plant Growth Substances; Mandava, N.;
ACS Symposium Series; American Chemical Society: Washington, DC, 1979.

Terpenoid lactones appear to follow the same mechanism of action. For example, the effect of heliangine on root growth was also due to the unsaturated lactone moiety acting as a sulfhydryl inhibitor (like coumarin).

4) Effect on Ethylene Production.

Release of ethylene has been implicated with the response of some growth substances. Phenylalanine ammonia-lyase (PAL) has



been reported to be an ethylene-releasing enzyme that appears to be involved in the formation of cinnamic acid from phenylalanine. Thus, there is a close relationship between ethylene and possible growth regulatory effect. The induction of PAL in citrus fruit peel is controlled by ethylene (291). Fuchs (292) has substantiated the PAL hypothesis by demonstrating that various phenol derivatives stimulate ethylene production in orange fruit peel. It was further shown by Morgan and Powell (293) that phenolic compounds such as coumarin stimulate ethylene production in other test systems such as an etiolated bean hypocotyl hook bioassay. According to Ilag and Curtis (294) who investigated ethylene production in 228 species of fungi, release of ethylene is a common factor for fungi, thus suggesting further that ethylene is involved in the growth phenomena of all plants. The results of Owens *et al*. (295) with rhizobitoxine indicate that the inhibition of ethylene production by this toxin in sorghum and apple tissues is mediated through methionine and not from the synthesis of methionine. Further work is needed to gain insight of ethylene involvement in growth regulation.

From the foregoing discussion, it is evident that we have explored only few parameters affecting plant growth processes. There are several others that may have either direct or indirect influence on growth and development. These include: 1) mineral uptake, 2) photosynthesis and respiration, 3) stomatal opening 4) protein synthesis and changes in lipid and organic acid metabolism, 5) hemoglobin synthesis, 6) membrane permeability, 7) specific enzymes such as cellulase, catalase, peroxidase,

In Plant Growth Substances; Mandava, N.;
ACS Symposium Series; American Chemical Society: Washington, DC, 1979.

phosphorylase, pectolytic and other enzymes, 8) flowering and 9) miscellaneous interactions. So far we have very limited knowledge available on natural products and hope that the future will uncover the implications of these substances in their mechanism of action, thereby allowing fuller appreciation of overall growth phenomena.

Problems and Prospects

When one considers the diverse occurrence of several thousand natural products from animal and plant sources and when some of these have shown great potential in palnt growth regulation, a few questions that often arise are:

1. What significant role do these natural products play in the metabolism of plants where they are produced (in the host plants)?
2. Are the secondary substances that exhibit growth-regulating properties actually involved in controlling the growth and developmental processes of the host plants?
3. What are the control mechanisms involved in growth for these substances?
4. How can one make use of these substances in improving the quality of crops and the environment in which we live? What research approach seems the most logical?

It seems difficult to answer these and many other related questions at this time because the field of plant growth substances is still in the stage of infancy. In the past, the foremost problem that had to be confronted in this field was the lack of suitable methods for isolation and characterization of the growth substances that were present in minute quantities, although the potential for such substances has long been recognized. As a result, progress in this field was greatly hampered. With recent developments of sensitive analytical methods and the introduction of new instrumentation for the characterization of organic compounds in trace amounts (at ppm and ppb levels), the future looks promising. We need a continued strong research effort in new detection systems. Particular attention should be given to developing new or modifying the existing plant bioassays in conjunction with specific analytical techniques. The new analytical methods will lead to the detection of many new substances, and will also enable us to learn more about the metabolic processes in the living intact systems. There is also a need for a continued effort in screening for growth-regulating activity among the several thousands of known secondary compounds which may reveal the presence of potentially useful growth substances.

Although the present guidelines are useful, our current tendency to explain all the known or yet-to-be discovered

In Plant Growth Substances; Mandava, N.;
ACS Symposium Series; American Chemical Society: Washington, DC, 1979.

secondary compounds through the 5 groups of primary growth substances, has to be revised as we discover many new natural products. However strong the foundations that were laid in the past 40 years in this field may be, our knowledge of fundamental interactions of the many biochemical processes at the molecular level that modify or regulate growth is meager indeed. In summary, there may be several other groups of primary growth substances and many more secondary compounds about which we yet have no knowledge.

The available information suggests that minor modifications or changes (sterochemical and configurational) in structure will either enhance or decrease biological activity. But we lack knowledge of how such changes in structure through a synthetic approach may modify activity. Another problem is that many of the growth substances so far identified have not been investigated in detail. Many examples cited in this review have been evaluated in only a single isolated bioassay; neither has their broad spectrum of activity been established, nor their mode of action studied. As a result, no basic knowledge on how they act endogenously is available, so that we have little information on how they can be useful to agriculture and the public.

Several biochemical processes involving secondary growth substances have to be studied. A few examples are cited here:

1) There have been implications that the changes in membrane permeability brought about by some of the growth substances represent an important mechanism of action. This field needs much more effort in the future.

2) The ability of growth substances to modify the activity of pectolytic enzymes appears to have great ecological significance. This is particularly true for allelopathic compounds.

3) It has been demonstrated that legume nodules have to contain hemoglobin in order to be effective in nitrogen fixation (3) and nitrogen deficiency also affects the production of chlorophyll (porphyrin synthesis) in the primary leaves of legume plants. Some growth substances (e.g. soil toxins) inhibit the free-living nitrogen-fixing bacteria such as Azobacter, Rhizobium and Clostridium sp., resulting in significant decrease in nodule formation. Problems concerning the effect of growth substances (particularly allelopathic agents) on the synthesis of hemoglobin and porphyrin need to be investigated in nitrogen-fixation studies.

4) Similarly, evidence directly points to inhibiting or altering the activity of other enzymes such as cellulose (tannins slow the decomposition of hemicellulose), catalase and peroxidase (phytotoxins affect the horseradish peroxidase breakdown of IAA), phosphorylase (polyphenols inhibit it in potatoes), and a host of other enzymes. All these appear to be very fertile areas for future research.

5) The fact that the activity of isolated respiratory enzymes, isolated mitochondria, one-celled organs and organs of plants has

In Plant Growth Substances; Mandava, N.;
ACS Symposium Series; American Chemical Society: Washington, DC, 1979.

been shown to be adversely affected by many compounds (e.g. phenolics) indicates that effects on respiratory systems probably represent an important mechanism of action of at least some growth substances.

6) At this stage, it is not clear how the growth substances affect the stomatal opening but this research area could be important with respect to water conservation. More work is needed to arrive at any definite conclusions.

7) Growth substances evidently affect protein synthesis and bring changes in lipids and organic acid metabolism. Undoubtedly, this is a fruitful area for future research.

In view of the increasing ecological concern and crop production requirements, future research should be directed to understanding the role of plant growth substances in controlling or modifying 1) radiation effects, 2) mineral deficiences, 3) stress-related phenomena, 4) photosynthesis and 5) nitrogen fixation. In this respect, the allelopathic effects of many of these natural compounds appear to have considerable potential for controlling disease, weeds, and other unwanted plants. Such trends could change future agricultural practices for increased efficiency of crop production.

Abbreviations

GA_3, Gibberellic acid A_3; IAA, Indole-3-acetic acid; NAA, Naphthalene acetic acid; ABA, Abscisic acid; BAL, 1,2-Dimercaptopropane; PAL, Phenylalanine ammonia-lyase.

Disclaimer Statement

Mention of a trademark or proprietary product does not constitute a guarantee or warranty of the product by the U.S. Department of Agriculture and does not imply its approval to the exclusion of other products that may also be suitable.

Acknowledgments

The author wishes to thank the Program Committee of the Middle Atlantic Regional Meeting for providing the necessary facilities for the symposium. Special thanks are due to Mrs. Betty Leishear and Mrs. Sunny Keithley for their ready cooperation and excellent help from the planning stages of the symposium to the finishing of this paper.

In Plant Growth Substances; Mandava, N.;
ACS Symposium Series; American Chemical Society: Washington, DC, 1979.

Literature Cited

1. Robinson, T., "The Organic Constituents of Higher Plants"; Burgess. Minneapolis, Minnesota, 1963; p. 8.
2. Whittaker, R. H.; Feeny, P. P. Science, 1971, 171, 757.
3. Rice, E. L., "Allelopathy"; Academic Press, New York, 1974; p. 246.
4. Hutchinson, S. A. Ann. Rev. Phytopathol., 1975, 11, 223.
5. Hutchinson, S. A.; Cowan, M. E. Trans. Brit. Mycol. Soc., 1971, 59, 71.
6. Takijama, Y. Soil Sci. Plant Nutrition (Tokyo), 1964, 10, 14.
7. Peters, K. E.; Lippincott, J. A.; Studier, M. Phytochemistry, 1974, 13, 2383.
8. Erner, Y.; Revveni, O.; Goldschmidt, E. E. Plant Physiol., 1975, 36, 279.
9. English, J.; Bonner, J.; Haagen-Smit, A. J. J. Am. Chem. Soc., 1939, 61, 3434.
10. English, J.; Bonner, J.; Haagen-Smit, A. J. Proc. Natl. Acad. Sci., 1939, 23, 323.
11. Bonner, J.; English, J. Plant Physiol., 1938, 13, 331.
12. Strong, F. E.; Kruitwagen, E. Nature (London), 1967, 215, 1380.
13. Haagen-Smit, A. J.; Vigilierchio, D. R. Recl. Tran. Chim. Pays-Bas Belg., 1955, 74, 1197.
14. Bittner, B.; Gazit, B.; Blumenfeld, A. Phytochemistry, 1971, 10, 1417.
15. Sassa, T.; Takemura, T. Ikeda, M.; Muira, Y. Tetrahedron Letters, 1973, 2334.
16. Sassa, T.; Tomizuka, K.; Ikeda, M.; Muira, Y. Agric. Biol. Chem., 1973, 37, 1221.
17. Kimura, Y.; Katagiri, K; Tumara, S. Tetrahedron Letters, 1971, 3137.
18. Ellstead, G. A.; McGahren, W. J.; Kunstmann, M. P. J. Org. Chem., 1972, 37, 2045.
19. Niwa, M.; Iguchi, M.; Yamamura, S. Tetrahedron Letters, 1975, 1539.
20. Shaw, E. J. Am. Chem. Soc., 1946, 68, 2510.
21. Evanari, M. Bot. Rev., 1949, 15, 153.
22. Haynes, L. J.; Jones, E. R. H. J. Chem. Soc., 1946, 954.
23. Crombie, L. J. Chem. Soc., 1955, 2535.
24. McInnes, A. G.; Yohida, S.; Towers, G. H. N. Tetrahedron, 1965, 21, 2939.
25. Tse, A.; Towers, G. H. N. Phytochemistry, 1967, 6, 149.
26. Siegel, S. M. Phytochemistry, 1976, 15, 566.
27. Camarda, L.; Merlini, L.; Nasini, G. Phytochemistry, 1976, 5, 537.
28. Raistrick, H. Lancet, 1943, 245, 625.
29. Norstadt, F. A.; McCalla, T. M. Science, 1963, 140, 410.
30. Bergel, F.; Klein, R.; Morrison, A. L.; Rinderknecht, H; Moss, A. R.; Ward, J. L. Nature, 1943, 152, 750.

In Plant Growth Substances; Mandava, N.;
ACS Symposium Series; American Chemical Society: Washington, DC, 1979.

31. Woodward, R. B.; Singh, G. B. J. Am. Chem. Soc., 1949, 71, 758.
32. Woodward, R. B.; Singh, G. B. J. Am. Chem. Soc., 1950, 72, 1428.
33. Strobel, G. A. Ann. Rev. Phytopathol., 1974, 75, 541.
34. Theophrastus: "De causis plantarum," Book 1 (3rd Century, (B.C.). Translated by Dengler, R. E. Ph.D. Thesis, University of Pennsylvania, Philadelphia, PA, 1927, p. 111.
35. Hirai, J.; Hirata, N.; Tada, H. J. Jpn. Soc. Hortic. Sci. 1966, 35, 354.
36. Stowe, B. B. "Plant Growth Substances"; Hirokama Publishing Co.:Tokyo, Japan, 1973; p. 997.
37. Stowe, B. B. "The 9th International Conference on Plant Growth Substances," Collected abstracts of the paper demonstrations: Lausanne, Switzerland, Edited by Pilet, P. E. 1976, p. 337.
38. Bruinsma, J. Chem. Weekbl., 1963, 59, 599.
39. Hemberg, T. Physiol. Plant, 1953, 6, 17.
40. Hemberg, T.; Lowen, B. Sven. Farm. Tidskr., 1954, 58, 938.
41. Le Poidevin, N. Phytochemistry, 1965, 4, 525.
42. Nieman, C. Bacteriol. Rev., 1954, 18, 147.
43. Tso, T. C. Nature, 1964, 202, 511.
44. Tso, T. C.; Steffens, G. L; Engelhaupt, M. E. J. Agric. Food Chem., 1965, 13, 78.
45. Steffens, G. L.; Tso, T. C.; Spaulding, D. W. J. Agric. Food Chem., 1967, 15, 972.
46. Cathey, H. M.; Steffens, G. L.; Stuart, N. W.; Zimmerman, R. H. Science, 1966, 153, 1382.
47. Cathey, H. M.; Steffens, G. L. "Plant Growth Regulators"; Soc. Chem. Ind. (London), Monograph 31, 1968, p. 224.
48. Mandava, N.; Chandra, G. R. "Pharmacologically Active Lipids"; Kabara, J. Ed. Am. Oil Chem. Soc., Champaign, Illinois, 1979, 133.
49. Crosby, D. G.; Vlitos, A. J. Contrib. Boyce Thompson Inst., 1959, 20, 283.
50. Mandava, N.; Mitchell, J. W. J. Sci, Food Agric., 1977, 22, 553.
51. Ries, S. K.; West, V. Sweeley, C. G.; Leavitt, R. A. Science, 1977, 195, 1339.
52. Gray, R.; Bonner, J. Am. J. Bot., 1948, 35, 52.
53. Gray, R.; Bonner, J. J. Am. Chem. Soc., 1948, 70, 1249.
54. Goodwin, R. H.; Kavaugh, F. Bull. Torray Bot. Club, 1949, 76, 255.
55. Sinha-Roy, S. P.; Chakraborty, D. P. Phytochemistry, 1976, 15, 2005.
56. Mayer, A. M.; Poljakoff-Mayer, A. "Plant Growth Regulation," 4th International Conference on Plant Growth Regulation: Iowa State Univ. Press, Ames, Iowa, 1961, p. 735.
57. Koshima, M.; Chi, H. J.; Hata, K. Chem. Pharm. Bull. 1968, 16, 1139.

In Plant Growth Substances; Mandava, N.;
ACS Symposium Series; American Chemical Society: Washington, DC, 1979.

58. Chi, H. J. J. Pharm. Soc. (Korea), 1969, 13, 47.
59. Yen, K. Y. Natl. Res. Inst. Chinese Med., 1970, 1.
60. Thimann, K. V. "Plant Physiology"; Steward, F. C. Ed. Vol. ViB, Academic Press: New York, 1972.
61. Isogi, Y.; Komoda, Y.; Okamoto, T. Chem. Pharm. Bull., 1970, 18, 1872.
62. Hendershott, C. H.; Walker, D. R. Science, 1959, 130, 798.
63. Greimal, A.; Koch, H. Biochem. Physiol. Pflanzen, 1977, 171, 425.
64. Shibata, K.; Kubota, T.; Kamishka, S. Plant and Cell Physiol. 1974, 15, 191.
65. White, G. A.; Starrat, A. N. Can. J. Bot., 1967, 45, 2087.
66. Gross, D. Phytochemistry, 1975, 14, 2105.
67. Valio, I. F. M.; Burden, R. S.; Schwabe, W. W. Nature, 1969, 223, 1176.
68. Pryce, R. J. Plants, 1971, 97, 354.
69. Pryce, R. J. Phytochemistry, 1971, 10, 2679.
70. Pryce, R. J. Phytochemistry, 1972, 11, 1355.
71. Letcher, R. M. Phytochemistry, 1973, 12, 2789.
72. Letcher, R. M.; Nhamo, L. R. M. J. Chem. Soc. (C), 1971, 3070.
73. Hashimoto, T.; Hasegaura, K. Phytochemistry, 1974, 13, 2849.
74. Sassa, T.; Aoki, H.; Namoki, M.; Munakata, K. Agric. Biol. Chem., 1968, 32, 1432.
75. Satomura, Y.; Sato, A. Agric. Biol. Chem., 1965, 29, 337.
76. Tokoroyama, T.; Maeda, S.; Nishikama, T.; Kubota, T. Tetrahedron, 1969, 25, 1047.
77. Morita, T.; Aoki, H. Agric. Biol. Chem., 1974, 38, 1501.
78. de Boer, J. J.; Bright, D.; Dallinga, G; Hewilt, T. G. J. Chem. Soc. (C), 1971, 2786.
79. Lavie, D.; Levy, E. C.; Coleau, A.; Evenari, M.; Guttermann, Y. Nature, 1974, 249, 388.
80. Cooper, R.; Levy, E. C.; Lavie, D. J. Chem. Soc. Comm. 1977, 794.
81. Kamikado, T.; Chang, C. F.; Murakoshi, S.; Sakurai, A.; Tamura, S. Agric. Biol. Chem., 1975, 39, 833.
82. Abe, H.; Uchiyama, M.; Sato, R. Agric. Biol. Chem., 1974, 38, 897.
83. Koizumi, T.; Isogai, Y.; Nomoto, S.; Shudo, K.; Okamoto, T. Phytochemistry, 1976, 15, 342.
84. Kimura, Y.; Tamura, S. Agric. Biol. Chem., 1973, 37, 2925.
85. Sigmund, W. Biochem. Z., 1924, 146, 380.
86. Muller, W. H.; Muller, C. H. Bull. Torrey Bot. Club, 1964, 93, 332.
87. del Moral, R.; Muller, C. H. Amer. Midl. Natur., 1970, 83, 254.
88. Al-Mousawa, A. H.; Al-Naib, F. A. G. Bull. Biol. Res. Cent. (Baghdad), 1976, 7, 17.
89. Asplund, R. O. Phytochemistry, 1968, 1, 1995.

In Plant Growth Substances; Mandava, N.;
ACS Symposium Series; American Chemical Society: Washington, DC, 1979.

90. Wellburn, A. R.; Ogunkaumi, A. B.; Fenton, R.; Mansfield, T. A. Planta, 1974, 120, 255.
91. Ogunkaumi, A. B.; Wellburn, A. R.; Mansfield, T. A. Planta, 1974, 117, 293.
92. Grummer, G. Symp. Soc. Expt. Biol., 1961, 15, 219.
93. Geismann, T. A.; Griffin, S.; Waddell, T. G.; Chen. H. H. Phytochemistry, 1969, 8, 145.
94. Miller, H. E.; Mabry, T. J.; Turner, B. L.; Payne, W. W. Amer. J. Bot., 1968, 55, 316.
95. Demole, E.; Lederer, E.; Mercier, D. Helv. Chim. Acta, 1962, 45, 675.
96. Crabalona, L. comp. rend., 1967, 264, 2074.
97. Aldridge, D. C.; Galt, M.; Giles, D.; Turner, W. B. J. Chem. Soc. (C), 1971, 1623.
98. Nakanishi, K.; Crouch, R.; Miura, I.; Dominguez, X.; Zamudio, A.; Villarreal, R. J. Am. Chem. Soc., 1974, 96, 609.
99. Owens, L. D. Science, 1969, 165, 18.
100. Iruchijima, S.; Kuyama, S.; Takahashi, N.; Tamura, S. Agric. Biol. Chem., 1966, 31, 240.
101. Nishikawa, M.; Kamiya, K.; Katabatake, A.; Oshio, H.; Tomile, Y.; Nitta, I. Tetrahedron, 1966, 22, 3601.
102. Morimato, H.; Sanno, Y.; Oshio, H. Tetrahedron, 1966, 22, 3173.
103. Sequeira, L.; Hemingway, R. J.; Kupchan, S. M. Science, 1968, 161, 789.
104. Kupchan, S. W.; Hemingway, R. J.; Werner, D.; Karim, A.; McPhail, A. T.; Sim, G. A. J. Am. Chem. Soc., 1968, 90, 3596.
105. Shibaoka, H.; Shimokoriyama, M.; Iriuchijima, S.; Tamura, S. Plant and Cell Physiol., 1967, 8, 297.
106. Osawa, T.; Suzuki, A.; Tamura, S. Agric. Biol. Chem., 1971, 35, 1966.
107. Romo, J.; Romo de Viver, A.; Trevino, R.; Joseph-Nathan, P.; Diaz, E. Phytochemistry, 1970, 9, 1615.
108. Yamazaki, S.; Tamura, S.; Marumo, F. Saito, Y. Tetrahedron Letters, 1969, 359.
109. Marshall, J. A.; Cohen, N. J. Org. Chem., 1964, 29, 3727.
110. Singh, G.; Paul, B.; Nanda, K. L. J. Sci. Ind. Res. (India), 1959, 18B, 351.
111. Dalvi, R. R.; Singh, B.; Salunkhe, D. K. Chem. Bid. Interactions, 1971, 3, 13.
112. Deuel, P. E.; Geissman, T. A. J. Am. Chem. Soc., 1957, 79, 3778.
113. Geissman, T. A.; Deuel, P. G.; Bonde, E. K.; Addicott, F. A. J. Am. Chem. Soc., 1954, 76, 685.
114. Bonde, E. K. Physiol. Plantarum, 1953, 6, 232.
115. Kanchan, S. D. Current Science, 1975, 44, 358.
116. Herz, W.; Watanabe, H. J. Am. Chem. Soc., 1959, 89, 688.

In Plant Growth Substances; Mandava, N.;
ACS Symposium Series; American Chemical Society: Washington, DC, 1979.

117. Garciduenas, M. R.; Dominguez, X. A. Turrialda, 1976, 26, 10.
118. Dominguez, X. A.; Sierra, A. Planta Medica, 1970, 18, 275.
119. Kalsi, P. S.; Vij, V. K.; Singh, O. S.; Wadia, M. S. Phytochemistry, 1977, 16, 784.
120. Paul, A.; Bawdekar, A. S.; Joshi, R. S.; Kulkarni, G. H.; Rao, A. S.; Kelkar, G. R.; Bhattacharyya, S. C. Perf. Ess. Oil Rec., 1960, 57, 115.
121. Kalsi, P. S.; Singh, O. S.; Chaabra, B. R. Phytochemistry, 1978, 17, 570.
122. Ballio, A.; Brufani, M.; Casinovi, C. G.; Cerrini, S.; Fedali, W.; Pellicciari, R.; Sauturbano, B.; Vaciago, A. Experientia, 1968, 24, 631.
123. Ballio, A.; Pocciari, F.; Russi, B.; Silano, V. Physiol. Plant. Pathol. 1968, 1, 95.
124. Lado, P.; Rosi-Caldogno, F.; Colombo, R. Physiol. Plant, 1974, 31, 149.
125. Sassa, T.; Negoro, T.; Ueki, H. Agric. Biol. Chem., 1972, 36, 2281.
126. Sassa, T. Agric. Biol. Chem., 1972, 36, 2037.
127. Sassa, T.; Takahama, A.; Shindo, T. Agric. Biol. Chem., 1975, 39, 1729.
128. Sassa, T.; Togashi, M.; Kittagushi, T. Agric. Biol. Chem., 1975, 39, 1735.
129. Sassa, T.; Takahama, A. Agric. Biol. Chem., 1975, 39, 2213.
130. Kato, T.; Kabuto, C.; Sasaki, N.; Tsunakaura, M.; Aizawa, H.; Fujita, K.; Kato, Y.; Kitahara, Y. Tetrahedron Letters, 1973, 3861.
131. Tsunakawa, M.;Ohba, A.; Sasaki, N.; Kato, Y.; Kitahara, Y.; Takahaski, N. Tetrahedron Letters, 1976, 1159.
132. Ellestad, G. A.; Evans, R. H.; Kunstmann, M. P.; Lancaster, J. E.; Morton, G. O. J. Am. Chem. Soc., 1970, 92, 5483.
133. Kakisawa, H.; Sato, M.; Ruo, T.; Hayashi, T. J. Chem. Soc. Chem. Comm., 1973, 802.
134. Crombie, R. C.; Russell, G. B. Phytochemistry, 1973, 12, 2057.
135. Sanchez, W. E.; Brown, K. B.; Nishida, T.; Darham, L. J.; Duffield, A. M. An. Acad. Brasil, Ciene, 1970, 42, 77.
136. Hayashi, T.; Kakisama, H.; Ito, S.; Chen, Y. P.; Hsu, H. Y. Tetrahedron Letters, 1972, 3285.
137. Galbraith, M. N.; Horn, D. H. S.; Sasse, J. M. Experientia, 1972, 28, 253.
138. Galbraith, M. N.; Horn, D. H. S.; Sasse, J. M.; Adamson, D. Chem. Comm., 1970, 170.
139. Ito, S.; Sunagawa, M.; Kodama, M.; Honma, H.; Takahashi, T. Chem. Comm., 1971, 91.
140. Adamson, D.; Low, V. H. K.; Adamson, H. "Biochemistry and Physiology of Plant Growth Substances"; Wightman, F.; Setterfield, G., Ed. Runge Press: Ottawa, 1968, p. 505.

In Plant Growth Substances; Mandava, N.;
ACS Symposium Series; American Chemical Society: Washington, DC, 1979.

141. Hayashi, Y.; Takahashi, S.; Ona, H.; Sakan, T. Tetrahedron Letters, 1968, 2071.
142. Ito, S.; Kodama, M.; Sunagawa, M.; Homna, H.; Hayashi, Y.; Takahashi, S.; Ona, S.; Sakan, T. Tetrahedron Letters, 1969, 295.
143. Galbraith, M. N.; Horn, D. H. S.; Ito, S.; Kodama, M.; Sasse, J. M. Agric. Biol. Chem., 1972, 36, 2393.
144. Galbraith, M. N.; Horn, D. H. S.; Sasse, J. M. Chem. Comm., 1971, 1362.
145. Ito, S.; Kodama, K.; Sunagawa, M.; Koreeda, M.; Nakanishi, K. Chem. Comm., 1971, 855.
146. Shrotria, A. Botanica, 1976, 26, 28.
147. Guha, J.; Sen, S. P. Nature, 1973, 244, 223.
148. Mandava, N.; Worley, J. F. (unpublished).
149. Ikeda, M.; Niwa, G.; Tohyama, K.; Sassa, T.; Miura, Y. Agric. Biol. Chem., 1977, 41, 1539.
150. Ikeda, M.; Watanabe, H.; Hayakawa, A.; Sato, K.; Sassa, T.; Miura, Y. Agric. Biol. Chem., 1977, 41, 1543.
151. Grunwald, C. Ann. Rev. Plant Physiol., 1975, 26, 209.
152. Kopcewiez, J. Naturwiss., 1971, 56, 287.
153. Biswas, P. K.; Paul, K. B.; Henderson, J. H. M. Nature, 1967, 213, 917.
154. Helmkamp, G.; Bonner, J. Plant Physiol., 1953, 28, 428.
155. Smith, A. R.; Van Staden, J. Z. Pflanzenphysiol., 1978, 88, 147.
156. Kopcewiez, J. Naturwiss., 1971, 65, 92.
157. Grunwald, C. Ann. Rev. Plant Physiol., 1975, 26, 209.
158. Heftmann, E.; Ko, S.; Bennett, R. D. Naturwiss., 1965, 52, 431.
159. Leboeuf, M.; Cave, A.; Bouterel, R. C. R. Acad. Sci. (Paris), 1964, 259, 3401.
160. Boiteau, P.; Ratsimamanga, A. R. Therapie, 1956, 11, 129.
161. Von Euler, H. Arkiv. Kemi. Mineral Geol., 1946, A22, 14.
162. Jonas, H. Z. Pflanzenphysiol., 1969, 60, 359.
163. Vendrig, J. C. Nature, 1964, 203, 1301.
164. Swain, T. Ann. Rev. Plant Physiol., 1977, 28, 479.
165. Roddick, J. G. Planta, 1972, 102, 134.
166. Boll, P. M. Acta Chem. Scand., 1958, 12, 358.
167. Schreiber, K. Der Zuchter, 1957, 27, 289.
168. Ceriotti, G. Nature, 1967, 213, 595.
169. Piozzi, F.; Fuganti, C.; Mondelli, R.; Ceriotti, G. Tetrahedron, 1968, 24, 1119.
170. Okamoto, T.; Torii, Y.; Isogai, Y. Chem. Pharm. Bull., 1968, 16, 1860.
171. Frost, G. M.; Hale, R. L.; Waller, G. R.; Zalkow, L. H.; Girotra, N. N. Chem. Ind., 1967, 320.
172. Waller, G. R.; Burstrom, H. Nature, 1969, 222, 576.
173. Kost, A. N.; Terene'ev, P. B.; Modyanova, L. V. "Some recent developments in the characterization of natural products"; Rangaswamy, S.; Subbarao, N. V. (Ed), Prentice-Hall, New Delhi, India, 1972, p. 153.

In Plant Growth Substances; Mandava, N.;
ACS Symposium Series; American Chemical Society: Washington, DC, 1979.

174. Matsushima, M.; Arima, K. Agric. Biol. Chem., 1973, 37, 1873.
175. Sheshi, Y. Biochem. Z., 1927, 189, 208.
176. Wilson, M. F.; Bell, E. A. Phytochemistry, 1978, 17, 403.
177. Hoffinger, M.; Coumans, M.; Ceulemans, E.; Gasper, Th. Planta Medica, 1976, 30, 303.
178. Isono, K.; Curtiss, R. W. Phytochemistry, 1964, 3, 277.
179. Bodansky, M.; Stahl, G. L. Bioorg. Chem., 1975, 4, 93.
180. Anderegg, R. J.; Biemann, K.; Buchi, G.; Cushman, M. J. Am. Chem. Soc., 1976, 98, 3365.
181. Hirota, A.; Suzuki, A.; Tamina, S. Agric. Biol. Chem., 1973, 37, 643.
182. Hirota, A.; Suzuki, A.; Aizama, K.; Tamina, S. Biomed. Mass. Spec., 1974, 1, 15.
183. Ube, H.; Uchiyama, M. Tanaka, Y.; Saito, H. Tetrahedron Letters, 1976, 3807.
184. Tanaka, Y.; Hashimoto, Y.; Yanagisawa, K.; Abe, H.; Uchiyama, M. Agric. Biol. Chem., 1975, 39, 1929.
185. Tanaka, Y.; Abe, H.; Uchiyama, M., Taya, Y. Nishimura, S. Phytochemistry, 1978, 17, 543.
186. Yanagawa, H.; Kato, T.; Kitahara, Y.; Takahashi, N.; Kato, Y. Tetrahedron Letters,1972, 2549.
187. Kitahara, Y.; Yanagawa, H.; Kato, T.; Takahashi, N. Plant and Cell Physiol., 1972, 13, 923.
188. Yanagawa, H.; Kato, T.; Kitahara, Y. Tetrahedron Letters, 1973, 1073.
189. Yanagawa, H.; Kato, T.; Sagami, H.; Kitahara, Y. Synthesis, 1973, 607.
190. Kato, A.; Takahashi, J. Phytochemistry, 1976, 15, 220.
191. Kato, A.; Okutani, T. Tetrahedron Letters, 1972, 2959.
192. Cregg, R. H.; Weston, A. F. Tetrahedron Letters, 1973, 655.
193. Kato, A.; Numata, M. Tetrahedron Letters, 1972, 203.
194. Loder, J. W.; Russel, G. B. Austr. J. Chem., 1969, 22, 1271.
195. Kato, A. Phytochemistry, 1975, 14, 1458.
196. Brian, P. W. Ann. Rev. Plant Physiol., 1957, 8, 413.
197. Wright, J. M. Ann. Bot., 1951, 15, 493.
198. Lynch, J. M. "Products of Soil Microorganisms in Relation to Plant Growth"; in CRC Critical Reviews in Microbiology, Laskin, A.I.; Lechevalier, H. Ed. CRC Press: Cleveland, Ohio, 1976, p. 1.
199. Strobel, G. A. Ann. Rev. Phytopathol., 1974, 25, 541.
200. Wood, R. K. S.; Ballio, A.; Fuchs, A. Ed. "Phytotoxins in Plant Disease"; Academic Press: London, 1972.
201. Barbalic, L. Qual. Plant Mater. Veg., 1969, 9, 286.
202. Santesson, J. Bot. Ges. N. F., 1970, 4, 5.
203. Huneck, S. in "Biochemistry and Chemistry of Plant Growth Regulators"; Schreiber, K.; Schutte, H. R.; Sembdner, G. editors, proceedings of the international symposium in cottbus, GDR, Sept. 24-26, 1974, Acad. Sci. GDR, 1976, p. 233.

In Plant Growth Substances; Mandava, N.;
ACS Symposium Series; American Chemical Society: Washington, DC, 1979.

204. de Mayo, P.; Spencer, E. Y.; White, R. W. Can. J. Chem., 1961, 39, 1608.
205. Tamura, S.; Sakurai, A.; Kainuma, K.; Takai, M. Agric. Biol. Chem., 1965, 29, 216.
206. Sakurai, A.; Tamura, S. Agric. Biol. Chem., 1965, 29, 407.
207. Tamura, S.; Sakurai, A. Agric. Biol. Chem., 1965, 29, 595.
208. Kato, J. Shiotani, Y.; Tamura, S.; Sakurai, A. Naturwiss. 1968, 51, 341.
209. Nitch, J. P. Ann. N. Y. Acad. Sci., 1967, 144, 279.
210. Nukina, M.; Hattori, H. Murumo, S. J. Am. Chem. Soc., 1975, 97, 2542.
211. Woods, Jr., H. B.; Allerton, R.; Diehl, H. W.; Fletcher, Jr., H. G. J. Org. Chem., 1955, 20, 875.
212. Rudd, M.; Land, A.; Mosettig, E. Naturwiss., 1963, 50, 23.
213. Valio, I. F. M.; Rocha, R. F. Z. Pflanzenphysiol., 1976, 78, 90.
214. Nakanishi, K.; Endo, M.; Naf, U.; Johnson, L. F. J. Am. Chem. Soc., 1971, 93, 5579.
215. Sharp, P. B.; Keitt, G. N. Clum, H. H.; Naf, U. Physiol. Plant, 1975, 34, 101.
216. Taylor, H. F.; Burden, R. S. Proc. Royal Soc. B., 1972, 80, 317.
217. Stuart, K. L.; Coke, L. B. Planta, 1975, 122, 307.
218. Leppik, R. A. Hollomon, D. W.; Bottomley, W. Phytochemistry, 1972, 11, 2055.
219. Mori, K. Agric. Biol. Chem., 1973, 2899.
220. Spalla, C.; Biffi, G. Experimentia, 1971, 27, 1387.
221. Lynch, J. M.; White, N. Proc. Gen. Microbiol., 1974, 2, 9.
222. Mitchell, Jr., E. D.; Tolbert, N. E. Biochem., 1968, 7, 1019.
223. Crow, W. D.; Nicholls, W.; Sterns, M. Tetrahedron Letters, 1971, 1353.
224. Aldridge, D. C.; Galt, S.; Giles, D.; Turner, W. B. J. Chem. Soc. (C), 1971, 1623.
225. Koshimizu, K.; Fukui, H.; Usuda, S.; Mitsui, T. "Plant Growth Substances"; Hirokowa: Tokyo, Japan, 1973, p. 126.
226. Cook, C. E.; Wichard, L. P.; Turner, B.; Wall, M. E.; Eglay, G. H. Science, 1966, 154, 1189.
227. Cook, C. E.; Wichard, L. P.; Wall, M. E.; Eglay, G. H.; Coggon, P.; Luhan, P. A.; McPhail, A. T. J. Am. Chem. Soc., 1972, 94, 6198.
228. Heather, J. B.; Mittal, R. S. D.; Sili, C. J. J. Am. Chem. Soc., 1974, 96, 1976.
229. MacAlpine, G. A.; Raphael, R. A.; Shaw, A.; Taylor, A. W.; Wild, H. J. J. Chem. Soc. Chem. Comm., 1974, 834.
230. MacAlpine, G. A.; Raphael, R. A.; Shaw, A.; Taylor, A. W.; Wild, H. J. J. Chem. Soc. Perkin Trans. I, 1976, 410.
231. Owens, L. D.; Wright, D. A. Plant Physiol., 1965, 40, 927.

In Plant Growth Substances; Mandava, N.;
ACS Symposium Series; American Chemical Society: Washington, DC, 1979.

232. Owens, L. D.; Guggenhein, S.; Hilton, J. L. Biochem. Biophys. Acta, 1968, 158, 219.
233. Owens, L. D.; Thompson, J. F.; Pitcher, R. G.; Owens, T. J. Chem. Soc. Chem. Comm., 1972, 714.
234. Owens, L. D.; Thompson, J. F.; Fennessy, P. V. J. Chem. Soc. Chem. Comm., 1972, 715.
235. Owens, L. D.; Lieberman, M.; Kunishi, A. Plant Physiol., 1971, 48, 1.
236. Owens, L. D. J. Weed Sci., 1973, 21, 63.
237. Cutler, H. G. Science, 1970, 170, 856.
238. Cutler, H. G.; Cole, R. J. Plant and Cell Physiol., 1974, 15, 19.
239. Springer, J. P.; Clady, J.; Cox, R. H.; Cutler, H. G.; Cole, R. J. Tetrahedron Letters, 1975, 2737.
240. Cutler, H. G.; Reid, W. W.; Detetang, J. Plant and Cell Physiol., 1977, 18, 711.
241. Cutler, H. G. Plant and Cell Physiol., 1975, 16, 181.
242. Cutler, H. G.; Eisner, T. Science, 1974, 183, 755.
243. Cutler, H. G.; LeFiles, J. H.; Crumley, F. G.; Cox, R. H. J. Agric. Food Chem., 1978, 26, 632.
244. Marchelli, R.; Vining, L. C. J. Antibiot., 1975, 28, 328.
245. Takahaski, C.; Yoshishira, K.; Natori, S.; Umeda, M. Chem. Pharm. Bull., 1976, 24, 613.
246. Lansden, J. A.; Cole, R. J.; Dorner, J. W.; Cox, R. H.; Cutler, H. G.; Clark, J. D. J. Agric. Food Chem., 1978, 26, 246.
247. Wells, J. M.; Cutler, H. G.; Cole, R. J. Can. J. Microbiol., 1976, 22, 1137.
248. Mitchell, J. W.; Mandava, N.; Smith, M. V.; Worley, J. F.; Plimmer, J. R. Nature, 1970, 225, 1065.
249. Steffens, G. L.; Buta, J. G.; Gregory, L. E.; Mandava, N.B. Meudt, W. J.; Worley, J. F. "Advances in Pesticides Science"; Geissbuhler, H.; Kearney, P. C.; Brooks, G. T.; Editors, IUPAC, 1979, 343.
250. Gregory, L. E.; Mandava, N,; Cina, D. 10th International Conference in Plant Growth Substances, Madison, Wisconsin July 1979. (Abstract).
251. Yopp, J.; Colclasure, C.; Mandava, N. Physiol. Plant., 1979, (in press).
252. Mandava, N.; Kozempel, M.; Worley, J. F.; Matthees, D.; Warthen, Jr., J. D.; Jacobson, M.; Steffens, G. L.; Kenney, H.; Grove, M. D. Ind. Eng. Chem. Prod. Res. Dev., 1978, 17, 351.
253. Grove, M. D.; Spencer, G. F.; Mandava, N.; Worley, J. F. Warthen, Jr., J. D.; Steffens, G. L.; Flippen-Anderson, J. L. 10th International Conference on Plant Growth Substances, Madison, Wisconsin, July 1979 (Abstract).
254. Mandava, N.; Mitchell, J. W. Chem. Ind. (London), 1972, 930.

255. Grove, M. D.; Spencer, G. F.; Pfeffer, P. E.; Mandava, N.; Warthen, Jr., J. D.; Worley, J. F. Phytochemistry, 1978, 17, 1187.
256. Pfeffer, P. E.; Rothman, E. S.; Moore, G. G. J. Org. Chem., 1976, 41, 2925.
257. Chandra, G. R.; Mandava, N.; Warthen, Jr., J. D. Biochem. Biophys. Acta, 1978, 526, 387.
258. Mandava, N.; Chandra, G. R. Glucolipids of rape (Brassica napus L.) pollen. In: "Pharmacologically Active Lipids"; Kabara, J. (Ed.), Amer. Oil. Chem. Soc.: Champaign, Ill., 1978, 133-143.
259. Mandava, N.; Anderson, J. D.; Dutky, S. R. Phytochemistry, 1974, 131, 2853.
260. Anderson, J. D.; Mandava, N.; Garrett, S. Plant and Cell Physiol., 1972, 49, 1024.
261. Anderson, J. D.; Mandava, N.; Garrett, S. Plant and Cell Physiol., 1975, 16, 233.
262. Mandava, N.; Orellana, R.; Warthen, Jr., J. D. Abstracts of the Amer. Chem. Soc. Fall Natl. Meeting, Miami, Florida, September 11-15, 1978, Abstract No. 59.
263. Mandava, N.; Mitchell, J. W. J. Sci. Food Agric., 1971, 22, 553.
264. Crosby, D. G.; Vlitos, A. J. Contrib. Boyce Thompson Inst., 1959, 20, 283.
265. Buta, J. G.; Worley, J. F. J. Agric. Food Chem., 1976, 24, 1085.
266. Buta, J. G.; Novak, M. J. Ind. Eng. Chem. Prod. Res. Dev., 1978.
267. Wall, M. E.; Wani, M. C.; Cook, C. E.; Palmer, K. H.; MacPhail, A. T.; Sim, G. A. J. Am. Chem. Soc., 1968, 88, 3888.
268. Buta, J. G.; Flippen, J. L.; Lusby, W. R. J. Org. Chem., 1978, 43, 1002.
269. Evanari, M. Chemical influence of other plants. "Encyclopedia of Plant Physiology"; Springer: Berlin, Vol. 16, pg. 691.
270. Molisch, H. "Der Einfluss einer Pflanze auf die andere - Allelopathic"; Gustav Fischer Verlag: Jena, 1937.
271. Rice, E. L. "Allelopathy"; Academic Press: New York, 1974, pg. 351.
272. Livingstone, B. E. Bull. U.S. Division Soils, 1907, No. 36.
273. Schreiner, O.; Reed, H. S. Bull. U.S. Division Soils, 1907, No. 40.
274. Audus, L. J. Plant Growth Substances. "Chemistry and Physiology"; Leonard Hill: London, Vol. 1, pg. 240.
275. Grummer, G. "Die gegenseitige Beeinflussung hoherer Pflanzen-Allelopathie"; Fischer: Jena, 1955.
276. Common, J. Am. J. Bot., 1964, 33, 217.

In Plant Growth Substances; Mandava, N.;
ACS Symposium Series; American Chemical Society: Washington, DC, 1979.

277. de Roubiax, J.; Lazar, O. The inhibitory substances contained in sugar beet glommerules. In: "Phenolics in Plants in Health and Disease"; Pergamon Press: Oxford, 1960, pg. 35.
278. Tomaszewski, M. The mechanism of synergistic effects between auxin and some natural phenolic substances. In: "Regulateurs Naturals de la Croissance Vegetate"; CNRS: Paris, 1964, pg. 335.
279. Tomaszewski, M.; Thimann, K. V. Plant Physiol., 1966, 41, 1443.
280. Ray, P. M. Ann. Rev. Plant Physiol., 1958, 9, 81.
281. Ray, P. M. Arch. Biochim. Biophys., 1960, 87, 19.
282. Jablonski, J.; Skoog, F. Physiol. Plant, 1954, 7, 16.
283. Thimann, K. V. The natural plant hormones. In: "Plant Physiology"; Vol. VI B, "Physiology of Development"; Steward, F. C. (Editor), Academic Press: New York, 1972, pg. 129.
284. Kefeli, V. I.; Turetskaya, R. K. Fiziol. Rast., 1967, 14, 796.
285. Wurzburger, J.; Leshem, Y. New Phytol., 1969, 68, 337.
286. Paleg, L. G.; West, C. A. The Gibberellins: The Natural Plant Hormones. In: "Plant Physiology"; Vol. VI B, "Physiology of Development. The Hormones"; Steward, F. C. (Editor), Academic Press: New York, 1972, pg. 363.
287. Corcoran, M. R. Plant Physiol., 1970, 46, 531.
288. Guha, J.; Sen, S. P. Nature, 1973, 244, 223.
289. Geissmann, T. A.; Phinney, B. O. Plant Physiol., 1972, 49, 323.
290. Thimann, K. V.; Bonner, Jr., W. D. Proc. Natl. Acad. Sci. U.S., 1949, 35, 272.
291. Riov, J.; Monselise, S. P.; Kahan, R. S. Plant Physiol., 1969, 44, 631.
292. Fuchs, Y. Plant Physiol., 1970, 45, 533.
293. Morgan, P. W.; Powell, R. D. Plant Physiol., 1970, 45, 553.
294. Ilag, L.; Curtis, R. W. Science, 1968, 159, 1357.
295. Owens, L. D.; Lieberman, M.; Kunishi, A. Plant Physiol., 1971, 48, 1.

RECEIVED June 19, 1979.

Advances in Analytical Methods for Plant Growth Substance Analysis

MARK L. BRENNER

Department of Horticultural Science and Landscape Architecture,
324 Alderman Hall, University of Minnesota, Saint Paul, MN 55108

There has been substantial progress in analytical methods for plant growth substance (PGS) research during this past decade. Previously, plant scientists had to primarily rely on the use of biological assays to determine the presence of the various compounds in which they were interested. Bioassays have helped in establishing the presence of PGS in plants. However, they have many inherent limitations in sensitivity and reliability. Bioassays may be somewhat imprecise in identifying active compounds, due to a test tissue's ability to perhaps alter an unknown compound to a form that would elicit the biological response even though the originally added compound was inactive. Bioassays often lack specificity in that an observed response can be the net result of inhibitory and promotive substances. Bioassays often take days to complete. Another limitation of bioassays is that their response curve usually extends over a logarithmic range; levels must differ by a factor of at least five to ten for the bioassay method to detect a significant separation.

Since many of the plant growth substances now have been structurally characterized, it is no longer necessary to rely on bioassays for PGS identification. In fact, physico-chemical techniques have become the methods of choice. There have been a number of reviews discussing some of the aspects of PGS analysis by physico-chemical procedures (1, 2, 3, 4, 5). This report will briefly survey the previous literature with special attention given to the advantages and disadvantages of the available approaches. Special emphasis will be given to the potential uses of high performance liquid chromatography (HPLC).

Qualitative vs. Quantitative Analysis.

Qualitative Analyses. A clear distinction must be made between qualitative and quantitative analyses. Qualitative analysis merely demonstrates the presence of a compound in the extracted sample while quantitative analysis determines the actual amount

0-8412-0518-3/79/47-111-215$07.50/0

In Plant Growth Substances; Mandava, N.;
ACS Symposium Series; American Chemical Society: Washington, DC, 1979.

of the specific compound being analyzed. Biological assays permit an assessment of the presence of compounds with activity "like" that of a given standard but for the reasons described above, they do not provide definite, qualitative or quantitative proof for the presence of a specific compound. They do provide proof that a compound with activity "like" that of the class being analyzed is present.

One of the key limitations to successful PGS analysis is the inherently low level of PGS found within plant tissue. Typical levels of many of these compounds range from 10 pg to 10 μg per gram fresh weight of tissue, with levels most often less than 10 ng per gram fresh weight. The recovery of the PGS during sample preparation thus becomes very critical.

Instrumental qualitative analysis of PGS in the past has primarily been attempted by gas liquid chromatography (GLC) fitted with either a flame ionization detector (FID) or an electron capture detector (ECD). However, the FID is essentially a nonselective detector and the ECD responds only to electronegative compounds. Thus, in the use of GLC-FID, one totally relies on prior cleanup, the gas chromatographic separation, and identification by co-chromatography for identification. When attempting to identify PGS that occur at trace levels, the likelihood of having multiple compounds within a given observed peak is very great (6). However, when one couples a very selective detector such as a mass spectrometer (MS) to the gas chromatograph, then the likelihood of valid identification of a given compound is greatly enhanced. One documented example in which peaks (presumed to be gibberellins) detected by an GLC-FID proved to incorrectly estimate the quantity of a PGS has been reported by Williams et al. (26). Their use of a GLC-MS provided substantial proof of the presence of GA_1 and GA_9 when the tissue extract was purified by thin layer chromatography. GLC-MS has been used to provide unequivocal, qualitative proof of the presence of many of the major PGS. Examples of some of the compounds identified by this method are listed in Table 1.

Although the use of GLC-MS remains one of the best methods of identifying trace biological compounds, relatively large amounts (0.1 to 1.0 kg) of tissue are required for analysis (Table 1) using full MS scan. For GLC-MS, one would ideally desire more than 1.0 μg of compound for positive identification. This amount is particularly necessary for trimethylsilyl ethers (TMS) derivatives of zeatin, which have unstable fragmentation patterns (22). However, the detection limit may be extended down to the ng range by use of multiple ion detection (MID). Detection of cytokinins by MID may be further aided by permethylation of the compounds (22) due to the greater stability of the methylated cytokinins.

Single ion detection (SID) also allows detection of PGS down to 10 ng or even lower (27, 28, 29, 30). However, focusing on one ion may introduce errors. The amount of the particular ion

In Plant Growth Substances; Mandava, N.;
ACS Symposium Series; American Chemical Society: Washington, DC, 1979.

Table I. Examples of plant growth substances qualitatively identified by gas chromatography-mass spectrometery.

Compound[1]	Source of PGS	Amount of tissue extracted	Type of derivative made	Detector	Ref.
ABA	standards	---	ME[2]	direct probe MS GLC-MS	(7)
ABA	*Ceratonia silique* L.	315 g fr wt	ME TMS[3]	GLC-MS	(8)
ABA	*Pisum sativum* L. chloroplasts	1 kg fr wt	ME	GLC-MS	(9)
ABA	*Humulus lupulus* L.	20 kg fr wt	ME	GLC-MS	(10)
GA_1-GA_{24}	*Phaseolus coccineus*	25.8 kg tissue	ME METMS[4]	GLC-MS	(11)
GA_1, GA_3, GA_5, GA_6, GA_8, GA_{17}, GA_{19}, GA_{21}	*Phaseolus vulgaris*	25.8 kg fr wt	METMS	GLC-MS	(12
GA_{20}, GA_{29}	*Pisum sativum*	68 g fr wt	ME METMS	GLC-MS	(13)

In Plant Growth Substances; Mandava, N.;
ACS Symposium Series; American Chemical Society: Washington, DC, 1979.

Table I. continued

Compound[1]	Source of PGS	Amount of tissue extracted	Type of derivative made	Detector	Ref.
GA glucosides and glucosyl esters	Pharbitis nil Cytisus scoparius Phaseolus vulgaris	---	METMS	direct probe MS	(14)
IAA	Zea mays	diffusate from 15,000 coleoptile tips	none	direct probe MS	(15)
IAA	Zea mays	25-255 g fr wt	TMS	direct probe MS after GLC	(16)
IAA	Pseudotsuga menziesii	300 g fr wt	ME METMS	MS	(17)
IAA-amino acid conjugates	standards	---	none	direct probe MS	(18)
di-o- & tri-o-IAA-myo-inositols	Zea mays	10.5 kg kernels	TMS	GLC-MS	(19)
Z, ZR	standards	---	TMS	GLC-MS	(20)
Z	malt extract	---	none	direct probe MS	(21)

In Plant Growth Substances; Mandava, N.;
ACS Symposium Series; American Chemical Society: Washington, DC, 1979.

Table I. continued

Compound[1]	Source of PGS	Amount of tissue extracted	Type of derivative made	Detector	Ref.
Z	Humulus lupulus L.	20 kg	TMS	GLC-MS	(10)
Z, ZR	Actinidia chinensis	90 g dry wt	permethyl-ated	GLC-MS	(22)
	Prunus cerasus	90 g dry wt			
ZR	Acer pseudoplatanus	2.5 l sap	TMS	GLC-MS	(23)
glycosyl Z	Vinca rosea	---	permethyl-ated	GLC-MS	(24)
glycosyl ZR			TMS		
Z, ZR Δ^2 - isopentyl-adenosine	Mercuriales ambigua	150-250 g fr wt	TMS	GLC-MS	(25)

[1]ABA = abscisic acid; GA = gibberellin; IAA - indole-3-acetic acid; Z = zeatin; ZR = zeatin riboside.

[2]ME = methyl ester.

[3]TMS = trimethylsilyl ethers.

[4]METMS = methyl ester trimethylsilyl ester.

In Plant Growth Substances; Mandava, N.;
ACS Symposium Series; American Chemical Society: Washington, DC, 1979.

of interest may be due to the PGS or to impurities. The application of MID reduces the possibility of error if one matches the ratio of intensity of several ions within the standard compound to the unknown compound.

Quantitative Analysis. A number of scientists have claimed that PGS analysis by GLC-MS with SIM or MID detection facilitates quantitative analysis. This is only true in the sense that one is able to obtain a reliable estimate of the quantity of the compound that is actually detected by the MS system. However, part of the sample may be lost during extraction and purification, before the sample reaches the final detector. The only apparent means of correcting for such loss is the addition of an internal standard to the sample at the start of sample preparation.

Use of internal standards. Mann and Jaworski (31) reported that when the recovery of {1-^{14}C} IAA is monitored during a sample purification procedure, considerable loss of IAA can be detected. Bandurski and Schulze (32) suggested the use of reverse isotope dilution to help quantify the actual loss of IAA during sample analysis. In this procedure, one adds a trace amount of radio-labeled compound which ideally is identical to the compound being monitored. High specific activity is required so that statistically significant amounts of isotope can be detected without having to add an excessive quantity (mass) of internal standard. The amount of internal standard must be less than the amount of PGS. One may then accurately determine the recovery efficiency of the internal standard and thus of the PGS (32).

Little et al. (30) found that the recovery efficiency of both IAA and ABA could vary up to fivefold. We have demonstrated the same type of variability (Table 2). Examination of the data from Little et al. (30) as well as from my research group (Table 2) shows that recovery variability for ABA is not as great as for IAA. Thus when analyzing for both compounds from the same sample, one needs to use two internal standards.

The number of internal standards required when monitoring several compounds should be carefully considered. For example, Cohen and Bandurski (33) demonstrated that the IAA conjugates are stable to oxidation by peroxidases, while free IAA is not. Thus, the addition of {1-^{14}C} IAA would not adequately monitor recovery of IAA conjugates. Another example is the use of kinetin as an internal standard for the estimation of zeatin recovery (34). The selection of kinetin for zeatin recovery estimation must be questioned on the basis of large differences in their partition coefficients (35). Another approach has been the use of nonlabeled isomers of a compound such as 6-(hydroxybenzylamino)-9-β-D-ribofuranosylpurine to estimate the recovery of 6-(o-hydroxybenzylamino)-9-β-D-ribofuranosylpurine (36). Saunders' group (37) has added 2-trans-ABA as a nonradioactive internal standard. Either internal standard must be shown to partition

In Plant Growth Substances; Mandava, N.;
ACS Symposium Series; American Chemical Society: Washington, DC, 1979.

Table II. Net recovery efficiency of internal standards of plant growth substances after extraction and purification.

Compound added	Tissue	Number of Samples	Recovery		
			Mean	Range	Std. dev.
				%	
{2-^{14}C} ABA	Glycine max. leaves	12	68	59-77	±0.042
{2-^{14}C} ABA	Cornus stolonifera leaves	27	72	61-93	±0.078
{1-^{14}C} IAA	Lycopersicon esculentum stems	45	64	10-85	±0.182
{1-^{14}C} IAA	Freesia hybrida corms	a. 16	60	28-90	±0.158
		b. 14	46	17-72	±0.187

In Plant Growth Substances; Mandava, N.;
ACS Symposium Series; American Chemical Society: Washington, DC, 1979.

exactly like the compound being followed for absolute confidence in its use.

What may prove to be the ultimate choice for an internal standard when using an MS (37) is the addition of a PGS standard as a deuterated compound to the initial sample preparation. The deuterated compound is quantified directly on the MS rather than having to subsequently subject the sample to conventional radioisotope detection methods. This procedure has been applied to ABA (29) and IAA (38, 39) analyses. A high deuterium content (labeled at five or more positions) should be sought to avoid confusion with naturally "heavy" isotopic compounds (39).

As summarized in Table 3, there are a number of examples in which internal standards have been effectively used for the quantitative analysis of PGS by GLC-MS. The prime limitation of this approach has been the high cost of the instrumentation. As will be discussed in a later portion of this manuscript, other selective detectors used on either a GLC or an HPLC should offer other choices for PGS analysis, but they are not as specific as GLC-MS.

Use of HPLC for PGS Analysis.

Preparative HPLC. Most current PGS analytical procedures have been optimized to examine a specific class of PGS. Therefore, several different procedures must be developed to analyze the major classes of PGS in a single plant sample. Exceptions to this approach have been reported by Shindy and Smith (44) and by Wightmann et al. (45) who attempted to identify the four major classes of PGS from the same plant sample with a single procedure. However, they offered no positive proof of purity of the compounds which were identified by GLC-FID. {Shindy and Smith confirmed the actual presence of several PGS by GLC-MS but did not determine the purity of the peaks (44).} The time consumed in the multistep processes necessary to obtain sufficiently purified extracts suitable for analytical chromatographic procedures has been a great limitation on PGS research. A desirable goal would be to reduce to a minimum the number of steps involved for quantitative recovery of multiple PGS from a single plant sample. Preparative HPLC (prep-HPLC) used to this end by a number of researchers (Table 4), greatly improves the separation and recovery efficiency of many PGS and substantially reduces the separation time compared to classical procedures.

Application of Prep-HPLC to PGS Analysis. Reverse phase liquid chromatography has proven to be well suited for cleanup of plant extracts by prep-HPLC (4, 46, 47, 48). When the mobile phase is initially an aqueous buffer at pH 2.8, all but the highly charged (e.g., zeatin ribotide with 5' AMP used as a representative compound for zeatin ribotide) plant hormones are retained at the head of the column (Fig. 1). Since the PGS are retained, samples can be injected onto the column in a dilute form. In-

In Plant Growth Substances; Mandava, N.;
ACS Symposium Series; American Chemical Society: Washington, DC, 1979.

Table III. Examples of quatitative analysis of PGS using gas chromatography-mass spectroscopy.

Compound identified[1]	Source of PGS	Internal standard	Type of derivative[2]	Method of analysis on GLC-MS	Ref.
ABA	Zea mays	{$^2H_6$-} ABA	ME	full scan dual ion monitoring	(29)
ABA	Picea sitchensis	{2-^{14}C-} ABA	ME	SIM[3]	(30)
ABA	Lactuca	{2-^{14}C-} ABA	ME	full scan	(40)
DPA	Phaseolus vulgaris	{2-^{14}C-} DPA	ME	GLC-EC[4]	(41)
PA		{2-^{14}C} PA	ME		
IAA	Avena sativa	{1-^{14}C} IAA	TMS	full scan	(32)
	Zea mays				
IAA	Phaseolus vulgaris	{5-^{3}H-} IAA	TMS	full scan SIM	(42)
IAA	Picea sitchensis	{1-^{14}C-} IAA	TMS	SIM	(30)
(o-OH BAP-riboside)	Populus	p-OH BAP riboside	TMS	SIM	(36)
Raphanatin	Rhaphanus sativa	{$^2H_2$} raphanatin {$^2H_{2-2}$} Z[5]	TMS	SIM	(43)

[1]ABA = abscisic acid; DPA = 4'-dihydroxyphaseic acid; PA= phaseic acid; o-OH-BAP riboside = 6-(o-hydroxybenylmino)-9-β-D ribofuranosylpurine; Raphanatin = 7-β-D glucopyranosylzeatin.

[2]ME = methyl ester; TMS = trimethylsilyl ether.

[3]SIM = single ion monitoring on GLC-MS.

[4]GLC-EC = gas liquid chromatography-electron capture detector.

[5]Z = zeatin.

In Plant Growth Substances; Mandava, N.;
ACS Symposium Series; American Chemical Society: Washington, DC, 1979.

Table IV. Example of the use of preparative HPLC for PGS purification.

Compound(s)[1]	Source of PGS	Prior sample cleanup	Column	Analytical identification	Ref.
ABA	*Glycine max.*	none	reverse phase $-C_{18}$	GLC-EC	(46)
Cytokinins	*Lycopersicon esculentum*	none	reverse phase $-C_{18}$	bioassay	(47)
IAA, ABA, DPA, PA	*Sorghum bicolor*	partition sephadex-G10	reverse phase $-C_{18}$	analytical HPLC GLC	(48)
gibberellins	*Phaseolus coccineus*	partition GPC[2]	adsorption silica	scintillation counting MS analytical HPLC	(49, 50)

[1]ABA = abscisic acid; IAA = indole-3 acetic acid; DPA = dihydrophaseic acid; PA = phaseic acid.

[2]GPC = gel permeation chromatography.

In Plant Growth Substances; Mandava, N.;
ACS Symposium Series; American Chemical Society: Washington, DC, 1979.

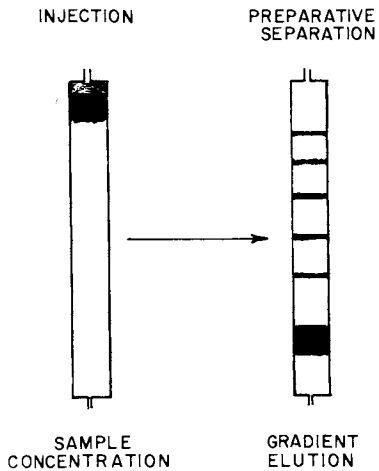


Figure 1. Diagram of sample concentration on a reverse-phase LC column (right) *followed by separation when the solvent strength of the mobile phase is increased. Column: μC18, 10 mm × 25 cm; solvent: 0.1N HAc to 50% ETOH in 0.1N HAc in 20 min; flow rate: 50 mL/min; detector: UV 254 nm.*

In Plant Growth Substances; Mandava, N.;
ACS Symposium Series; American Chemical Society: Washington, DC, 1979.

jection volumes as large as 4.0 ml are quite practical because large quantities of solute may be injected without solubility problems. In addition, the transfer of solute to the column is more efficient when made in a large injection.

The PGS are separated and eluted from the column by the addition of a water-miscible organic solvent to the mobile phase. We have found that ethanol works well for this purpose. When microparticle-packed HPLC columns are used, the solvent change must be accomplished as a gradual, continuous gradient. Discontinuous increments (step gradients) tend to cause rapid viscosity and thermal changes along the column bed; these are known to cause destruction of the bed.

The gradient profile dramatically affects the separation on the column. A linear gradient of 0.1 N aqueous acetic acid to 0.1 N acetic acid in 50% (v/v) ethanol/water delivered in a 25 minute period has been used to separate many PGS standards (Fig. 2). Ciha et al. (46) have established that a similar gradient sequence permits rapid recovery of a fraction containing ABA from crude plant extracts. The recovery efficiency of {2-^{14}C}-ABA and of the endogenous plant ABA was greatest when all conventional partitioning was by-passed and the crude plant extract was injected directly onto the chromatograph. We have extended that separation technique to allow recovery of multiple PGS from a single sample. Now, from the same plant extract, specific fractions containing zeatin, zeatin riboside, IAA, IAA-acetyl-aspartate, ABA, phaseic acid and dihydrophaseic acid can be recovered.

The resolution shown in Figure 2 was accomplished by a highly efficient column which we packed with 10 μm diameter Bondapak μC$_{18}$ particles (Waters Associates). This column (10 mm I.D. x 25 cm) has over 5000 theoretical plates as compared to 600 for the two 1.0 m columns packed with 35 to 75 μm diameter particles previously used (46, 47).

To protect the column from compounds that irreversibly adhere to or partition into the column packing, a precolumn may be used. A pellicular packing coated with C$_{18}$ material (C$_{18}$ Corasil II, Waters Associates) has proven beneficial, yet has a minimally detrimental effect on compound resolution.

Fractions are collected on the basis of the retention times of the respective PGS standards. The remaining portions of the column effluent are diverted to waste. Figure 3 diagramatically represents the sequence of events used for prep-HPLC of PGS samples.

The addition of an acidic buffer (0.1 N acetate) that serves as a polar modifier in the mobile phases (in both water and ethanol) is required for consistent results. It serves to protonate all of the PGS and helps to minimize adsorptive properties of the column, thereby facilitating reproducible results (47). The buffer accomplishes this by saturating the exposed silicic acid sites. Ethanol, rather than methanol, is the preferred organic

In Plant Growth Substances; Mandava, N.;
ACS Symposium Series; American Chemical Society: Washington, DC, 1979.

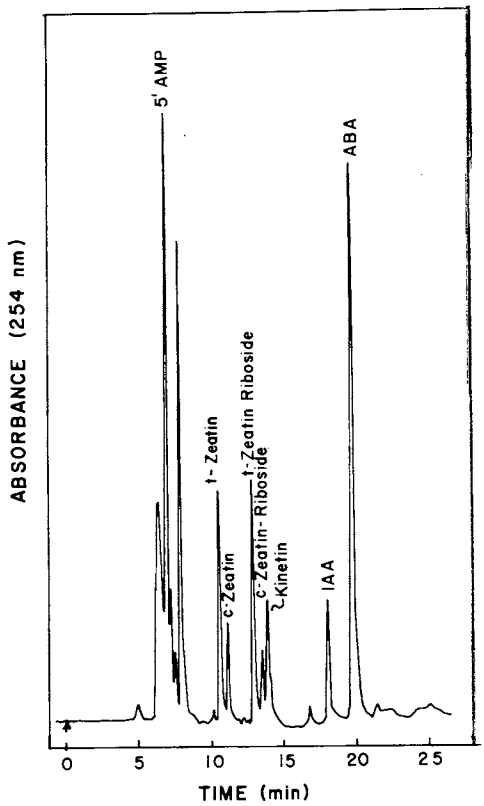


Figure 2. Separation of PGS standards on a preparative HPLC column. Note that the retention time of 5′ AMP would be representative of cytokinin ribotides.

In Plant Growth Substances; Mandava, N.;
ACS Symposium Series; American Chemical Society: Washington, DC, 1979.

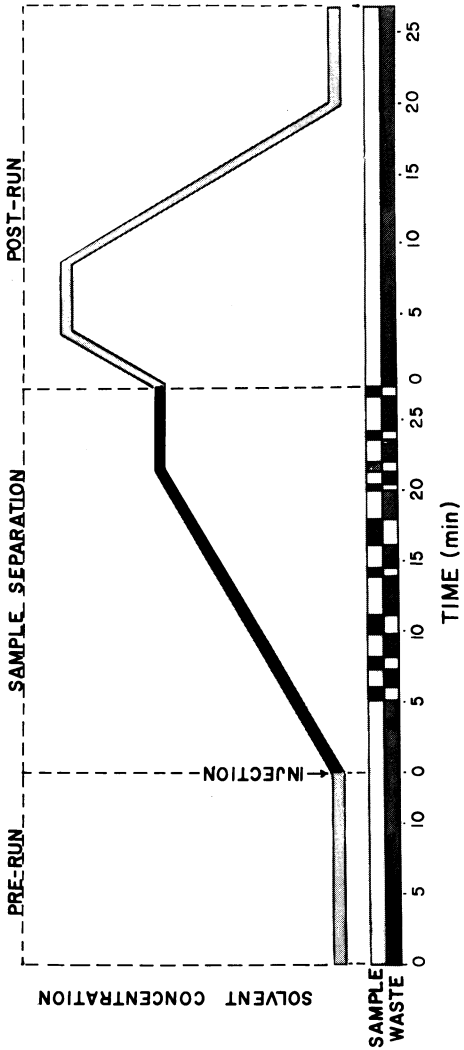


Figure 3. Diagram of the sequence of events during the preparative HPLC separation. The boxes on the sample line indicate retention times of fractions that may be collected for further analysis.

In Plant Growth Substances; Mandava, N.;
ACS Symposium Series; American Chemical Society: Washington, DC, 1979.

mobile phase because it more effectively reduces peak tailing. Acetonitrile also works well but much greater care must be taken to avoid operator exposure to toxic solvent vapors.

After the compounds of interest are eluted from the column, the concentration is linearly increased to 0.1 N acetic acid in 95% (v/v) ethanol/water to remove the more nonpolar components retained on the column. Following an adequate equilibration (greater than 10 column volumes) at maximum solvent strength (95% ethanol), the solvent is linearly programmed to return to the initial conditions of 0.1 N aqueous acetic acid.

Automation of Prep-HPLC. Reverse phase prep-HPLC separation has proven to be a very reproducible technique. For this reason, the process can be automated. In addition to the standard components of an HPLC system, the following are required for automation: an autoinjector capable of large volume injections (2.0 to 5.0 ml), a programmable controller (a microprocessor controller), a fraction collector, and a waste valve (3-way valve) controlled by a solenoid (Fig. 4). The microprocessor should allow programming of the solvent flow rate, the sequence for solvent gradient formation, the time of injection, advancement of the fraction collector (to collect specific fractions rather than just uniformly incremented advancement), and control of a waste valve.

Automation of the prep-HPLC system offers several advantages over manual operation. Time use efficiency increases several fold. For example, we have been able to quadruple the number of samples separated per day. Greater precision is also obtained due to the accurate timing of the microprocessor-controlled functions. The other obvious advantage is more effective use of labor. However, automation without the proper controls has definite limitations. That is, if one of the components of the system fails while everything else continues to operate, then all of the samples injected while the system is malfunctioning may be lost. From our experience the following functions must be monitored for unattended operation (closed-loop control): high pressure limit (to detect a plugged liquid path), low pressure limit (to detect a leak), pressure pulsation (to monitor uniform solvent flow), sample injection (to verify sample injection), liquid level sensors (to verify adequate reserve of solvents), and advancement of the fraction collectors (to verify that the fraction collector actually advances and that a new tube is ready to collect the next sample). Thus, if any of the monitored items indicates a faulty system, the microprocessor should either correct the problem or should shut the system down.

Analytical HPLC of PGS. A number of reports are currently available on the use of HPLC for the analytical identification of native PGS (Table 5). These techniques have primarily relied on purification by prior partitioning and chromatographic separation

In Plant Growth Substances; Mandava, N.;
ACS Symposium Series; American Chemical Society: Washington, DC, 1979.

Table V. Examples of the use of analytical HPLC for PGS.

Compound	Source of PGS	Prior Sample Clean Up	Column	Detector	Ref
ABA[1]	Vitis vinifera	partition & TLC	anion exchange	UV-254 nm	(52, 53)
ABA	Malus Gossipium	partition and Sephadex-G25	cation exchange	UV-254 nm	(54)
ABA	Glycine max	prep HPLC	adsorption partition NH_2	UV-254 nm	(45)
ABA	Sorghum bicolor	partition and Sephadex-G10	reverse phase-C_{18}	UV-254 nm bioassay	(47)
Cytokinins (multiple)	Standards	none	cation exchange	UV-254 nm	(55)
N^6-(Δ^z-isopentenyl) -adenine	Agrobacterium tumefaciens	TLC	reverse phase-C_{18}	UV-254 nm	(56)
Cytokinins	Standards	none	cation exchange partition	UV-254 nm	(57)
Zeatin Zeatin riboside	Sorghum bicolor	partition; PVP Sephadex-G10	reverse phase-C_{18}	UV-254 nm	(58)

In Plant Growth Substances; Mandava, N.;
ACS Symposium Series; American Chemical Society: Washington, DC, 1979.

Table V. continued

	Source of PGS	Prior Sample Clean Up	Column	Detector	Ref.
Cytokinins (multiple)	*Phaseolus vulgaris*	partition; LH-20	reverse phase-C_8	UV-260 nm	(59)
GA_1, GA_3, GA_4, GA_5, GA_7, GA_{20}	Standards	none-all derivatized as p-nitrobenzyl esters	adsorption $AgNO_3$-silica	UV-265 nm	(60)
IAA	*Vitis vinifers*	partition TLC	anion exchange	UV-254	(53)
IAA	*Sorghum bicolor*	partition Sephadex-G10	reverse phase-C_{18}	UV-254	(47)
IAA	*Glycine max* *Gossypium hirsutum* *Phaseolus vulgaris* others	partition Sephadex-G10	anion exchange reverse phase-C_{18} adsorption	fluorescence electrochemical	(61)

[1]See abbreviations for Table I.

In Plant Growth Substances; Mandava, N.;
ACS Symposium Series; American Chemical Society: Washington, DC, 1979.

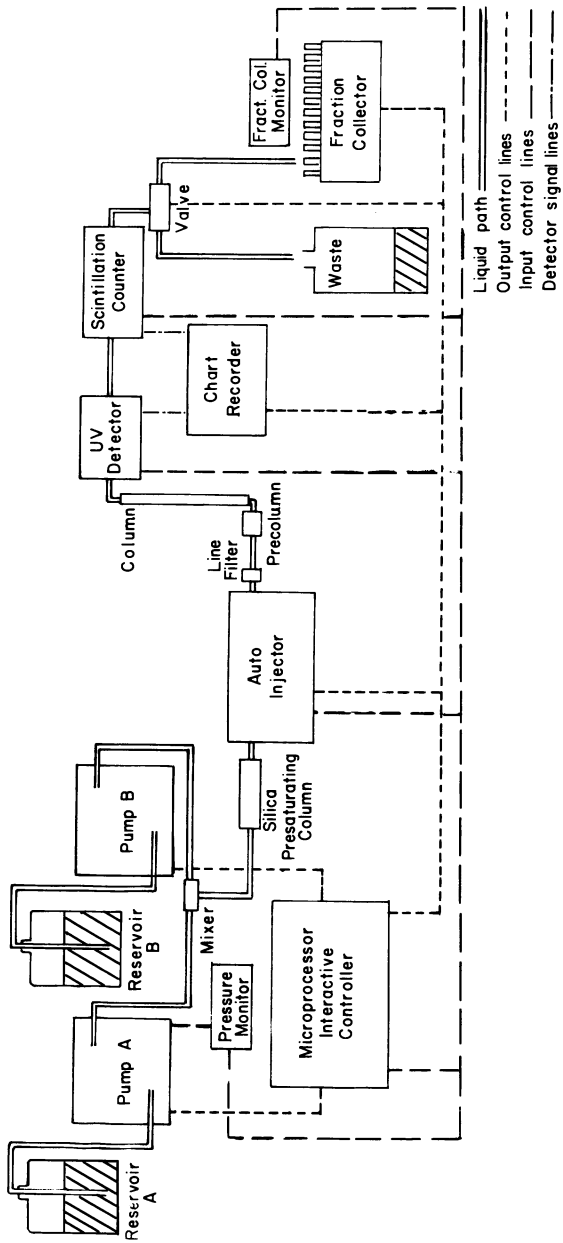


Figure 4. Schematic diagram of an automated preparative HPLC system.

In Plant Growth Substances; Mandava, N.;
ACS Symposium Series; American Chemical Society: Washington, DC, 1979.

on one or two HPLC columns. Identification has generally been accomplished with a UV detector at 254 nm. However, this method is relatively nonselective, since most aromatic compounds absorb radiation at 254 nm. The use of a UV detector on an HPLC system is only slightly more selective than a flame ionization detector on a GLC system.

Selective Detectors for Analytical HPLC. Sweetser and Swartzfager (61) demonstrated that either fluorescence or electrochemical detectors are efficient for selective identification of IAA. Fluorescence detection is much more selective than UV detection since fewer compounds fluoresce than absorb UV radiation. Electrochemical detection is also specific because only compounds that may be oxidized or reduced are detected. IAA is oxidized at a low voltage potential (0.7 to 0.9 V) relative to other compounds. In our hands, these two methods of detection appear to be quite accurate, since the same plant sample yields the same quantitative data by both methods. Since they are quite different, using both detection method adds credibility to the assays.

Future Selective Analytical HPLC Methods for PGS. The IAA analytical procedure developed by Sweeter and Swartzfager (61) is an approach that should be extended to the other PGS.

Several other selective analytical techniques are promising but still need to be proven suitable for PGS analysis. One technique is the simultaneous monitoring of UV absorbance at several different wavelengths. The ratio of absorbance at the respective wavelengths has proven to be unique for many compounds (62, 63). As with MID on MS, the more wavelengths that are simultaneously monitored, the greater is the likelihood of valid identification. Another analytical technique is the formation of derivatives which are fluorescent or absorb UV radiation at unique wavelengths. The compound of interest may be derivatized and injected onto the HPLC system; the column separates the reactants and then passes them through the detector. The compound may also be derivatized "post column" as done by amino acid analyzers. The derivatizing reactant is metered to mix with the column effluent and is then sent to the detector. Ideally, only the derivatized products should be detectable.

Derivative formation is essential for analysis of gibberellins because they only absorb radiation below 230 nm, which is an extremely nonspecific region. Benzyl esters (49) and p-nitrobenzyl esters (60) of gibberellins have successfully been synthesized prior to injection to permit their detection as they elute from HPLC columns. Unfortunately, these derivatives have added little selectivity to the analytical procedure. The derivatives are monitored at 254 or 265 nm which, as previously mentioned, is a nonspecific region.

A new method of detection of PGS by HPLC has been introduced

In Plant Growth Substances; Mandava, N.;
ACS Symposium Series; American Chemical Society: Washington, DC, 1979.

with the development of on-line liquid scintillation counters that now are commercially available. Reeve et al. (49) and Reeve and Crozier (50) described a system in which the scintillator is added to the column eluant which then passes through a liquid scintillation counter. This approach is destructive and thus only a portion of the column effluent should be diverted to the scintillation counter if further work is to be done on a portion of the effluent. Another approach is the use of a scintillation counter with a special flow cell packed with scintillator beads. This new technology is advantageous because it is nondestructive to the sample, yet offers detection efficiency comparable with the conventional liquid scintillation system. For a valid comparison of efficiency, one should recognize that the system that adds scintillant must dilute the sample by a factor of 4 to 10, while the packed cell system with solid scintillant does not dilute the sample. This normalizes the difference between the two types of scintillation counting since adding the scintillator is usually three to four times more efficient than using the system packed with a flow cell scintillator.

Radio-labeled PGS can be detected in the presence of many other compounds and thus can be fractionated from crude samples (Fig. 4) on the basis of radioactivity of eluting peaks. This procedure is ideal for the separation and identification of PGS metabolites and for analysis of PGS recovery efficiency.

Other Selective Detection Procedures for PGS.

The use of GLC-EC has become a well accepted method for the analysis of ABA as described by Saunders (37). The purity of ABA (methyl ester) detected on this system may be confirmed by forming the trans-ABA isomer methyl ester in sunlight while in acetone and rerunning the sample. We have found that prep-HPLC is useful in the purification of plant extracts for ABA analysis by GLC-ED (45). Another unique identification method takes advantage of the extreme cotton effect that ABA exhibits. The degree of optical rotation can be used for quantification of ABA if the sample is highly purified (37).

Specific monitoring of nitrogen or phosphorus containing compounds may be accomplished with an alkaline-flame ionization detector on a GLC with substantially greater sensitivity than an FID. The alkaline-FID has recently been reported (64) to detect IAA-methyl esters from plant samples.

Another analytical procedure that has drawn considerable attention is the conversion of IAA to indole-α-pyrone (65, 66, 67, 68). The limitations of this technique are that it is specific to free IAA, the assay is destructive, and the limit of detection is approximately 1 ng. However, for those studies that only require quantification of IAA, the procedure should be seriously considered. As Bandurski documented in this volume, there are many other forms of IAA that occur in substantial amounts. The

In Plant Growth Substances; Mandava, N.;
ACS Symposium Series; American Chemical Society: Washington, DC, 1979.

indole-α-pyrone procedure would fail to detect these other auxins.

Futher Refinement of PGS Analysis.

Selection of Appropriate Method of Sampling. As reviewed by Dennis (69), the appropriate sampling of tissue represents a significant challenge when attempting to relate PGS level with function. Even if the correct tissue (cells or organelles) is selected for extraction, the problem of determining the best solvent for PGS extraction still exists. Methanol has most commonly been used (1). However, natural PGS esters can undergo transesterification such as the formation of methyl abscisate (70). When the extraction is done with acetone, no methyl abscisate is recovered. Acetone has also been used for recovery of natural IAA esters (18, 32). Another approach to minimize formation of methyl esters has been to extract with hot water (53). Dichloromethane has been used (71) in preference to methanol or ethanol to minimize the conversion of indole-3-pyruvic acid to IAA.

The use of Triton X-100 to disperse Triticum chloroplast membranes has been reported to increase the recoverable yield of GA_9 by 1000 as compared with methanol extraction. The authors suggest that the methanol causes irreversible binding of GA_8 to the plastid membrane (72). However, the enhanced recovery using Triton X-100 has been disputed (73) and was not beneficial in the extraction of chloroplasts of Pisum. Now that physico-chemical procedures are available for many of the PGS, more attention should be directed at improving the extraction procedures for PGS.

Minimization of Impurities. The presence of solvent impurities may be one of the most common sources of analytical error. Even high purity solvents have been documented to contain plasticizers (74) and careful purification is required before use (75). The purity of the solvents should be examined by the detection procedures that will be used for the PGS (75).

For reverse phase HPLC, removal of volatile organic compounds from water can be especially troublesome. The problem may be diminished by filtering the water through activated charcoal. Pumping the aqueous mobile phase through a scrubber column (packed with reverse phase material) located between the pump and injector also has been useful.

Maximization of HPLC Column Efficiency. Many of the available HPLC bonded phase column packings are sold as single function packings, such as cation exchange materials. However, these packings often have several types of functions. Many ion exchange packing materials were made by covalently attaching the ion exchange group to the silic support by means of an organic link (Fig. 5). The organic phase also serves to protect the silica support from solubilization by aqueous buffers. However, charged molecules that also are nonpolar, such as most PGS, will be sep-

In Plant Growth Substances; Mandava, N.;
ACS Symposium Series; American Chemical Society: Washington, DC, 1979.

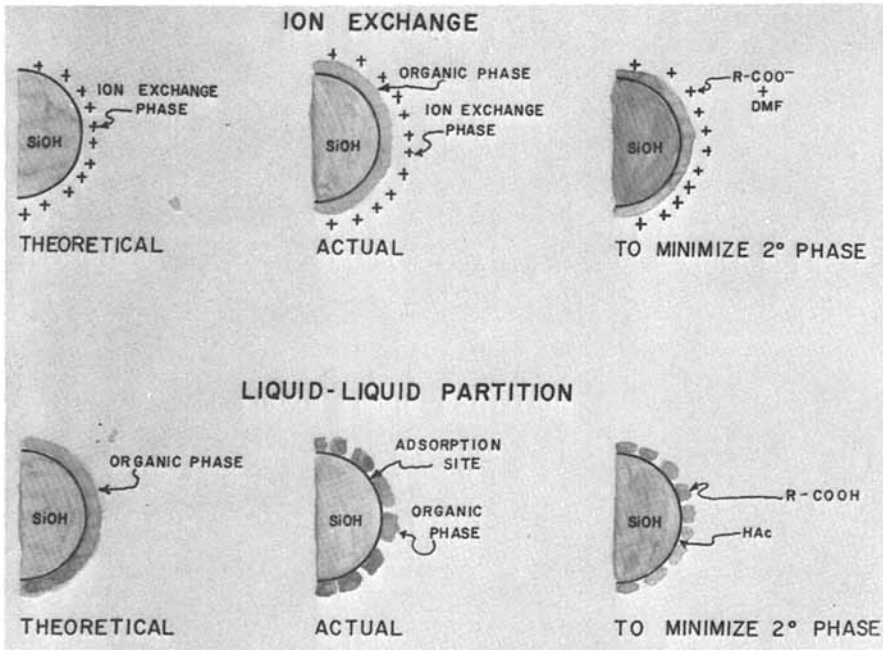


Figure 5. Diagram of the dual functionality of ion exchange and liquid-liquid partition of HPLC column packing

In Plant Growth Substances; Mandava, N.;
ACS Symposium Series; American Chemical Society: Washington, DC, 1979.

arated both by partitioning in the organic phase and by ion exchange on the same column.

We have found that the organic phase of Vydac anion exchange material has a higher affinity than the anion exchange sites for IAA. Thus, at low pH which maintains IAA in the protonated form, most of the IAA partitions into the column packing and results in a nonlinear concentration response curve (Fig. 6). However, the partitioning may be minimized by increasing the pH to 6, which converts IAA to the charged, more polar form, resulting in a linear concentration response curve (Fig. 6). Another method to reduce the dual separation process is the addition of a miscible organic solvent to the mobile phase to overcome partitioning into the column packing. Acetonitrile added to the mobile phase substantially increases the efficiency of the IAA separation even though it also reduces the k' (Fig. 7). The separation may be optimized by decreasing the buffer strength in the presence of the acetonitrile.

Similar dual functionality exists for many of the reverse phase packing materials (Fig. 5). Due to steric hindrance, the silica support is incompletely coated with the octadecyl molecules. The exposed silica groups serve as strong adsorption sites but the effect may be minimized by the addition of an organic acid to the mobile phase (46).

Determination of Correction for Sample Loss Due to Adsorption. Adsorption of the PGS to glassware can be a cause of significant losses of samples. The problem is particularly pronounced for relatively pure samples of PGS. The adsorption process described by the Langmuir isotherm indicates that, at very low concentrations, most material is adsorbed to the glassware. As the concentration is increased, the adsorption sites are saturated and thus, a greater percentage of sample is recovered. The recovery of PGS at the nanogram level is sensitive to losses by adsorption, while at the microgram level adsorption is insignificant. This also indicates that estimation of recovery by use of internal standards requires that the standard should be added at levels approximating those of the sample.

The silylation of all glassware that contacts the plant extract has proven to effectively reduce adsorption losses. As diagrammed in Figure 8, the hydroxyl adsorption sites on the silica surface can be coated with dichlordimethyl silane. The unreacted chloride groups are then displaced with methanol in a substitution reaction. A secondary advantage of the silyation process is that water will not adhere to the glass surface. Aqueous residues bead together, which allows more efficient sample transfers.

In Plant Growth Substances; Mandava, N.;
ACS Symposium Series; American Chemical Society: Washington, DC, 1979.

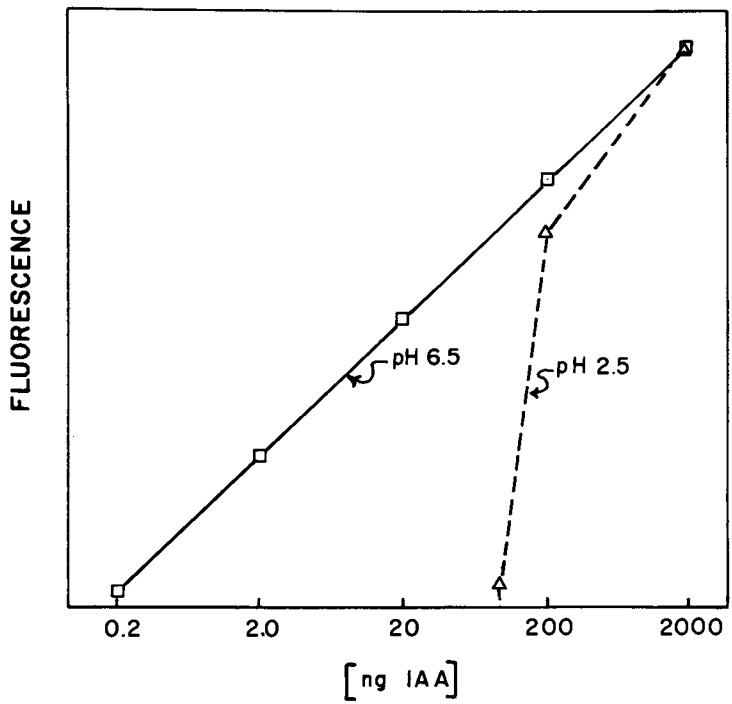


Figure 6. Effect of pH on anion exchange of IAA. Column = Vydac AX — TP (10μm); 3.9 mm ID × 20 cm, flow = 2.0 cm^3 min^{-1}, mobile phase = 0.1 m, *NaH_2PO_4 — Na_2HPO_4 buffer. Pump = Waters Associates 6000A, solvent programmer, Waters Associates 600.*

In Plant Growth Substances; Mandava, N.;
ACS Symposium Series; American Chemical Society: Washington, DC, 1979.

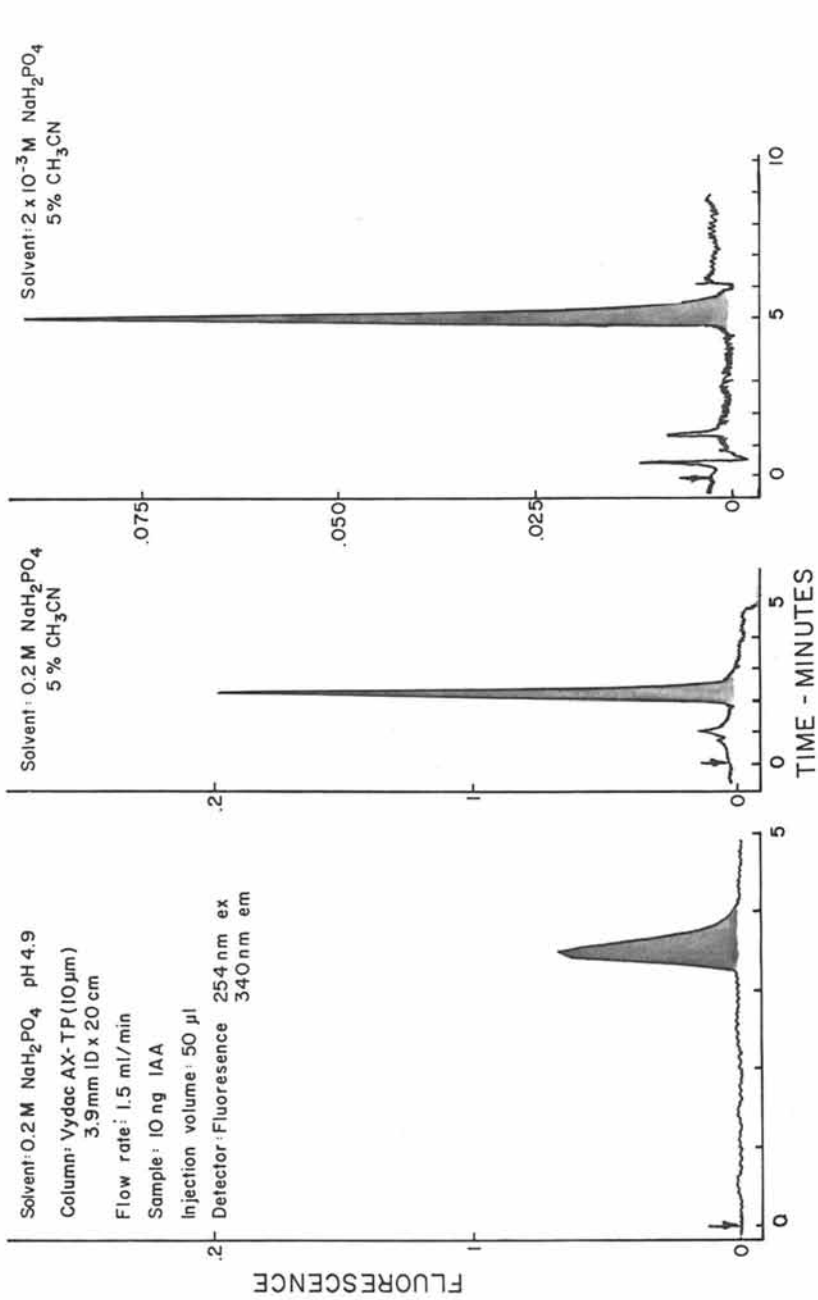


Figure 7. Effect of acetonitrile on anion exchange of IAA. Conditions—see Figure 6.

In Plant Growth Substances; Mandava, N.;
ACS Symposium Series; American Chemical Society: Washington, DC, 1979.

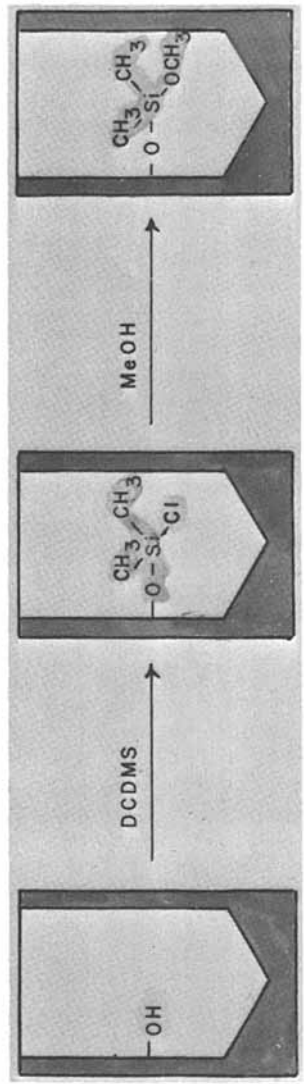


Figure 8. Diagrams of the silylation reaction of glassware

Conclusion

Analytical methods for PGS research have been greatly improved during this past decade. GLC-MS analysis has proven to be the method of choice, particularly when appropriate internal standards are used for accurate assessment of PGS recovery. HPLC, the most rapidly developing form of separation science, should substantially enhance present PGS analytical efforts. One advantage of HPLC is the substantial purification obtained for PGS compounds from crude plant extracts. For analytical identification by instrumentation, scrupulous purification is required, along with selective identification of the PGS. Preferably, two different analytical procedures should be utilized for positive identification of a given compound.

Manuscript no. 10,818 of the Agricultural Experiment Station, University of Minnesota, Saint Paul, MN 55108.

Literature Cited.

1. Hillman, J. R., Ed. "Isolation of Plant Growth Substances"; Cambridge University Press: London, 1978; p. 157.
2. Sweetser, P. B., in "Proceedings of the Fifth Annual Meeting of the Plant Growth Regulator Working Group", Abdel-Rahman, M., Ed., 1978; pp. 1-19.
3. Hedden, P., in "Proceedings of the Fifth Annual Meeting of the Plant Growth Regulator Working Group", Abdel-Rahman, M., 1978; pp. 33-44.
4. Brenner, M. L., in "Proceedings of the Fifth Annual Meeting of the Plant Growth Regulator Working Group", Abdel-Rahman, M., Ed., 1978; pp. 20-32.
5. Russel, S., in "Gibberellins and Plant Growth", Krishnamoorthy, N. J., Ed., John Wiley and Sons: New York, 1978; pp. 1-34.
6. Cram, S. P.; Risby, T. H. Anal. Chem., 1978, 50:213R-243R.
7. Gray, R. T.; Mallaby, R.; Ryback, G.; Williams, V. J. Chem. Soc. Perkin II, 1974:919-924.
8. Most, B. H.; Gaskin, P.; MacMillan, J. Planta (Berl.), 1970, 92:41-49.
9. Railton, I. D.; Reid, D. M.; Gaskin, P,; MacMillan, J. Planta (Berl.), 1974, 117:179-182.
10. Watanabe, N.; Yokota, T.; Takahashi, N. Plant and Cell Physiol., 1978, 19:1263-1270.
11. Binks, R.; MacMillan, J.; Pryce, R. J. Phytochem., 1969, 8: 271-284.

12. Durley, R. C.; MacMillan, J.; Pryce, R. J. Phytochem., 1971, 10:1891-1908.
13. Frydman, V.M.; MacMillan, J. Planta, 1973, 115:11-15.
14. Yokota, T.; Hiraga, K.; Yamane, H. Phytochem., 1975, 14: 1569-1574.
15. Greenwood, M. S.; Shaw, S.; Hillman, J. R.; Ritchie, A.; Wilkins, M. B. Planta, 1972, 108:179-183.
16. Bridges, I. G.; Hillman, J. R.; Wilkins, M. B. Planta, 1973, 115:189-192.
17. DeYoe, D.; Zaerr, J. B. Plant Physiol., 1976, 58:299-303.
18. Feung, C.; Hamilton, R. H.; Mumma, R. D. J. Agri. Food Chem., 1975, 23:1120-1124.
19. Ehmann, A.; Bandurski, R. S. Carbohyd. Res., 1974, 36:1-12.
20. Upper, C. D.; Helgeson, J. P.; Schmidt, C. J., in "Plant Growth Substances 1970", Carr, D. J., Ed.; Springer-Verlag: New York, 1972; pp. 798-807.
21. van Staden, J.; Drewes, S. E. Plant Sci. Lett., 1975, 4: 391-394.
22. Young, H. Anal. Biochem., 1977, 79:226-233.
23. Horgan, R.; Hewett, E. W.; Purse, J. G.; Horgan, J. M.; Wareing, P. F. Plant Sci. Lett., 1973, 1:321-324.
24. Morris, R. O. Plant Physiol., 1977, 59:Mass spectroscopic identification of cytokinins, pp. 1029-1033.
25. Dauphin, B.; Teller, G.; Durand, B. Planta, 1979, 144:113-119.
26. Williams, P. M.; Bradbeer, J. W.; Gaskin, P.; MacMillan, J. Planta (Berl.), 1974, 117:101-108
27. Gaskin, P.; MacMillan, J., in "Isolation of Plant Growth Substances", Hillman, J. R., Ed.; Cambridge University Press: London, 1978, pp. 79-96.
28. McDougall, J.; Hillman, J. R., in "Isolation of Plant Growth Substances", Hillman, J. R., Ed.; Cambridge University Press: London, 1978; pp. 1-26.
29. Rivier, L.; Milon, H.; Pilet, P. E. Planta, 1977, 134:23-27.
30. Little, C. H. A.; Heald, J. L.; Browning, G. Planta, 1978, 139:133-138.
31. Mann, J. D.; Jaworski, E. G. Planta (Berl.), 1970, 92:285-291.
32. Bandurski, R. S.; Schulze, A. Plant Physiol., 1974, 54:257-262.
33. Cohen, J. D.; Bandurski, R. S. Planta, 1978, 139:203-208.
34. Dekhuijzen, H. M.; Gevers, E. C. T. Physiol. Plant., 1975, 35:297-302.
35. Letham, D. S. Planta (Berl.), 1974, 118:361-364.
36. Thompson, A. G.; Horgan, R.; Heald, J. K. Planta, 1975, 124: 207-210.
37. Saunders, P. F., in "Isolation of Plant Growth Substances", Hillman, J. R., Ed.; Cambridge University Press: London, 1978; pp. 115-134.

In Plant Growth Substances; Mandava, N.;
ACS Symposium Series; American Chemical Society: Washington, DC, 1979.

38. Caruso, J.; Smith, R. G.; Smith, L. M.; Cheng, T.; Daves, G. D., Jr. Plant Physiol., 1978, 62:841-845.
39. Magnus, V.; Bandurski, R. S. Plant Physiol., 1978, 61 (Suppl.):63.
40. McWha, J. A.; Hillman, J. R. Z. Pflanzenphysiol., 1974, 74: 292-297.
41. Walton, D. C.; Dorn, B.; Fey, J. Planta, 1973, 112:87-90.
42. Hillman, J. R.; Math, V.B.; Medlow, G.C. Planta, 1977, 134: 191-193.
43. Summons, R. E.; McLeod, J. K.; Parker, C. W.; Letham, D. S. FEBS Let., 1977, 82:211-214.
44. Shindy, W. W.; Smith, O. E. Plant Physiol., 1975, 55:550-554.
45. Wightman, F., in, "Plant Growth Regulation", Pilet, P. E., Ed.; Springer-Verlag: Berlin, 1977, pp. 77-90.
46. Ciha, A.; Brenner, M. L.; Brun, W. A. Plant Physiol., 1977, 59:821-826.
47. Carnes, M. G.; Brenner, M. L.; Andersen, C. R. J. Chromatogr., 1975, 108:95-106.
48. Durley, R. C.; Kannangara, T.; Simpson, G. M. Can. J. Bot., 1978, 56:157-161.
49. Reeve, D. R.; Yokota, T.; Nash, L.; Crozier, A. J. Exp. Bot. 1976, 27:1243-1258.
50. Reeve, D. R.; Crozier, A., in, "Isolation of Plant Growth Substances", Hillman, J. R., Ed.; Cambridge University Press: London, 1978; pp. 41-78.
51. Suttle, J.; Zeevart, J. A. D. Plant Physiol., 1978, 61 (Suppl.):49.
52. Düring, H.; Bachmann, O. Physiologia Pl., 1975, 34:201-203.
53. Düring, H. Experentia, 1977, 33:1666-1667.
54. Sweetser, P. B.; Vatvars, A. Anal. Biochem., 1976, 71:68-78.
55. Challice, J. S. Planta (Berl.), 1975, 122:203-207.
56. Hahn, H. Plant Cell Physiol., 1976, 17:1053-1058.
57. Cole, D. L.; Leonard, N.; Cook, J. C., Jr., in, "Recent Developments in Oligonucleotide Synthesis and Chemistry of Minor Bases of tRNA," International Conference, Poland, 1974, Uniwersytet Im. Adama Mickiewicza Press: Poland, 1974, pp. 153-174.
58. Kannangara, T.; Durley, R. C.; Simpson, G. M. Physiol. Plant., 1978, 44:295-299
59. Holland, J. A.; McKerrell, E. H.; Fuell, K. J.; Burrows, W.J. J. Chromatogr., 1978, 166:545-553.
60. Heftmann, E.; Saunders, G. A.; Haddon, W. F. J. Chromatogr., 1978, 156:71-77.
61. Sweetser, P. B.; Swartzfager, D. G. Plant Physiol., 1978, 61:254-258.
62. Baker, J. K.; Skelton, R. E.; Ma, C. J. Chromatogr., 1979, 168:417-427.
63. Li, K.; Arrington, J. Anal. Chem., 1979, 51:287-291.

In Plant Growth Substances; Mandava, N.;
ACS Symposium Series; American Chemical Society: Washington, DC, 1979.

64. Swartz, H. J.; Powell, L. E. Plant Physiol., 1978, 61 (Suppl.):63.
65. Stoessl, A.; Venis, M. A. Anal. Biochem., 1970, 34:344-351.
66. Knegt, E.; Bruinsma, J. Phytochem., 1973, 12:753-756.
67. Mousdale, D. M. A.; Butcher, D. H.; Powell, R. G., in, "Isolation of Plant Growth Substances," Hillman, J. R., Ed.; Cambridge University Press: London, 1978, pp. 27-40.
68. Kamisaka, S.; Larsen, P. Plant and Cell Physiol., 1977, 18: 595-602.
69. Dennis, F. G., Jr. HortScience, 1977, 12:217-220.
70. Milborrow, B. V.; Mallaby, R. J. Exp. Bot., 1975, 26:741-748.
71. Atsumi, S.; Kuraishi, S.; Hayashi, T. Planta (Berl.), 1976, 129:245-247.
72. Browning, G.; Saunders, P. F. Nature, 1977, 265:375-377.
73. Railton, I. D.; Rechav, M. Plant Sci. Lett., 1979, 14:75-78.
74. De Zeevw, R. A.; Jonkman, J. H. G.; van Mansvelt, F. J. W. Anal. Biochem., 1975, 67:339-341.
75. Martin, G. C.; Nishijima, C. HortScience, 1977, 12:212-216.

RECEIVED June 19, 1979.

Hormonal Regulation of Genome Activity in Higher Plants

G. RAM CHANDRA

Seed Research Laboratory, Beltsville Agricultural Research Center, USDA, Beltsville, MD 20705

S. MUTHUKRISHNAN and E. S. MAXWELL

Laboratory of Molecular Biology, National Institute of Arthritis, Metabolism, and Digestive Diseases, NIH, Bethesda, MD 20205

Hormones are substances that are produced in one part of an organism and then transferred to another part, where they influence specific physiological processes (1). In this paper, we consider hormones to be naturally occurring substances that regulate growth processes. The classic list of plant hormones includes indole acetic acid or auxin (IAA), gibberellic acids (GA), cytokinins, abscisic acid (ABA), and ethylene. Some fatty acids, sterols, and other substances of plant origin (2) can be added to this list because they can also elicit growth responses in plants. Recent publications have reviewed the chemical and physiological activities of gibberellins (3, 4), auxins (5), cytokinins (6), abscisic acid (7), ethylene (8), fatty acids (9, 10), and sterols (11). For analytical procedures, the reader is referred to a recent monograph (12). Taylorson and Hendricks (13) have summarized the apparent relationships between phytochrome and gibberellins.

We say that a hormone has "physiological activity" if, when applied in low concentrations (10^{-6}M to 10^{-12}M), it induces a physiological response (cell division, cell elongation) in the cell. In plants, these responses ultimately result in organogenesis, flowering, fruiting, senescence, and the total growth of the plant. In general, the latent times for growth responses of individual plants to hormones range from 5 to 23 minutes (14). Many synthetic compounds such as 2,4-dichlorophenoxy acetic acids (2,4-D), naphthalene acetic acids (NAA), and benzyl adenine (BA) elicit physiological responses comparable to those of naturally occurring auxins and cytokinins. For the sake of convenience, we have considered specific studies with synthesized hormones to be representative of studies with the naturally occurring hormones. The biosynthesis and metabolism of hormones have been reviewed (15, 16) and are also discussed in the other chapters of this monograph.

Growth is the sum of innumerable genetically determined cellular and metabolic processes, each influencing the other. The real question in plant hormone research is which biological

In Plant Growth Substances; Mandava, N.;
ACS Symposium Series; American Chemical Society: Washington, DC, 1979.

processes are specifically under the control of which specific hormone. Each cell must clearly differentiate the hormones acting upon it. Some studies have questioned whether the biologically active form of a hormone is the form supplied to the tissue or the hormone after it is metabolized by the tissue. Such questions have been raised for the catabolic products of auxins, abscisic acid, conjugates of auxins, and gibberellin, and for the hormonal role of cytokinins and auxins in tRNA molecules, but are not reviewed in this article (17). A satisfactory answer to this question requires elucidation of the cellular mechanisms (discussed in this paper) that are believed to be primarily under hormonal regulation.

Hormone Receptors

The subject of receptors for plant hormones was recently reviewed (5, 18). Many studies (18) have elucidated the relationship between a hormone's structure and its physiological activity: generally, growth-promoting activity depends on a stereo-specific interaction of the hormone and a site-specific cell constituent or receptor protein. Hormone receptor sites are separate from the sites of synthesis, transport, and catabolism of the hormones.

With the availability of labeled hormones of high specific activity and the application of the principles of affinity chromatography, researchers were able to isolate cellular proteins that bind to plant hormones *in vitro*. Such proteins have been referred to as receptor proteins, binding proteins, or acceptor proteins. Tacit in the concept of hormone receptor proteins is the stereo-specific interaction of the hormone and the receptor protein (19). The resulting hormone-protein complex participates in growth processes that depend on new or enhanced protein synthesis. Advances in molecular biology and related sciences have enabled many researchers to study the role of receptors in the control of nuclear functions or other activities and to determine the site of primary hormonal action.

Matthyssee and Phillips (20) isolated two nuclear proteins, from tobacco cells, that bound specifically to 2,4-D. Receptor proteins for auxins, kinetins, and GA have been found (21). Subcellular fractions from bean leaves were recently shown to bind abscisic acid (22). Preliminary experiments (22) indicated that maximum ABA binding activity coincides with the activities of membrane-bound Mg^{++}-dependent, K^{+}-stimulated ATPase and glucan synthetase. Table I of Biswas and Roy (21) lists hormone receptor proteins reported in plant tissue. For a protein to qualify as a receptor molecule, it should have a high stereo-specific binding capacity (Kd 10^{-6} to 10^{-8}M) for its particular hormone. In corn coleoptiles, both IAA and NAA are equally effective in inducing cell elongation; fractions of plasma membrane and endoplasmic reticular membrane contain receptor proteins with Kd values of 10^{-6}M to 10^{-7}M for auxins (5, 18). When one considers procedural

In Plant Growth Substances; Mandava, N.;
ACS Symposium Series; American Chemical Society: Washington, DC, 1979.

variations between laboratories, it is remarkable that the values for the binding constants fit the concept that the auxin receptors are membrane bound and such protein molecules possess a high stereo-specific binding capacity for the hormone. Reports about receptor proteins for other hormones are limited and do not allow us to generalize about the cellular site of interaction of hormones. The cytosol of the pea contains receptor proteins for 2,4-D (23), GA (24, 25), and kinetin (26). Hormone receptor proteins isolated from various plant tissues are listed in Table II (21). Venis (5) summarized the micro-environmental properties of auxin receptor sites: a) the presence of anionic function (-COOH for auxin activity) and b) the availability of functional groups (depending on the pk values) containing one or more amino acids such as tyrosine, lysine, or histidine in the receptor molecules. Wardrop and Polya (27) purified a soluble auxin receptor protein with a molecular weight of 315,000 daltons from bean seedlings. Yoshida and Takegami (28) isolated a low-molecular-weight (4000-5000 daltons) cytokinin-binding protein from tobacco leaves that lacks tryptophan. Unlike the hypocotyl, the cytosol from soybean cotyledon was shown to contain auxin-binding protein (29).

Detailed information on receptor proteins is crucial for the elucidation of the cellular mechanisms for the actions and interactions of hormones. It would be valuable to know if there are different receptor proteins for different hormones at different cellular sites, at different stages of maturity, particularly in the genetic variants of the corn tissue. The discovery that exogenous application of GA to corn seedlings can overcome genetic "dwarfness" (30) has been the basis for studies on hormones and hormone receptor complexes as regulators of genome function(s). Continuation of investigations into organ specificity of hormone-receptor-chromatin interactions (31, 32) is highly desirable.

Regulation of Nuclear RNA Polymerase Activity

A large number of studies suggest that hormone-induced growth processes require RNA-dependent protein synthesis. In a recent review (33) on regulation of RNA metabolism by plant hormones, Jacobson concluded that "although there is much information to be obtained, the apparent uniformity of the responses from hormone to hormone leads one to think that the regulation of RNA metabolism is a specific and necessary event either for the initiation or for the perpetuation of all hormone responses although it is not necessarily the sole controlling element." Therefore, we will not review studies that demonstrate effects of hormones on general RNA metabolism, including the formation of polysomes. Rather, we will focus on a few studies in which hormone effects are evident at the level of chromatin activity and especially on studies that demonstrate the formation of the

In Plant Growth Substances; Mandava, N.;
ACS Symposium Series; American Chemical Society: Washington, DC, 1979.

specific mRNA for an enzyme. By restricting the scope of this discussion, we do not imply that the formation of a specific enzyme necessarily represents the primary mechanism of hormone action. We have also not reviewed studies that deal with the effect of hormones on membrane structure and their resultant pH effects, for there is no satisfactory information in the literature (5) to relate these findings to the question of regulation of genome activity. We have focused on studies that relate extranuclear activities to the hormone-induced formation of mRNA and enzymes in higher plants, so that any similarities to the effects of steroid on mammalian transcription (34) would be evident.

Auxin treatment stimulates the growth of soybean hypocotyl essentially by cell enlargement (35). Auxin-induced growth depends on the formation of mRNA's, for researchers have found that growth responses are sensitive to actinomycin D and insensitive to 5-fluorouracil, an inhibitor believed to be specific for ribosomal RNA's (36). This conclusion is consistent with the finding that auxin stimulates polysome formation well before the increase in ribosomal RNA synthesis and growth are established (37). These findings form the basis for studies (38) of the role of auxins in regulating the activity of nuclei/chromatin by limiting the number of sites that are available for transcription and the activity of RNA polymerases.

The activity of polymerase I in nuclei from soybean hypocotyl treated *in vivo* with auxin is about five to eight times the activity in nuclei isolated from control tissue, whereas the activity of polymerase II is, at most, only slightly higher in treated tissue (39). The ratio of the activities of the polymerases *in vivo* is consistent with the proportions of ribosomal and messenger RNA's synthesized *in vitro* by preparations of nuclei or chromatin (40, 41). However, auxin treatment did not result in any qualitative change in RNA synthesis (41, 42, 43). To determine whether the increase in the activity of polymerases is due to a) increase in the amount of the enzyme, b) activation of the enzyme, and/or c) increase in the number of template sites, workers (39, 40, 44) measured the activity of chromatin in soybeans in the presence of excess amounts of *E. coli* RNA polymerases. Although treatment of intact soybean hypocotyl with auxin increased the activity of polymerase I and slightly increased the activity of polymerase II in nuclei (39, 40), treatment did not increase the amount of polymerase I molecules actively transcribing chromatin *in vitro* (44). From solubilization studies (39, 42, 43, 44), researchers concluded that the activity of polymerase I increased because enzyme molecules were activated, not because the number of template sites increased.

Stimulatory effects of auxins on the activity of polymerases of lentil roots have been reported (45). Hardin's model (46), in which factors that "modify" RNA polymerases are released as a result of the auxin-plasma membrane interaction, has not been confirmed, but the finding that auxin action involves regulation

In Plant Growth Substances; Mandava, N.;
ACS Symposium Series; American Chemical Society: Washington, DC, 1979.

of proton extrusion strengthens the possibility that the hormone-membrane interaction is related to cell elongation. This relationship was elegantly demonstrated in many plant tissues with fusicoccin, a fungal toxin (47) that mimics the early action of auxin. More recently, it was shown (48) that chromatin preparations from auxin-treated soybean hypocotyl tissue, unlike intact nuclei, do not contain α-amanitin-sensitive RNA polymerase II. Similarly, in developing pea cotyledons, there is an apparent lack of gene dosage effects on rRNA production (49). Whether stimulatory effects of auxins on the synthesis of ribosomal and messenger RNA's are the primary event of hormone action remains to be determined. Although many studies show that auxin effects mRNA (33), no data demonstrate specific hormone effects on the activity of polymerase II or on the formation of qualitatively different mRNA molecules. It is possible that new mRNA's are made within a short period after auxin treatment and that such events are masked by turnover of mRNA and relatively large changes in the rates of synthesis of rRNA's. In tobacco, peas, and coconut endosperm cells, the auxin-receptor complex stimulated the activity of homologous chromatin preparations (21). A few studies have reported that treatment with cytokinins and gibberellins increased the activity of polymerases and stimulated chromatin activity (21). In all these studies, the activity of polymerase I increased significantly but the number of available sites for template activity did not. In a study of the in vitro effects of GA on pea nuclei, Johri and Varner (50) found that GA enhanced (by 10-15%) synthesis of messenger-like RNA molecules required an extra-nuclear specific hormonal interaction. In this system, GA_3 was active and GA_8 inactive. The system holds promise for further studies on the role of hormone receptors in eliciting qualitative changes of genome activity. By studying the α-amanitin-sensitive and α-amanitin-insensitive synthesis of RNA by nuclei isolated from GA-treated leaves and roots of pea plants, Dzhokhadze and Goglidze (51) concluded that polymerase activities are unequally stimulated in different tissues. However, they present no data that support their conclusion that the relative activities of the polymerases are a mechanism of hormone influence on specific transcription.

Abscisic acid is a negative regulator in that it primarily antagonizes the action of cytokinins, auxins, and in particular, gibberellins. Abscisic acid decreased the activity of polymerase in radishes (52), peas (53), maize coleoptiles (54), and pear embryos (55). More detailed studies are needed before the question of ABA-induced "modification" of RNA polymerase (54) or "alterations" in the number of sites for template activity (56) can be answered. In barley aleurone cells, ABA-induced suppression of GA-induced α-amylase formation was presumed to involve the continuous synthesis of a short-lived RNA (57).

In Plant Growth Substances; Mandava, N.;
ACS Symposium Series; American Chemical Society: Washington, DC, 1979.

Regulation of the Formation of Messenger RNAs for Specific Enzymes

For a hormone to have a specific effect on gene activity, any increase in enzyme activity must result from de novo synthesis by newly formed mRNA. This increase in enzyme activity may or may not precede any general increase in metabolic activity. From the foregoing discussion on chromatin activity, it is clear that plant hormones largely either increase the activity of polymerase I or increase the synthesis of total RNA's. Claims that the hormones "activate" chromatin-bound polymerases and "modulate" the number of active sites on the chromatin (21) have not been substantiated. There are only two known examples of hormone-induced synthesis of specific mRNA's. The classic example is the barley aleurone cells, in which GA treatment induces de novo synthesis and release of α-amylase (58, 59, 60), protease (61), and possibly as many as ten proteins (62).

A large number of studies (33) support the thesis (59) that the function of GA may be that of a derepressor of gene activity. Giberellic acid has significant effect on the synthesis of all species of RNA's (63, 64), but the formation of α-amylase does not depend on new synthesis of ribosomal and transfer RNA's. Unequivocal proof for GA-induced formation of transcripts was provided by the in vitro synthesis of peptides that are immunologically similar to α-amylase on poly A+RNA templates that were isolated from hormone-treated aleurone cells (65, 66, 67). Of particular significance is the finding that detectable levels of α-amylase mRNA's were formed within 2 hr of treatment with GA. The amounts of mRNA and amylase that are formed in vivo in the first 12 hr of incubation with GA correlate well (65). However, questions were not answered in this study. Levels of α-amylase mRNA's were measured during the first 15 hr of incubation with GA, when only 10 to 15% of the total α-amylase molecules are formed. Correlation of the level of mRNA and the level of α-amylase produced during a particular period of incubation implies that for every molecule of template, a constant amount of peptide is formed. The template is either recycled at a constant rate or destroyed after the formation of the first molecule of peptide.

We (68) measured α-amylase mRNA in aleurone tissue treated with GA for 2 to 24 hr at 25°C. The level of α-amylase mRNA increased to a maximum value at 12 hr of incubation and decreased thereafter. The mRNA level of a tissue treated with GA for 24 hr is comparable to the level of α-amylase mRNA in tissue treated with GA for 4 hr. From previous studies (69, 70), we know that α-amylase synthesis is maximum between 8 to 24 hr of incubation with GA. Thus, the rate of synthesis of α-amylase mRNA's reaches its maximum in the first 12 hr of incubation and reflects the later rate of α-amylase synthesis during the 12-24 hr incubation. A direct correlation of the level of mRNA with the amount of α-amylase produced at any given time interval (65) would imply that the template molecules either remain intact after translation

In Plant Growth Substances; Mandava, N.;
ACS Symposium Series; American Chemical Society: Washington, DC, 1979.

or turnover at a constant rate. However, neither is true, for the net level of translatable mRNA for α-amylase declines (68). By measuring the level of mRNA by in vitro synthesis of peptides that are immunologically similar to α-amylase and by in vitro methylation of the 5' ends of mRNA molecules with vaccinia virus methyltransferase, we ruled out the possibility of inactive transcripts in the purified poly A + RNA preparations. We believe that the synthesis of GA-dependent α-amylase mRNA's reaches its maximum by the twelfth hour of incubation with the hormone, after which the levels of mRNA's not attached to polysomes decline. Furthermore, at some point during the synthesis of mRNA, the tissue establishes another rate for the formation of polysomes that, in turn, determines the rate of synthesis of α-amylase in vivo. Therefore, during the 12-24 hr of incubation with GA, the net amount of translatable mRNA's declines as a result of the decline in free, excess translatable mRNA's, and the amount of polysome with α-amylase mRNA's no longer changes. Therefore, late in the GA-evoked formation of α-amylase (58), the rate-limiting step is the translation, not the transcription, of mRNA's. The rate of turnover of "free" α-amylase mRNA's is probably different from that of transcripts that are bound to the polysomes. The level of transcripts in the polysomes and the rate of in vivo synthesis of α-amylase are probably in a steady state during the 12-24 hr of incubation of cells with GA.

Control of the synthesis of α-amylase mRNA's in barley aleurone cells and the synthesis of cellulase mRNAs in pea epicotyl cells are similar in some respects. The control of cellulase activity in pea epicotyl is the only known example of auxin-induced formation of specific mRNA molecules. The formation of cellulase mRNA was demonstrated by the isolation of poly A + RNA's and in vitro synthesis of cellulase (71) using the protein-synthesizing system of wheat germ (72). The formation of cellulase mRNA precedes the increase in cellulase levels by more than 12 hr. Thus, it appears that the increase in rate of synthesis of translatable cellulase mRNA's in the pea epicotyl (71) and that of α-amylase mRNA's in barley aleurone cells (65, 67, 68) precedes the increase in rate of synthesis of protein molecules. There may be other regulatory factors influencing the translational rate of synthesis of cellulase and amylase. The events between transcription and translation are probably not controlled by GA, for the net amount of specific transcripts declines (68).

From a critical study of the metabolism of poly (A) in auxin-treated pea epicotyl, Verma and Maclachlan (73) showed that discreet classes of poly (A) (presumably part of mRNA's) are differentially associated with free and membrane-bound polysomes. The induction of specific mRNA's, the decline in the rate of synthesis of mRNA's, the polysome content per cell, and the formation of cellulase were all related to the membrane-bound polysomes. Although the rate of in vivo enzyme synthesis is

In Plant Growth Substances; Mandava, N.;
ACS Symposium Series; American Chemical Society: Washington, DC, 1979.

related to the translational activity of the polysomes, other factors such as processing, maturation, transport, and stability of poly (A) RNA may be modulated by hormones.

Attachment of poly A chains to the 3' terminus, capping of the 5' terminus, and methylation of the polynucleotide chain are three processes known to determine the activity or turnover of RNA's. Hormones may regulate such post-transcriptional modifications (74, 75, 76), and thereby determine the level of translatable mRNA's. There is some correlation between stimulation of tRNA methylation by gamma-irradiation and induction of invertase in sugar beet (77). This correlation does not rule out the possibility of post-transcriptional modification of invertase mRNA. Key and Silflow (78) could not detect poly (A) sequences in the DNA-like RNA fraction isolated from soybean hypocotyl tissue that was not treated with auxin. By treating the tissue with auxins, Schmid et al (79) were able to isolate DNA-like RNA fractions containing poly A^+ sequences. If the DNA-like RNA is of nuclear origin, the data (79) suggest that auxin promotes the polyadenylation of precursor mRNA's in the nucleus. Recently, Okita et al (80) studied the in vitro synthesis of α-amylase catalyzed by mRNA's isolated from GA-treated wheat aleurone cells and confirmed that the formation of α-amylase mRNA's is GA dependent (66, 67, 68) and that the enzyme is derived from a precursor peptide that is approximately 1500 daltons larger than native α-amylase (42,000 daltons) molecules. Since the amount of the putative precursor was a small part of the total amount of peptides synthesized in vitro, the possibility of hormonal regulation of post-transcriptional proteolysis is not clear. The membrane-associated latent α-amylase molecules are either a) the putative precursors (80) destined for release as active enzymes or b) intermediates of the secretory processes (81).

Do Hormones Regulate Genome Activity?

Although we have no prior reason to expect that the modes of action of plant hormones and steroid hormones are similar, we can expect that the regulation of genome activity is similar in all eucaryotic cells. Evidence for the presence of adenyl cyclase specific for 3',5'-cyclic AMP in plants is controversial (82). It appears that cyclic AMP does not mediate the action of GA (83). Two reports (85, 86) rule out the involvement of cyclic adenosine 3',5'-monophosphate and adenylate cyclase in the hormonal regulation of plant growth processes. Although barley aleurone cells metabolize GA's, attempts to isolate GA-binding proteins have not been successful (84).

The question whether hormones regulate genome activity can only be answered by speculations for evidence is limited. We know of only two instances of hormonal induction of synthesis of specific mRNA's. The cellular mechanisms that allow an increase in the amount of translatable mRNA's at any given time of hormone

In Plant Growth Substances; Mandava, N.;
ACS Symposium Series; American Chemical Society: Washington, DC, 1979.

action must take into account post-transcriptional modifications of the transcripts and degradation of the transcripts. Since the increase in rate of synthesis of translatable mRNA's precedes increase in rate of formation of enzymes (65, 67, 68, 71), the rate of turnover of the transcripts could be controlled. Varner and Ho (87) presented two possible explanations for the relationship between the effects of GA on membrane proliferation and enhanced synthesis of α-amylase and other hydrolases in barley aleurone cells: (1) The enhanced synthesis of α-amylase depends on the faster rate of formation (transcription and/or processing) of its specific mRNA. Membrane proliferation would be a separate effect of GA_3. 2) The GA_3-enhanced membrane proliferation forms more sites for the synthesis of α-amylase. Thus, the amount of amylase-specific mRNA need not be increased in the presence of GA_3.

Studies (66, 67) on the rate of formation of α-amylase mRNA's support the conclusion that the GA-induced synthesis of α-amylase depends on the formation of α-amylase mRNA's.

Studies on the effect of auxins on the activity of chromatin and intact nuclei suggest that the hormone acts by affecting the activity of polymerases rather than by increasing the amount of polymerases or the number of sites that are available for transcription (44). The rate of rRNA synthesis far exceeds the rate of mRNA synthesis. This difference in rates of turnover could be a reason why no major qualitative change in the synthesis of mRNA's has been found in the in vitro studies (41, 42, 43). For the same reason, stimulatory effects on the activity of polymerase II could have been masked by the increase in the activity of polymerase I. Thus, we are led to conclude that auxins (44) and, possibly, other hormones activate the polymerases. However, no data have been found that support the hypotheses that a) hormones participate in the activation of inactive zymogen molecules, or b) solubilization of chromatin-bound polymerases activates polymerase. Hormones could regulate the activity of polymerases by regulating endoribonuclease activity. Auxin treatment suppresses the ribonuclease activity in pea stems, wheat coleoptile (88) and lens roots (89), and kinetin suppresses ribonuclease in barley leaves, particularly those enzymes associated with chromatin (90, 91). In contrast, the negative regulator ABA causes an increase in nuclease activity in barley leaf chromatin (91), lentil root (92), barley aleurone cells (70), and tomato (93). These findings suggest that hormones may control chromatin activity by regulating the activity of site-specific endoribonucleases. The standard in vitro transcription assays measure template activity without considering endogenous nuclease activity. Aurintricarboxylic acid (ATA) is widely used in enzymatic reactions of protein and nucleic acid biosynthesis and is also a potent inhibitor of nucleases (94). Levy et al (95) have proposed that poly (A) inhibits endonuclease activity in eukaryotic cells. Thus, any suppression of endonuclease activity

In Plant Growth Substances; Mandava, N.;
ACS Symposium Series; American Chemical Society: Washington, DC, 1979.

by hormones would be measured as an increase in the activity of polymerases and increased synthesis of RNA's. We conjecture that, as compared to free hormones, the hormone receptor complexes are site-specific inhibitors of endonucleases. This possibility is consistent with the observations that the addition of hormone receptor complex to a chromatin preparation causes an increase in RNA synthesis (21). Thus, it can be hypothesized that stereo-specific interactions of hormones with receptors produce hormone receptor complexes that are site-specific inhibitors of endoribo-nucleases associated with chromatin. At a second level of regulation, other cellular mechanisms would either catabolize the hormone, thus returning the receptor proteins to their original site for recycling or destroy both the hormone and the receptors. The hypothesis of hormonal regulation of chromatin activity by site-specific inhibition of endoribonucleases with hormone-receptor complex is attractive, simple, and experimentally verifiable. It is possible that the hormone receptor complex has site-specific DNase activity. In this case, in hormone-treated cells, endonucleases selectively may nick single strands in the DNA of specific genes, thus opening up the chromatin for transcription by RNA polymerases.

The subject of plant nucleases, last reviewed in 1975 (96), needs to be updated. The significance of reports describing the influence of plant hormones on the DNA composition (97) role of endonuclease (DNase I) in "active" chromatin structure (98) and phosphorylation of nuclear proteins (99) in the regulation of gene transcription (100) needs to be elucidated. For the GA-induced α*-amylase synthesis in barley aleurone cells and the auxin-induced cellulase synthesis in pea epicotyl, the available reports support the thesis (59) that plant hormones induce selective gene activities. Knowledge of the chemistry of plant hormones has provided little clues to their action. Future research on the synthesis of specific gene sequences (cDNA) (101) should provide a greater understanding of the mechanism(s) of hormonal regulation of mRNA synthesis.*

In Plant Growth Substances; Mandava, N.;
ACS Symposium Series; American Chemical Society: Washington, DC, 1979.

LITERATURE CITED

1. Went, F. W.; Thimann, K. V. "Phytohormones"; Macmillan: New York, 1937.
2. Gross, D. Growth regulating substances of plant origin. Phytochemistry, 1975, 14, 2105-2112.
3. Hedden, P.;MacMillan, J.; Phinney, B. O. The metabolism of the gibberellins. Ann. Rev. Plant Physiol., 1978, 29, 149-192.
4. MacMillan, J. Some aspects of gibberellin metabolism in higher plants. In Plant Growth Regulation, Proceedings of the 9th International Conference on Plant Growth Substances; Springer-Verlag: Berlin, Heidelberg, New York, 1977; pp 129-138.
5. Venis, M. A. Receptors for plant hormones. In Advances in Botanical Research, Vol. 5, pp 53-85.
6. Skoog, F.; Armstrong, D. J. Cytokinins. Ann Rev. Plant Physiol., 1976, 21, 359-384.
7. Milborrow, B. V. The chemistry and physiology of abscisic acid. Ann. Rev. Plant Physiol., 1974, 25, 259-307.
8. Abeles, F. D."Ethylene in Plant Biology"; Academic Press: New York, 1973.
9. Iwata, T.; Stowe, B. B.. Probing a membrane matrix regulating hormone action. II. The kinetics of lipid-induced growth and ethylene production. Plant Physiol., 1973, 51, 691-701.
10. Mandava, N.; Mitchell, J. W. Structural elucidation of brassins. Chem. Ind., 1972, 930-931.
11. Granwald, C. Plant Sterols. Ann. Rev. Plant Physiol., 1975, 26, 209-236.
12. Hillman, J. R., Ed. "Isolation of Plant Growth Substances"; Society for Experimental Biology Seminar Series 4: Cambridge Univ. Press, 1978.
13. Taylorson, R. B.; Hendricks, S. B. Dormancy in seeds. Ann. Rev. Plant Physiol., 1977, 28, 331-354.
14. Warner, H. L.; Leopold, A. C. Timing of growth regulator responses in peas. Biochem. Biophys. Res. Comm, 1971, 44, 989-994.
15. Sheldrake, A. R. The production of hormones in higher plants. Biol. Rev., 1973, 48, 509-559.
16. Bearder, J. R.; Sponsel, V. M. Selected topics in gibberellin metabolism. Biochem. Soc. Trans., 1977, 5, 569-582.
17. Lea, P. J.; Norris, R. D. tRNA and aminoacyl-tRNA synthetases from higher plants. In Progress in Phytochemistry, Vol. 4, 1977; Pergamon Press: New York, pp. 121-167.
18. Kende, H.; Gardner, G. Hormone binding in plants. Ann. Rev. Plant Physiol., 1976, 27, 267-290.
19. Ray, P. M. Specificity of auxin-binding sites on maize coleoptile membranes as possible receptor sites for auxin action. Plant Physiol., 1977, 60, 585-591.
20. Matthysse, A. G.; Phillips, C. Protein intermediary in the interaction of a hormone with genome. Proc. Natl Acad. Sci., 1969, 63, 897-903.

In Plant Growth Substances; Mandava, N.;
ACS Symposium Series; American Chemical Society: Washington, DC, 1979.

21. Biswas, B. B.; Roy, P. Plant growth substances as modulators of transcription. Chapter 4, Subcellular Biochemistry, 1978, 5, 187-219.
22. Hocking, T. J.; Clapman, J.; Cattell, K. J. Abscisic acid binding to subcellular fractions from leaves of Vicia faba. Planta, 1978, 138, 303-304.
23. Venis, M. A. Stimulation of RNA transcription from pea and corn DNA by protein retained on sepharose coupled to 2,4-dicholorphenoxyacetic acid. Proc. Natl. Acad. Sci. USA, 1971, 68, 1824-1827.
24. Stoddart, J.; Breidenbach, R. W.; Nadeau, R.; Rappaport, L. Selective binding of ^{3}H gibberellin A_1 by protein fractions from dwarf pea epicotylis. Proc. Natl. Acad. Sci. USA, 1974, 71, 3255-3259.
25. Konjevic, R.; Grubisic, D.; Markovic, R.; Petrovic, J. Gibberellic acid-binding proteins from pea stems. Planta, 1976, 131, 125-128.
26. Matthysee, A. G.; Abrams, M. A factor mediating interaction of kinins with the genetic material. Biochem. Biophys. Acta., 1970, 190, 511-518.
27. Wardrop, A. J.; Polya, G. M. Properties of soluble auxin-binding protein from dwarf bean seedlings. Plant Sci. Lett., 1977, 8, 155-163.
28. Yoshida, K.; Takegami, T. Isolation of cytokinin binding protein from tobacco leaves by bioaffinity chromatography and its partial characterization. J. Biochem., 1977, 81, 791-799.
29. Ihl, M. Indole-acetic acid binding proteins in soybean cotyledon. Planta, 1976, 131, 223-228.
30. Phinney, B. O. Growth response of single-gene dwarf mutants in maize to gibberellic acid. Proc. Natl. Acad. Sci. USA, 1956, 42, 185-189.
31. Matthysse, A. G. Organ specificity of hormone-receptor-chromatin interactions. Biochem. Biophys. Acta., 1970, 199, 519-521.
32. Voges, B.; Fellenberg, G. Organ-specific properties of chromatin in pea plants. Biochem. Physiol. Pflanzen, 1976, 169, 507-510.
33. Jacobsen, J. V. Regulation of ribonucleic acid metabolism by plant hormones. Ann. Rev. Plant Physiol., 1977, 28, 537-564.
34. Yamamoto, K. R.; Alberts, B. M. Steroid receptors: Elements for modulation of eukaryotic transcription. Ann. Rev. Biochem. 1976, 45, 721-746.
35. Nooden, L. D.; Thimann, K. V. Evidence for a requirement for protein synthesis for auxin-induced cell enlargement. Proc. Natl. Acad. Sci. USA, 1963, 50, 194-200.
36. Key, J. L. Hormones and nucleic acid metabolism. Ann. Rev. Plant Physiol., 1969, 20, 449-474.
37. Davies, E.; Larkins, B. A. Polyribosomes from peas. Plant Physiol., 1973, 52, 339-345.

In Plant Growth Substances; Mandava, N.;
ACS Symposium Series; American Chemical Society: Washington, DC, 1979.

38. Duda, C. T. Plant RNA polymerases. Ann. Rev. Plant Physiol., 1976, 27, 119-132.
39. Guilfoyle, T. J.; Lin, C. Y.; Chen, Y. M.; Nagao, R. T.; Key, J. L. Enhancement of soybean RNA polymerase I by auxin. Proc. Natl. Acad. Sci. USA, 1975, 72, 69-72.
40. Chen, Y.; Lin, C.; Chang, H.; Guilfoyle, T. J.; Key, J. L. Isolation and properties of nuclei from control and auxin-treated soybean hypocotyl. Plant Physiol., 1975, 56, 78-82.
41. O'Brien, T. J.; Jarvis, B. C.; Cherry, J. H.; Hanson, J. B. Enhancement by 2,4-dichlorophenoxyacetic acid of chromatic RNA polymerase in soybean hypocotyl tissue. Biochem. Biophys. Acta., 1968, 169, 35-43.
42. Johnson, L. K.; O'Brien, T. J.; Strehler, B. L. Developmental restrictions on hormone modulated gene transcription. I. Effects of auxin on template capacity and chromatin-bound RNA polymerase. Mech. Ageing Dev., 1978, 8, 97-112.
43. Johnson, L. K.; Strehler, B. L.; O'Brien, T. J. Developmental restrictions on hormone modulated gene transcription. II. Hormone induced interactions of RNA polymerase with chromatin. Mech. Ageing Dev., 1978, 8, 113-130.
44. Guilfoyle, T. J.; Hanson, J. B. Greater length of ribonucleic acid synthesized by chromatin-bound polymerase from auxin-treated soybean hypocotyls. Plant Physiol., 1974, 53, 110-113.
45. Teissere, M.; Penon, P.; Van Huystee, R. B.; Azou, Y.; Richard, J. Hormonal control of transcription in higher plants. Biochem. Biophys. Acta., 1975, 402, 391-402.
46. Hardin, J. W.; Cherry, J. H.; Morré, D. J.; Lembi, C. A. Enhancement of RNA polymerase activity by a factor released by auxin from plasma membrane. Proc. Natl. Acad. Sci. USA, 1972, 69, 3146-3150.
47. Marrè, E.; Labo, P.; Rasi-Caldogno, F.; Colombo, M. Cocucci, R.; DeMichelis, M. I. Regulation of proton extrusion by plant hormones and cell elongation. Physiol. Veg., 1975, 13(4), 797-811.
48. Gurley, W. B.; Lin, C. Y.; Guilfoyle, T. J.; Key, J. L. Analysis of plant RNA polymerase I transcript in chromatin and nuclei. Biochem. Biophys. Acta., 1976, 425, 168-174.
49. Cullis, C. A. Chromatin-bound DNA-dependent RNA polymerase in developing pea cotyledons. Planta, 1976, 131, 293-298.
50. Johri, A. A.; Varner, J. E. Enhancement of RNA synthesis in isolated pea nuclei by gibberellic acid. Proc. Natl. Acad. Sci. USA, 1968, 59, 269-276.
51. Dzhokhadze, D. I.; Goglidze, R. I. Comparative effect of gibberellic acid on RNA polymerase activity of cell nuclei in leaves and roots of pea. Fiziol. Rast., 1977, 24(4), 746-750.

In Plant Growth Substances; Mandava, N.;
ACS Symposium Series; American Chemical Society: Washington, DC, 1979.

52. Pearson, J. A.; Wareing, P. F. Effect of abscisic acid on activity of chromatin. Nature, 1969, 221, 672-673.
53. McComb, A. J.; McComb, J. A.; Duda, C. T. Increased ribonucleic acid polymerase activity associated with chromatin from internodes of dwarf pea plants treated with gibberellic acid. Plant Physiol., 1970, 46, 221-223.
54. Bex, J. H. M. Effects of abscisic acid on the soluble RNA polymerase activity in maize of coleoptiles. Planta, 1972, 103, 11-17.
55. Khan, A. A. ABA- and kinetin-induced changes in cell homogenates, chromatin-bound RNA polymerase and RNA composition. Int. Conf. Plant Growth Subst., 7th, Canberra, pp. 207-215, 1972.
56. Basak, S.; Roy, P.; Biswas, B. B. Interaction of indole acetic acid and abscisic on transcription. Ind. J. Biochem. Biophys., 1977, 14, 147-150.
57. Ho, D. T. Response of barley aleurone layers to abscisic acid. Plant Physiol., 1976, 57, 175-178.
58. Varner, J. E. Gillerellic acid controlled synthesis of α-amylase in barley endosperm. Plant Physiol., 1964, 39(3), 413-415.
59. Varner, J. E.; Chandra, G. R. Hormonal control of enzyme synthesis in barley endosperm. Proc. Natl. Acad. Sci. USA, 1964, 52, 100-106.
60. Filner, P.; Varner, J. E. A test for de novo synthesis of enzymes: density labeling with H_2 180 for barley -amylase induced by gibberellic acid. Proc. Natl. Acad. Sci. USA, 1967, 58, 1520-1526.
61. Jacobsen, J. V.; Varner, J. E. Gibberellic acid-induced synthesis of protease by isolated aleurone layers of barley. Plant Physiol., 1967, 42, 1596-1600.
62. Jacobsen, J. V.; Know, R. B. The proteins released by isolated barley aleurone layers before and after gibberellic-acid treatment. Planta, 1974, 115, 193-206.
63. Chandra, G. R.; Duynstee, E. E. Hormonal regulation of nucleic acid metabolism in aleurone cells. In Biochemistry and Physiology of Plant Growth Substances. Ed. F. Wightman, F. G. Setterfield. Runge Press, Ottawa, 723-745, 1968.
64. Jacobsen, J. V.; Zwar, J. A. Gibberellic acid and RNA synthesis in barley aleurone layers: metabolism of rRNA and tRNA and of RNA containing polyadenylic acid sequences. Aust. J. Plant Physiol., 1974, 1, 343-356.
65. Higgins, T. J. V.; Zwar, J. A.; Jacobsen, J. V. Gibberellic acid enhances the level of translatable mRNA for α-amylase in barley aleurone layers. Nature, 1976, 260, 166-169.
66. Higgins, T. J. V.; Zwar, J. A.; Jacobsen, J. V. Hormonal control of the level of translatable mRNA for α-amylase in barley aleurone layers. In Nucleic Acids and Protein Synthesis in Plants, Ed. J. H. Well; L. Bogorad, Paris: CNRS, 1977.

In Plant Growth Substances; Mandava, N.;
ACS Symposium Series; American Chemical Society: Washington, DC, 1979.

67. *Ho, D. T.; Varner, J. E. Hormonal control of messenger ribonucleic acid metabolism in barley aleurone layers. Proc. Natl. Acad. Sci. USA, 1974, 71, 4783-4786.*
68. *Muthukrishnan, S.; Maxwell, E. E.; Chandra, G. R. Messenger RNA for α-amylase in aleurone layers of barley seeds treated with gibberellic acid. Abstracts of the Eleventh International Congress of Biochemistry, 1979.*
69. *Chrispeels, M. J.; Varner, J. E. Gibberellic acid-enhanced synthesis and release of α-amylase and ribonuclease by isolated barley aleurone layers. Plant Physiol., 1967, 42(3), 398-406.*
70. *Chrispeels, M. J.; Varner, J. E. Hormonal control of enzyme synthesis: On the mode of action of gibberellic acid and abscisin in aleurone layers of barley. Plant Physiol., 1967, 42(7), 1008-1016.*
71. *Verma, D. P. S.; MacLachlan, G. A.; Byrne, H.; Ewings, D. Regulation and in vitro translation of messenger ribonucleic acid for cellulase from auxintreated pea epicotyls. J. Biol. Chem., 1975, 250, 1019-1026.*
72. *Marcus, A.; Efron, D.; Weeks, D. P. The wheat embryo cell free system. In Methods in Enzymology, Volume 30. Ed. S. P. Colowick, N. O. Kaplan, Academic Press, New York, 749-754, 1974.*
73. *Verma, D. P. S.; MacLachlan, G. A. Metabolism of poly(A) in plant cells. Discrete classes associated with free and membrane-bound polysomes. Plant Physiol. 1976, 58, 405-410.*
74. *Chandra, G. R.; Duynstee, E. E. Methylation of ribonucleic acids and hormone-induced α-amylase synthesis in the aleurone cells. Biochem. Biophys. Acta., 1971, 232, 514-523.*
75. *Chandra, G. R. Effect of gibberellic acid on the tRNA methylase activity of barley aleurone cells. In Plant Growth Substances. Ed. D. J. Carr, Springer-Verlag, Berlin, Heilelberg, New York, 365-370, 1970.*
76. *Jankowski, J.; Wielgat, B.; Kleczkowski, K. Gibberellin-affected methylation and synthesis of ribosomal RNA in isolated maize seed scutellum. Plant Sci. Lett., 1975, 5, 347-350.*
77. *Stone, B. P.; Cherry, J. H. Induced production of invertase in sugar-beet root by gamma-irradiation: Role of RNA. Planta, 1972, 102, 179-189.*
78. *Key, J. L.; Silflow, C. The occurrence and distribution of poly(A) ribonucleic acid in soybean. Plant Physiol., 1975, 56, 364-369.*
79. *Schmid, B. D.;Siegel, N. R.; Vanderhoef, L. N. The isolation and characterization of adenosine monophosphate-rich polynucleotides synthesized by soybean hypocotyl cells. Plant Physiol., 1975, 55, 277-281.*

In Plant Growth Substances; Mandava, N.;
ACS Symposium Series; American Chemical Society: Washington, DC, 1979.

80. Okita, T. W.; DeCaleya, R.; Rappaport, L. Synthesis of a possible precursor of α-amylase in wheat aleurone cells. Plant Physiol., 1979, 63, 195-200.
81. Locy, R.; Kende, H. The mode of secretion of α-amylase in barley aleurone layers. Planta, 1978, 143, 89-99.
82. Amrhein, N. The current status of cyclic AMP in higher plants. Ann. Rev. Plant Physiol., 1977, 28, 123-132.
83. Keates, R. A. B. Evidence that cyclic AMP does not mediate the action of gibberellic acid. Nature, 1973, 244, 355-356.
84. Musgrave, A.; Kays, S. E.; Kende, H. Uptake and metabolism of radioactive gibberellins by barley aleurone layers. Planta, 1972, 102, 1-10.
85. Yunghans, W. N.; Morre, D. J. Adenylate cyclase activity not found in soybean hypocotyl and onion meristem. Plant Physiol. 1977, 60, 144-149.
86. Bressan, R. A.; Ross, C. W. Attempts to detect cyclic adenosine 3': 5'-monophosphate in higher plants by three assay methods. Plant Physiol., 1976, 57, 29-37.
87. Varner, J. E.; Ho, D. T. The role of hormones on the integration of seedling growth. In the molecular biology of hormone action. Ed. John Papaconstantinou, Academic Press, New York, 173-194, 1976.
88. Truelsen, T. A. Indoleacetic acid-induced decrease of the ribonuclease activity in vivo. Physiol. Plant, 1967, 20, 1112-1119.
89. Pilet, P. E.; Braun, R. Riboneclease activity and auxin effects in the Lens root. Physiol. Plant, 1970, 23, 245-250.
90. Srivastava, B. I. S. Acceleration of senescence and of the increase of chromatin-associated nucleases in excised barley leaves by abscisin II and its reversal by kinetin. Biochem. Biophys. Acta., 1968, 169, 534-536.
91. Srivastava, B. I. S. Increase in chromatin associated nuclease activity of excised barley leaves during senescene and its suppression by kinetin. Biochem. Biophys. Res. Commun., 1968, 32, 533-538.
92. Pilet, P. E. The effect of auxin and abscisic acid on the catabolism of RNA. J. Exp. Bot., 1970, 21, 446-451.
93. Puri, J.; Tal, M. Abnormal stomatal behavior and hormonal imbalance in flacca, wilty mutant of tomato. Plant Physiol., 1977, 59, 173-177.
94. Hallick, R. B.; Chelm, B. K.; Gray, P. W.; Orozco, Jr., E. M. Use of aurintricarboxylic acid as an inhibitor of nucleases during nucleic acid isolation. Nucleic Acids Res., 1977, 4(9), 3057-3064.
95. Levy, C. C.; Schmukler, M.; Frank, J. J.; Karpetsky, T. P.; Jewett, P. B.; Hieter, P. A.; LeGendre, S. M.; Dorr, R. G. Possible role for poly(A) as an inhibitor of endonuclease activity in eukaryotic cells. Nature, 1975, 256, 340-341.

In Plant Growth Substances; Mandava, N.;
ACS Symposium Series; American Chemical Society: Washington, DC, 1979.

96. *Wilson, C. M. Plant nucleases. Ann. Rev. Plant Physiol., 1975, 26, 187-208.*
97. *Schafer, A.; Neuman, K. H. The influence of gibberellic acid on reassociation kinetics of DNA of Daucus carota L. Planta, 1978, 143, 1-4.*
98. *Lilley, D. M. J. Active chromatin structure. Cell Biol. Int. Rep., 1978, 2(1), 1-10.*
99. *Murray, M. G.; Key, J. L. 2,4-dichlorophenoxyacetic acid-enhanced phosphorylation of soybean nuclear proteins. Plant Physiol., 1978, 61, 190-198.*
100. *Jungmann, R. A.; Kranias, E. G. Nuclear phosphoprotein kinases and the regulation of gene transcription. Int. J. Biochem., 1977, 9, 819-830.*
101. *Rosen, J. M. Synthesis and processing of eukaryotic messenger RNA. In Receptors and Hormone Action, Vol. 1, Ed. Bert W. O'Malley, Lutz Birnbaumer, Academic Press, New York, 237-266, 1977.*

RECEIVED June 19, 1979.

10

Controlling Biological Behavior of Plants with Synthetic Plant Growth Regulating Chemicals

LOUIS G. NICKELL

Research & Development, Velsicol Chemical Corporation, 341 E. Ohio Street, Chicago, IL 60611

Plant growth regulators usually are defined as organic compounds, other than nutrients, that, in small concentrations, affect the physiological processes of plants. For practical purposes, plant growth regulators can be defined as either natural or synthetic compounds that are applied directly to a plant to alter its life processes or structure in some beneficial way so as to enhance yields, improve quality, or facilitate harvesting. Herbicides, when applied to induce a specific beneficial change, also can be considered plant growth regulators.

The response of a plant or a plant part to a plant growth regulator may vary with the variety of plant. Even a single variety may respond differently, depending on its age, environmental conditions, physiological state of development (especially its natural hormonal content), and its state of nutrition. Thus, whenever a general rule is suggested concerning the action of a specific growth regulator on plants, exceptions almost always can be found.

Uses of Growth Regulants

The regulation of plant growth can be economically useful in a great many ways. Among other things, it can:

- Promote rooting and propagation of the plant.
- Initiate or terminate the dormancy of seeds, buds, and tubers.
- Induce or retard aging (senescence).
- Promote, delay, or prevent flowering.
- Induce or prevent leaf and/or fruit drop (abscission).
- Control fruit set and further fruit development.
- Control plant or organ size.
- Prune the plant chemically.

0-8412-0518-3/79/47-111-263$05.00/0

In Plant Growth Substances; Mandava, N.;
ACS Symposium Series; American Chemical Society: Washington, DC, 1979.

- Modify sex expression.
- Increase plant resistance to pests.
- Enhance plant resistance to such environmental factors as temperature, water, and air pollution.
- Prevent postharvest spoilage.
- Regulate the chemical composition of plants and the color of fruit.
- Influence mineral uptake from the soil.
- Change the timing of crop development
- Control weeds.

As far back as the 1940's, chemicals that we now classify as plant growth regulators were used experimentally to root cuttings and to promote flowering in pineapple. The first important commercial application of a plant growth regulator was in the 1940's, when naphthalene acetic acid was applied, as it still is, to prevent the preharvest drop of apples.

One of the oldest and best known uses for plant growth regulators has been in initiating and/or accelerating the rooting of cuttings. One of the best chemicals for this purpose is indolebutyric acid. This compound is decomposed relatively slowly by the hormone destroying enzyme systems in plants. Because the chemical also moves very slowly in the plant, much of it is retained near the site of application - another desirable characteristic. Although a host of other chemicals have been evaluated for their effect on rooting, and some of them have desirable actions, indolebutyric acid still remains the compound of choice for this purpose.

In the early 1950's, 1,2-dihydro-3,6-pyridazinedione (maleic hydrazide) was first marketed to prevent the sprouting of onions in storage. Soon after, maleic hydrazide also was used to inhibit turf growth and prevent the sprouting of potatoes during storage.

Controlling Flowering

Pineapple.

In the late 1920's, scientists found that the long-recognized fact that pineapple could be forced to flower by smoke from fires (used to prevent growth from stopping during cold weather) was caused by its content of unsaturated gases, such as ethylene. By the mid-1930's, acetylene gas was used commercially in Hawaii to force the initiation of flowering. Later, plant hormones were shown to produce this effect, and 1-naphthaleneacetic acid was the next forcing agent to be used commercially on pineapples.

Although hydrazines are more commonly thought of as growth retardants, several of them, especially B-hydroxyethylhydrazine, were shown in the mid-1950's to induce flowering in Hawaiian pineapples by Dr. Donald P. Gowing and Dr. Robert W. Leeper at the Pineapple Research Institute (1).

The latest group of compounds demonstrated to be effective forcing agents for pineapples are the haloethanephosphonic acids

In Plant Growth Substances; Mandava, N.;
ACS Symposium Series; American Chemical Society: Washington, DC, 1979.

such as ethephon. Levels of 1 to 2 lb of ethephon per acre produce total floral induction.

Fruit Trees.

In many fruit trees, especially apple, pear, and peach, the the extent of floral induction is increased by spraying them with inhibitors such as succinic acid-2,2-dimethyl hydrazine (sold by Uniroyal as Alar) and 2,3,5-triiodobenzoic acid.

Vegetables.

Many annual vegetables, such as lettuce, radish, mustard, and dill, which normally flower only when days are long, can be made to flower early by treatment with gibberellins. Many biennial vegetables, such as carrots, beets, and cabbage, which require low temperatures to flower, also do so after treatment with gibberellins.

Sugarcane.

To improve the yields of some crops, such as sugarcane, it is commercially desirable to prevent flowering. In other crops, among them almond, peach, and tung oil trees, a delay in the onset of flowering may be desirable to avoid adverse weather conditions, such as extremes in temperature and moisture. Such a delay also can bring two plant varieties with different flowering dates into synchronization for breeding purposes (as in the case of varieties of almond trees) or control the timing of flowering of plants such as carnation or poinsettia to coincide with major holidays, when selling prices are higher.

Fundamental studies of flowering in a large variety of plants have shown that nighttime length is the critical factor in many cases. Sugarcane belongs to a group of short-day plants that initiate flowers only within a critical range of day-lengths (2). Members of this group must have an uninterrupted dark period if they are to flower. The briefest interruption (for sugarcane as little as 50 foot-candle-minutes of incandescent light) is usually sufficient to prevent flowering. Dr. George O. Burr and his colleagues at the Hawaiian Sugar Planters' Association Experiment Station determined in the mid-1950's that night interruption from Sept 1 to 20 would inhibit flowering of the sugarcane varieties propagated in Hawaii at that time. Field experiments later showed that suppression of flowering resulted in increases in the yield of sucrose averaging 1.3 tons per acre.

In the years immediately following these studies, the factors affecting flowering, as well as methods for preventing it, were studied extensively. Several methods were found to be effective in preventing flowering: interrupting night with light, lowering temperature, leaf and spindle trimming, withdrawing water, or applying chemicals. Because temperature cannot be controlled in the field and because leaf trimming and light interruption on a commercial scale are not economically feasible, emphasis has

In Plant Growth Substances; Mandava, N.;
ACS Symposium Series; American Chemical Society: Washington, DC, 1979.

been placed on water withdrawal and the application of chemicals. Withdrawal of water is possible only on irrigated plantations. Because this practice causes various operational problems, the use of chemicals eventually became the standard practice in Hawaii.

Maleic hydrazide was the first potentially useful commercial chemical for preventing the flowering of sugarcane but, at best, it gave only about 60% control. Rapid developments in the 1950's led to the use of 3-(p-dichlorophenyl)-1,1-dimethylurea (monuron) as the chemical of choice and later to the use also of 3-(3,4-dichlorophenyl)-1,1-dimethylurea (diuron). When properly applied, 4 lb per acre of either chemical provides virtually complete control of flowering in the heavy-tasseling cane varieites used in the 1950's and early 1960's in Hawaii.

Continued testing for more active chemicals to prevent flowering led the late Tyrus T. Tanimoto and me to the discovery in the early 1960's that 6,7-dihydrodipyrido (1,2-a:2',1'-c) pyrazidinium dibromide (diquat), is active at rates of 0.125 lb per acre. Thus, this newer chemical is one of the most active compounds yet used for this purpose. The cost of controlling flowering, per unit area, is decreased a substantial 70%. The compound also has been found to be highly effective in preventing the flowering of sugarcane in Guyana, Mexico, the Philippines and Taiwan, in addition to Hawaii (3).

Controlling Abscission

The control of abscission, (the separation of shedding of a plant part, such as a leaf, flower, fruit, or stem from the parent plant) is extremely important in agriculture. To ensure the most effective crop growth, leaves should be retained in a healthy, green state. On the other hand, to simplify the mechanical harvesting of certain crops, such as cotton, it is highly desirable to have the leaves removed. The same is true for fruit. In tree crops that have a large number of fruit started, it is sometimes desirable to thin the fruit by using an abscission-inducing compound, thereby increasing the size and quality of the remaining fruit. During crop growth, the fruit should be retained on the tree for maximum development and maturity. However, at harvest, for many if not most crops, the use of an abscission-inducing agent can be highly profitable in reducing labor requirements and costs. This is particularly true of citrus.

Citrus.

An active abscission research program for citrus has been underway for a number of years in Florida by the Florida State Citrus Commission in collaboration with a number of chemical companies. This program was initiated originally because of a shortage of labor for handpicking citrus. Chemicals to speed up the removal of fruit by mechanical harvesters and to increase

In Plant Growth Substances; Mandava, N.;
ACS Symposium Series; American Chemical Society: Washington, DC, 1979.

the productivity of handpickers have been developed (4).

The active compounds used earliest were termed "mass action" compounds because they were applied at high rates (up to 100 lb per acre). Unfortunately at these levels, the chemicals damaged both the fruit and the tree. The first breakthrough came when the antifungal antibiotic 3-(2-[3,5-dimethyl-2-oxocyclohexyl] -2-hydroxyethyl)glutarimide (cycloheximide or Upjohn's Acti-Aid) was found to loosen citrus fruits at application rates of less than 0.1 lb per acre (5).

Although cycloheximide is used commercially in Florida on most orange varieties, it is not used during the harvesting of Valencia oranges because it damages the flowers and the immature fruit. For a long time, efforts to find suitable abscission chemicals for Valencia oranges faced a number of problems. Recently, a selective abscission material, 5-chloro-3-methyl-4-nitro-1H pyrazole (Abbott's Release), has been developed that effectively induces abscission in mature fruit without damaging new twig growth and immature fruit (6).

Olives.

Man-power availability for olive picking is becoming an increasing problem for olive growers. Considerable research is being carried out in the Mediterranean region and in California, the main goal of which is to decrease the amount of labor needed for olive harvesting. Efforts to decrase labor consumption are directed toward a mechanical solution, a chemical solution, or a combination of both. It has been known for some time that ethephon is one of the best compounds for decreasing the fruit removal force needed to facilitate olive harvesting. More recently a new material, Alsol [2-chloroethyl-tris-(2-methoxy-ethoxy) silane], has been developed which is more effective for this purpose than ethephon. However, buffered ethephon can be used to facilitate a rapid, easy and relatively inexpensive harvest of olives. Now growers have two chemicals of similar efficiency to choose between. The data so far indicate that this decision will be based on economic calculations (7).

Cotton

We all know of the old practice of "pickin' cotton" - harvesting cotton bolls by hand. In general, this practice has been replaced by machine harvesters. Since introduction of the harvest machine, use of chemical aids has become common-place. Cotton leaves are defoliated by chemical harvest aids on more than 75% of the cotton acreage in the U.S. - representing more than 7 million acres of cotton.

Historically cotton bolls have been harvested by hand. More recently, however, the development of machine harvesters has reduced production costs. For efficient use of both types of pickers (the spindle type with rotating spindles that picks the cotton only from open bolls, and the stripper type which strips

In Plant Growth Substances; Mandava, N.;
ACS Symposium Series; American Chemical Society: Washington, DC, 1979.

the entire plant except the main stems) most of the foilage must be removed before harvest. This has been accomplished for sometime by harvest-aid practices consisting of chemically treating the cotton plant at the proper time to induce defoliation before harvest.

Chemical harvest aids are presently used on more than 75% of the cotton acreage in the U.S.. The amount of foliage may be reduced either by the use of defoliants or the use of desiccants. Defoliants induce leaf fall and must be applied one to two weeks before harvest so that the abscission process may be complete. Desiccants cause the foliage to lose water and sometimes the leaves and stems are killed so rapidly by desiccants that an abscission layer has insufficient time to develop and the drying leaves remain attached to the plant. Desiccants usually require one to three days to act before harvest can be started. The obvious advantage of desiccants over defoliants is that they may be applied at a later date, thus gaining additional time during which the leaves continue to function and to contribute to see the fiber quality. Hundreds of chemicals have been evaluated as defoliants and desiccants. Only a few are in commercial use. The best known of these defoliants are sodium chlorate which has the danger of starting fires, tributyl-phosphorotrithioite (merphos, Folex) its oxygenated relative tributylphosphorotrithioite (DEF), and endothall (Accelerate). Recent research reports claim that amino methyl phosphonic acid and certain of its relatives were found to be more active than current commercial products. The best known desiccant used for defoliation of cotton is paraquat (1,1'-dimethyl-4,4'-bipyridinium) (8).

Fruit.

The thinning of fruit on various specied of fruit trees and grapes is necessary commercially. Because this is difficult and costly to do by hand, however, growers have turned to the use of chemicals. Chemicals have been used to thin fruit set in apples, for example, since the early 1930's. In addition to permitting crops to be produced annually be eliminating or reducing alternate-year bearing, this practice also enhances the size, color and quality of the fruit.

Of the early compounds used for this purpose, the most important was 1-naphthaleneacetic acid and its amide. More recently, ethephon has shown promise as a thinning agent. 3-Chlorophenoxy- - propionamide has produced good thinning in several varieties of peach. Both gibberellic acid and ethephon are effective thinning agents for grapes.

The preharvest drop of citrus fruit can be reduced or prevented with 2,4-dichlorophenoxyacetic acid, which delays development in the abscission zone of the fruit stem, thus allowing the fruit to remain on the tree longer. Because of 2,4-D's herbicidal properties, however, care must be taken in spraying to prevent the chemical from damaging other crops, as well as the target crop

In Plant Growth Substances; Mandava, N.;
ACS Symposium Series; American Chemical Society: Washington, DC, 1979.

itself.

Control of Fruit Development

In recent years, scientists have found that many synthetic growth regulators will develop fruit in plants. The best of these are 4-chlorophenoxyacetic acid and 2-naphthoxyacetic acid. These chemicals are most effective on fruits that have many ovules, such as tomato, squash, egg-plant, and fig. They are usually rather ineffective, however, on peach, cherry, plum, and other stone fruits. Many fruits that can be set by such hormonal compounds also can be set by the gibberellins. In addition, gibberellins can set fruit in some species that do not respond to the other chemicals.

In the San Joaquin Valley, several grape varieties yield much below the vine capacity because of poor fruit set. Yield of Malvasia bianca grapes was more than doubled by pre-bloom spray of the plant growth regulator Cycocel (chlormequat). The increase of 4.4 tons per acre (20 pounds per vine) was primarily due to improved berry set. Clusters from treated vines were well filled; those from the controlled vines were loose and straggly, with some clusters setting no fruit at all. To date, Cycocel has not been registered for this use (9).

Experiments in Australia show advantages in spraying prune trees before harvest with selected growth regulating chemicals. This has been done over a period of four years and recent reports suggest significant financial benefits from the use of growth regulators. The recommendations are for use of gibberellic acid at 10 ppm or napthalene acetic acid at 20 ppm yielding an extra $260.00 per hectare (10,11).

Gibberellins

Infection of rice by the fungus Gibberella fujikuroa, causing the elongated "foolish seedling" effect, has been known for generations. Not until 1938, however, was a crystalline fungal metabolite isolated as the causative agent at the University of Tokyo by Dr. Teijiro Yabuta and Dr. Yusuke Sumimi. Their work led to the discovery of a new class of hormones, the gibberellins. Because of wartime secrecy, little was published about these new "wonder compounds" until the 1950's, when their spectacular effects on both ornamental and edible crop plants received enormous publicity.

In most plants, the outstanding effct of the gibberellins is to elongate the primary stalk. This effect occurs in the young tissues and growth centers and is caused either by an increase in cell length, an increase in the rate of cell division, or a combination of both, depending on the specific type of plant treated.

Gibberellins can induce flowering in many plant species, such as carrots, that require low temperatures to initiate this process. The ability of a chemical to promote flowering is

In Plant Growth Substances; Mandava, N.;
ACS Symposium Series; American Chemical Society: Washington, DC, 1979.

valuable both in controlling the timing of flowering to match holiday markets and for the production of seed.

Gibberellins have remarkable effects on many dwarf plants, such as dwarf pea, dwarf corn, and bush beans. When treated with gibberellins, these plants grow to full size. Gibberellins also affect the extent to which a plant develops side branches. In addition, they increase the size of many young fruits, expecially grapes. Because gibberellins induce the production of the enzyme amylase in barley, they also are commonly used in the malting of barley.

At present, the biggest uses of gibberellins are in increasing the size of grapes and in stimulating the growth of sugarcane. Treatment of sugarcane with as little as 2 oz of gibberellin per acre increases the yield of cane more than 5 tons per acre and raises the output of sugar 0.2 to 0.5 ton per acre.

Gibberellins are used in grapes for several different purposes. The present commercial practice in California is to spray twice, once at bloom for a loosening and sizing effect and again at fruit-set stage for an additional sizing effect.

Although gibberellins have been used on a small scale for over a decade in Hawaii (12,13), it was not until 1976 that one of the sugar plantations on the island of Oahu utilized gibberellins on a large commercial scale and also studies the interaction with ripeners. The test results show that treatments of split applications of gibberellins in combination with a chemical ripener gave the best response.

The overall yield comparisons throughout the entire season were based on 42 field blocks of approximately 40 acres each, in 18 different locations. An increase of 5% in total crop tonnage is credited to this program. The economic benefit of this program for the application of growth regulators has been considerable. An outlay of $200,000 for gibberellins and the ripener Polaris for this one plantation during 1976 added over 3,500 tons of sugar to the crop at a cost of about $60 per ton of sugar produced. This sugar reduced the average production cost by nearly $10 per ton and returned approximately $400,000 net profit after taxes to the plantation for 1976 (14).

The gibberellins also have been tested on a wide range of vegetable crops, with results to date that are spectacular but often not beneficial. For example, in many species, such treatment can induce premature flowering, which is undesirable in crops such as cabbage, since it is the vegetative leaves that are commercially valuable.

Control of Plant Size

A plant growth regulator that reduces stem length in cereal crops, 2-chloroethyl trimethylammonium chloride (American Cyanamid's Cycocel) has become an important factor in farming, especially in growing of wheat. This compound prevents, or at least greatly reduces, the possibility that wheat will "lodge"

In Plant Growth Substances; Mandava, N.;
ACS Symposium Series; American Chemical Society: Washington, DC, 1979.

(fall over in heavy winds and rain). About half of the wheat grown in West Germany, for example, is treated with Cycocel. In fact, that country's increased yield of wheat in recent years is largely attributed to this plant growth regulator.

Dr. Sylvan H. Wittwer, director of the Michigan Agricultural Experiment Station, says that the use of Cycocel eliminates the need for genetically developed lodging-resistant varieties of wheat. Treatment with Cycocel produces a shorter plant with thicker stems, greener leaves, more side shoots, and better-filled heads. Cycocel is among the most widely used plant growth regulators in the world (15).

Mowing turf grass is a time consuming and costly maintenance procedure both for professional turf managers and for homeowners. The use of plant growth regulators to inhibit grass growth has been a practice for years. This approach has been of considerable interest both to industry and to landscape maintenance people.

Maleic hydrazide)1,2-dihydro-3,6-pyridazinedione, (MH) has been utilized for 20 years in grass growth regulation. More recently chlorflurenol and mefluidide (Embark) have been shown to exhibit considerable potential in the control of a number of grasses.

The plant growth regulator 1,1-dimethyl piperidinium chloride can be used to manage the vegetative development of cotton plants to offset the effect of excessive rain water or nitrogen by decreasing both overall plant height and length of lateral branches. This maintains a plant form which can facilitate crop protection and mechanical harvesting practices. The use of this same material at rates lower than that to inhibit size of plant causes increases in yield which appears to be partly due to less shedding of flowers and/or bolls, increases in boll weight, and number of open bolls at harvest.

Modify Sex Expression

Because they can affect both the determination of sex and the time of flowering, growth regulators now are commonly used to aid in seed production in vegetables and in the breeding of several types of crops. Crops whose sex has been manipulated through growth regulators include begonia, cotton, cucumber, grape, hops, pumpkin, squash, and tomato.

Biochemistry and Cell Biology

For more than two decades, maleic hydrazide has been used extensively on tobacco to prevent the formation of suckers (auxiliary buds), which decrease leaf quality. Before this material was developed, large amounts of hand labor were required to remove the buds. This use for maleic hydrazide is one of the great success stories in the plant growth regulator field.

In Plant Growth Substances; Mandava, N.;
ACS Symposium Series; American Chemical Society: Washington, DC, 1979.

Chemical pinching of azaleas has been practiced commercially for many years. A number of compounds are now available for use in the chemical pruning of azaleas and other plants. One of these is methyldecanoate (sold under the trade name Off-Shoot-O) (16). Recently it has been shown that apical dominance can be successfully overcome with the proper application of the chemical dikegulac. The savings in labor and accompanying costs are substantial with the use of the chemical pinching or chemical pruning (17). A group of diphenyl ethers has recently been reported as being effective in controlling undesirable sucker growth in tobacco and undesirable secondary growth in general in a number of plants. The inhibition of bud growth has been shown also to be accomplished by substituted 2,6-dinitroanalines.

An understanding of the postharvest physiology of plant tissues is of great importance because of the large spoilage losses caused by plant overripening and aging. For many years, some scientista have believed that the deterioration of a crop if associated with the action of the ethylene that the plant produces internally. A more up-to-date concept is that aging in plant tissues is not only a deteriorative process but also a developmental process in which growth regulators besides ethylene play important roles. As Dr. Morris Lieberman, head of the post-harvest plant physiology laboratory at USDA's Agricultural Research Center in Beltsville, MD., says, "Although ethylene is still considered a major influence on postharvest metabolism, the other plant hormones - the auxins, gibberellins, cytokinins, and abscisic acid - also are thought to significantly influence the aging process. Most likely, ethylene action results from interactions with these hormones" (18).

Today, another exciting research area involves the bioregulation of plant composition. Such bioregulation is the process of controlling specific metabolic pathways (or a series of such pathways) by externally supplied synthetic chemicals. These bioregulators are low-molecular-weight compounds, in contrast to the high-molecular-weight biological polymers (proteins and nucleic acids) through which the synthetic bioregulators appear to exert their control on metabolism. Bioregulation is more than just a theoretical approach; already, color in citrus and tomatoes and vitamin content in a number of vegetables and fruits has been controlled and the sugar yield in the cane plant increased.

Several companies, in cooperation with the U.S. Forest Service, have been studying the effectiveness of paraquat in enhancing the quantity of chemicals produced in slash and loblolly pine trees. Results over the last 6 years show that there can be a positive effect and that beta-pinene is preferentially produced in slash pines by treatment with paraquat. Results reported on loblolly pine show an increase of 50% in tall oil content when correctly applied. The expected increase

In Plant Growth Substances; Mandava, N.;
ACS Symposium Series; American Chemical Society: Washington, DC, 1979.

is promising enough that most companies involved are continuing their efforts with this approach.

Growth regulators can influence a plant's absorption of minerals from the soil. The uptake of potassium by wheat, for example, is accelerated by treating the plant with gibberellic acid. The uptake of both nitrogen and phosphorus by wheat and soybean plants is enhanced by treatment with 2,4-D.

There are a number of recent reports from the Russion literature on the increase of corn yields through weed control by atrazine and 2,4-D amine. Although they do not distinguish between weed control and its specific positive effects on yield, they clearly show increased uptake of nutrients in the absence of weeds (19).

Growth retardants have been found to increase the drought resistance of a number of plants. The mechanism by which growth retardants accomplish this is not known. However, the effect of Cycocel and SADH in increasing the ability of a plant to withstand drought is thought to be related to the ability of these chemicals to delay the senescence of detached leaves. Also, in the case of brussel sprouts, treatment by Cycocel reduces the number of stomata per unit area which could decrease the rate of water loss from the leaf and contribute to the plants drought tolerance. Also, application of this growth retardant increases leaf thickness in certain other plants which might also contribute to drought resistance. The best success with increasing either drought resistance or tolerance has been demonstrated in wheat, barley, grapes, beans, apples, sunflower, and gladiolas. Treatment with certain growth retardants also increases a related physiological phenonomen, i.e., solt tolerance. The use of plant growth retardants to increase the tolerance of plants to high concentrations of salt has been most successful with wheat, soybean and spinach. The use of inhibitors or plant growth retardants to increase resistance to low temperatures has been most successful with cabbage, tomato, woody plants, apples, pears, wheat, citrus, raspberry, grapes, mulberry, strawberry, and azalea.

Applied to certain crops, plant growth regulators can change the timing of crop development. Such developmental change might enable a crop to avoid the effects of adverse environmental factors, such as abnormally low temperatures or lack of water. It also might interfere with the rate of plant development, thereby disrupting the normal life cycle of insect pests. This, in turn, may offer a new method of insect control.

The termination of cotton fruiting by the use of chemicals is a new approach for insect control. Several plant growth regulators have been found which are effective in this treatment. They are two types: 1) fast-acting and non-persistent, and 2) slow-acting and persistent. The slow acting persistent growth regulator type is represented by chlormequat (Cycocel or CCC) and chlorflurenol (methyl-2-chloro-9-hydroxy-flourene-9-

In Plant Growth Substances; Mandava, N.;
ACS Symposium Series; American Chemical Society: Washington, DC, 1979.

carboxylate). The fast-acting type includes 2,4-D and 3,4-dichloroisothiazole-5-carboxylic acid. Mixtures of 2,4-D with either of the two persistent growth regulators is more effective than 2,4-D applied alone. All of these treatments have been tested extensively and found to be effective. It is now felt that they are suitable for use by entomologists in large scale testing of chemical termination of fruiting in cotton for insect control.

Another area which might be considered of a plant growth regulatory nature is the use of "safening agents" with herbicides and other pesticides in order to reduce the toxic effects on crops under conditions where it is necessary to use the pesticides (20,21,22). Recent Japanese work shows that such polyamines as 1,8-diaminooctane or their salts can be used as safening agent for phenylcarbamoyl-amino acid herbicides. For example, the application of one of the alanine phenylcarbamoyl herbicides decreased the final yield of wheat by 39% while the simultaneous application of a polyamine resulted in only a 4% decrease in yield (23).

Increasing Crop Yields

Corn

An exciting development has been the use of the herbicide 2,4-dinitro-6-sec-butylphenol (dinoseb) as a spray applied to leaves at low concentrations to increase the yield of corn. Dr. Alvin J. Ohlrogge of Purdue University reported in 1969 that only a few grams per acre of this compound boosts the yield of corn 5 to 10%. Since his findings were announced, however, investigators in various parts of the country have published conflicting reports on the effectiveness of the compounds, possibly because of variations in the genetic background of the different corn varieties used. Nevertheless, three companies (Dow Chemical, Helena Chemical, and Agway) are now marketing formulations of this chemical for this use. The most recent report on dinoseb for its effects on sweetcorn yields in the midwest show positive effects on three varieties but decreased yields with a fourth variety (24).

Soybeans

In the 1960's, 2,3,5-triiodobenzoic acid was considered a spectacular plant growth regulator. When applied to the leaves of soybeans, it shortens the plants, increases their branching, stiffens them, and increases pod set (thus increasing yield). Unfortunately, the compound is not always effective. Scientists have found that the timing of its application is very critical and that different varieties respond differently. Hence, results are disappointingly inconsistent, and the use of this compound to increase soybean yields has been discontinued.

In Plant Growth Substances; Mandava, N.;
ACS Symposium Series; American Chemical Society: Washington, DC, 1979.

Rubber.

The use of 2-chloroethyl phosphonic acid (ethephon) to boost the yield of latex from rubber trees has become standard estate practice in recent years. This compound increases the flow of latex and the yields of dry rubber from commercially important tree varieties as much as 100%. In addition, ethephon helps to preserve the bark, which normally is cut regularly to permit the flow of latex. As a result, the economic life of the tree is prolonged. Ethephon breaks down into ethylene, which is probably the basis for its activity.

When 100% of the tapped trees are treated, the yield increase varys from 36% to 130% depending on the time of year. The highest yields are obtained from November through March. Starting in April there is a dip in the yield curve which reaches a low in July. At that time the curve starts going up, reaching the high level by November. The profitability is even higher than the yield return. This is due primarily to the fact that a doubling of yield was accomplished with no increase in land, number of trees and their cultivation, or work force.

Guayule.

Because it can be grown in the United States, because it can grow under extreme arid conditions as in desert areas good for little else at the present time, guayule has attracted increasing interest since the original work done on it during World War II. Rubber made from guayule is equal in every respect to that made from rubber trees and commercially valuable by-products are also obtainable, including bagasse, resins, and leaves. Two tons of bagasse and one ton of leaves can be obtained for each ton of rubber. Bagasse may be used in paper pulp-making. The resins contain volatile and nonvolatile terpenoids, a high melting wax, a shellac-like gum, drying oils, and succinic acid. The leaves contain a valuable hard wax with a higher melting point than that of high quality cornuba. Recent reports from the USDA show that low cost triethylamines, particularly 2-(3,4-dichlorophenoxy triethylamine), sprayed about three weeks before harvest increased yields two to six times the normal 500 lbs./acre. Investigators carrying out the work state that the compound works by causing the plants to express genetic traits more efficiently -- rubber producing cells in the plants become more active and produce more rubber. Estimates are that the process of using such chemicals could increase overall yields 30-35% and cut the growing time by 1-2 years. It is important to note that estimates suggest that a yield of 1,000 lbs./acre could support a rubber inductry.

Temperate Fruits.

Among the most widely used plant growth regulators is succinic acid-2,2-dimethyl hydrazine (diaminozide), commonly referred to as Alar or SADH. Originally developed to reduce the

In Plant Growth Substances; Mandava, N.;
ACS Symposium Series; American Chemical Society: Washington, DC, 1979.

vegetative growth of flower crops, it may have far greater value in the future as a regulator of flowering and fruiting. Treated with Alar, trees bear fruit after only four years, instead of the usual seven to 10. Because it is a growth retardant, however, it tends to dwarf the fruit trees.

Alar promotes fruit setting of grapes and accelerates by several days the maturing of cherries. It also is used on apples, prunes, tomatoes, peanuts, and various ornamental crops. On apple trees, Alar increases the firmness of the fruit and prevents it from dropping permaturely. On tomato transplants, the compound retards stem elongation.

Tropical Fruits.

With the exception of 2,4-D and gibberellic acid treatments of a variety of citrus crops, where improved fruit set, reduced fruit drop, and the ability to delay harvesting without loss of fruit quality are recognized benefits, few applications have been developed for the use of hormone-type regulators in tropical fruits. Recent work has shown that gibberellic acid will delay postharvest ripenining and improve the quality of bananas and that napthaleneacetic acid treatment of oil palm to delay fruit abscission has increased both yield and oil content.

Sugarcane

One of the most important developments in recent years has been the use of chemicals as ripeners in sugarcane. Work begun in Hawaii in the early 1960's has shown that a surprising number of chemicals increase the sucrose content of cane at harvest. Some casual observers have suggested that, because such a diverse array of chemical structures effectively ripens sugarcane, "almost anything will do so." Actually, of course, this is not the case. Many thousands of compounds of a wide range of chemical types have been tested for this effect, with only a few dozen giving enough positive results to warrant serious further study (25).

The first material seriously considered as a candidate ripener for increasing sugar yields was the dimethylamine salt of 2,3,6-trichlorobenzoic acid. However, because of a number of technical, environmental, and legal proglems, this material did not prove successful commercially. Nevertheless, it served as a standard for comparison in screening tests aimed at finding better sugarcane ripeners.

Currently, only one product is registered in the U.S. for use as a sugarcane ripener. This is N,N-bis (phosphonomethyl) glycine, known generically as glyphosine and marketed by Monsanto as Polaris. Although it has been registered with the Environmental Protection Agency for only about three years, it already has been used to treat several hundred thousand acres of sugarcane throughout the world. It now is used commercially in Hawaii, Texas, Florida, Guyana, Brazil, Guatemala, Jamaica,

In Plant Growth Substances; Mandava, N.;
ACS Symposium Series; American Chemical Society: Washington, DC, 1979.

and Mauritius.

The ethylene-producing compound ethephon is used commercially on sugarcane in South African and Rhodesia. Both Cycocel and 7-oxabicyclo (2,2,2,1)heptane-2,3-dicarboxylic acid (sold by Pennwalt as Ripenthol) have been registered for experimental use on sugarcane in the U.S. and are under test in several other countries. Experimental registration is expected in the near future for evaluating N-2,4-dimethyl-5-trifluoromethyl)-sulfonyl-aminophenyl acetamide (mefluidide), in the field as a sugarcane ripener. It is available from 3M as Embark, and several other companies have potential products for this use nearing this stage (26).

The financial return to the grower is still substantial, despite the low current price of sugar. The increased sugar yield produced by such compounds varies from 10 to 20%, depending on the variety of sugarcane treated, as well as prevailing weather and soil conditions. Fortunately for the acceptance of chemical ripening of sugarcane, the first such chemical (Polaris) was introduced commercially in 1974, when the price of sugar was high and the potential return to the grower was enormous. The chemically stimulated ripening of sugarcane is definitely here to stay, even though the price of sugar has dropped to an extremely low level.

Discussion and Remarks

Compounds that regulate crop maturity are especially likely to have a dramatic impact on agriculture in the years ahead. In many instances, these chemicals might not increase yields directly but might prevent losses caused by failure to achieve maturity. The use of ripening compounds in sugarcane is a good example. This already has resulted in a 10 to 20% increase in yields in certain parts of the world. Changes in cultural practices to adjust the use of ripeners probably will increase sugar yields even more.

Chemical companies have considerable interest in the plant growth regulator field, and they have great ability for discovering and developing new growth regulators. Their capacity starts with their current inventory of hundreds of thousands of organic compounds, many of which have never been tested as plant growth regulators. In addition, it is known that almost any bioregulated process can be influenced by organic chemicals. The secret is to find the chemical that has great enough technical and commercial possibilities.

One of the limitations is the lack of a single, simple test by which thousands of compounds can be evaluated. Such tests are available for herbicides, but the field of plant growth regulation is much more complex. For plant growth regulation, the rate of application of the chemical and the stage of plant growth are two variables that must be considered almost from the beginning. Also, for many responses, it is necessary to grow

In Plant Growth Substances; Mandava, N.;
ACS Symposium Series; American Chemical Society: Washington, DC, 1979.

a plant for its entire life cycle, which is an expensive, time-consuming procedure. Major efforts are being made in commercial and academic research centers to tackle the difficult problem of how best to evaluate growth regulation in the initial stages of experimentation.

Literature Cited

1. Gowing, D. P., Leeper, R. W., "Induction of flowering in pineapple by beta-hydroxyethylhydrazine", Science (1955) 122,1267.
2. Burr, G. O., Hartt, C. E., Brodie, H. W., Tanimoto, T., Kortschak, H. P., Takahashi, D., Ashton, F. M., Coleman, R. E. "The sugarcane plant", Ann. Rev. Plant Physiol. (1957) 8, 275.
3. Tanimoto, T., Nickell, L. G., "Field control of sugarcane flowering in Hawaii with diquat", Proc. 12th Cong. ISSCT, Puerto Rico (1967) 113-116.
4. Wilson, W. C., "Four years of abscission studies on oranges", Proc. Fla. State Hort. Soc. (1969) 81,75.
5. Wilson, W. C., "Field testing of cycloheximide for abscission of oranges in the Indian River area", Proc. Fla. State Hort. Soc. (1971) 84,67.
6. Clark, R. K., Ellis, M. T., "The use of Release, an abscission agent, to increase the productivity of pickers on processing oranges", Proc. Fla. State Hort. Soc. (1976) 89,72.
7. Ben-Tal, Y., Lavee, S., "Increasing the effectiveness of ethephon for olive harvesting", Hort. Science (1976) 11,489.
8. Cooper, W. C., Rasmussen, G. K., Rogers, B. J., Reece, D.C., Henry, W. H., "Control of abscission in agricultural crops and its physiological basis", Plant Physiol. (1968) 43,1560.
9. Peacock, W. L., Jensen, F. L., "Chlormequat doubles yield of Malvasia bianca grapes", Calif. Agri. (1977) 31(6),9.
10. Dirou, J. F., Logan, B. J., "Benefits from growth regulators on prune trees", Agri. Gaz. of NSW (1978) 89(1),47.
11. Dirou, J. F., Logan, B. J., "Regulating prune maturity using chemicals", Agri. Gaz. of NSW (1977) 88(6),34
12. Tanimoto, T., Nickell, L. G., "Re-evaluation of gibberellin for field use in Hawaii", 1966 Rpts. Haw. Sugar Technol. (1967) 184-190.
13. Tanimoto, T., Nickell, L. G., "Effect of gibberellin on sugarcane growth and sucrose production", 1967 Rpts. Haw. Sugar Technol.(1968) 137-146.
14. Sowers, R., "Yield response of gibberellin at Waialua Sugar Company", 1976 Rpts. Haw. Sugar Technol.(1977) 274-277.
15. Wittwer, S. H., "Growth regulants in agriculture", Outlook Agri.(1971) 6,205

In Plant Growth Substances; Mandava, N.;
ACS Symposium Series; American Chemical Society: Washington, DC, 1979.

16. Cathey, H. M., Steffens, G. L., Stuart, N.W., Zimmerman, R. H., "Chemical pruning of plants", Science (1966) 153,1382.
17. Larson, R. A., "Stimulation of lateral branching of azaleas with dikegulac-sodium (Atrinal)", Jour. Hort. Sci. (1978) 53,57.
18. Lieberman, M., "Post harvest responses and plant growth regulators",ACS Symp. Series No. 37 (Pesticide Chemistry in the 20th Century, J. R. Plimmer, ed.) (1977) 280-292.
19. Vas'Kovskii, G.P., "Role of herbicides in the use of nutrients by corn plants", Khim Sel'sk. Khoz. (1978) 16,58. [Russian].
20. Hoffman, O. L. "Chemical seed treatments as herbicide antidotes", Weeds (1962) 10, 322.
21. Hoffman, O. L., "Herbicide antidotes: concept to crop", Chemtech (1978) 8, 488.
22. Leavitt, J. R. C., Penner, D., "Potential antidotes against acetanilide herbicide injury to corn (Zea mays)", Weed Res. (1978) 18, 281.
23. Okii, M., Onitake, T., Kawai, M., Takematsu, T., Konnai, M., "Polyamine safening agents for phenylcarbomoylamine acid herbicides", Japan. Kokai Tokkyo Koho 78,94,035. January 24, 1977.
24. Oplinger, E. S., "Response of sweet corn to foliar applications of dinoseb", Proc. 5th Ann. Mtg. Plant Growth Regulator Working Group (1978) 100-105.
25. Nickell, L. G., "Chemical enhancement of sucrose accumulation in sugarcane", ACS Advances in Chemistry Series No. 159 (Plant Growth Regulators, C.A. Stutte, ed.) (1977) 6-22.
26. Nickell, L. G., "Chemical growth regulation in sugar cane", Outlook Agri. (1976) 9 (2), 57.

RECEIVED June 19, 1979.

11

Factors Affecting Commercialization of Specialty-Use Plant Growth Regulating Chemicals

ALDO J. CROVETTI

Agricultural Chemicals Research, Chemical and Agricultural Products Division, Abbott Laboratories, North Chicago, IL 60064

Plant growth regulators, whether natural occurring or synthetic have been used commercially since the 1950's. Their uses have generally been to modify growth, enhance yields of food and fiber, adapt plants to scheduled harvest patterns and make certain crops more adaptable to mechanical harvesting.

The progress with plant growth regulators has taken us through the periods of auxins, maleic hydrazide, the gibberellins, cytokinins, abscisic acid to the newer generation of ethylene releasers and ripeners. The major plant growth regulators in use include Alar (apples and cherries), MH-30 (tobacco), other sucker control agents, ethephon for a variety of uses, gibberellic acid, CCC (grains) and Polaris® (sugarcane) (1, 2, 3, 4).

There are an estimated two-three million acres of U.S. cropland treated with plant growth regulators (5). This is not a large treatment pattern when considering cotton, corn, peanuts and soybeans total 144 million acres. Of the 20-30 formulated products available, the growth regulators mentioned account for >90% of the current market. Tobacco accounts for one-half the consumption with apples a close second, employing chemicals for thinning, pre-harvest drop control and promotion of return bloom. Tomatoes, grapes, cherries and citrus account for a majority of the remaining uses.

Several materials have commercial use as abscission agents (6). In cotton, the products Accelerate (endothall), Folex (merphos) and DEF (oxidized form of merphos) have been identified as defoliants. Amid-thin continues to be used to thin fruit set. 3-CPA and Peach-thin 322, formerly used as peach thinners, serve as reminders of the disadvantages of highly specialized markets. RELEASE and PIK-OFF are under development as citrus abscission agents.

Ethephon is a recent example of a new generation plant growth regulator. Among the registered uses, the abscission of cherries has been of major importance. The technology of using ethephon, grower interest and need, synchronized nicely to

0-8412-0518-3/79/47-111-281$05.00/0

In Plant Growth Substances; Mandava, N.;
ACS Symposium Series; American Chemical Society: Washington, DC, 1979.

provide a case where 95% of the cherries in Michigan are now harvested mechanically. Alsol , another example of an ethylene release abscission agent, has been useful in the harvesting of olives.

Growth regulators, because of their relatively high cost and methodological complexity have been utilized in high-value specialty crops. Most attempts to penetrate larger agronomic crops have been disappointing. In recent years, companies have been directing new research towards plant growth regulators for large acreage crops such as: corn, wheat, soybeans, peanuts and cotton (7). Although some plant growth regulators are commercially available many others of potential value and those providing measurable technical success are not. Whether a plant growth regulator, for example an abscission agent, becomes a commercially available product depends on a variety of interrelated scientific and economic factors.

The specific criteria used by private industry to evaluate a potential new product include a description of product characteristics and marketing factors that corporations must weigh before spending dollars to support a project. The objectives of business are quite clear; profit and market potential must be considered because profit is key to our economic system. For industry to engage in the development and manufacture of a new plant growth regulant, an acceptable return on investment must be assured. The ultimate criterion is: "how many dollars will be returned for an investment, how fast and within what framework."

This must be the real incentive for continued research on any new product whether a new abscission agent of any other plant growth regulator. Thus unless there is a good chance of adequate return on investment to support the increasingly expensive business of agricultural chemicals R and D, a plant growth regulator material can be extremely risky and subject to management deletion.

Over the past several years, we have included within the scope of agricultural R and D efforts the search for abscission agents with prime emphasis on citrus abscission. My comments in part will be drawn from factors which have effected the development of the potential abscission agent RELEASE by Abbott Laboratories. Examples relative to the general concerns and obstacles in the development of a plant growth regulator will also be discussed.

Scientific Factors

Synthesis and Screening. One of the key problems for industry is the question "How does one screen for new plant growth regulants?" (8). In the case of herbicides and insecticides, they either work or they don't. The field effects of plant growth regulators are harder to see, predict or test for.

In Plant Growth Substances; Mandava, N.;
ACS Symposium Series; American Chemical Society: Washington, DC, 1979.

For the most part, plant growth regulator screening has been based upon non-specific general herbicide screens where experience and intuition in interpreting non-lethal responses have been the main driving forces leading to further plant growth testing. Without a series of detailed tests, it is essentially impossible to identify or predict useful or commercial activity. The procedure of modifying a crop property, such as enhancing yield, is much more difficult than killing weeds or insects. Inconsistency of results is characteristic of plant growth regulators and a major problem in their evaluation.

Initial efforts aimed to identify a specific activity may, also, lead to a secondary response which may be unanticipated and eventually more useful and economically important. Many plant growth regulators have been developed in this way after accidental observations or *via* serendipity. Alar and ethephon are examples (5,6).

In the case of citrus abscission, screening has involved several approaches: (9)

1. Explant tests - leaf and fruit
2. Related whole plant species - calamondin
3. Tree, limb testing

In our experience, the explant test was highly variable and inconsistent. RELEASE would not have been found *via* this route. The calamondin test was useful in developing data with good carry-over to field screening. The chemical-biological relationships developed by use of this whole plant had good agreement and offered guidelines for the chemist to follow.

As illustrated (Fig. 1), synthesis of related compounds to maximize activity (and incidental balancing of phytotoxicity and potential costs) can involve the preparation of several hundred materials - an expensive proposition. For pesticides generally, only 1/200 compounds survives the initial synthesis and screening stage (10, 11, 12, 13). (There was no exception in the case of RELEASE -related materials). In expanded confirmatory tests, questionable chemicals are constantly discarded because of narrow toxicity/efficacy ratios or performance is too erratic. Synthesis or formulation difficulties often compound this picture.

A sterling example of industry and the research community cooperation in developing sophisticated plant growth regulators is the very energetic citrus abscission screening program established by the Department of Citrus in Florida. Support by industry growers, has made possible the screening of compounds at rates of up to 9,000 compounds per year. This partnership between growers and industry has allowed the screening and evaluation of approximately 50,000 compounds (9).

In Plant Growth Substances; Mandava, N.;
ACS Symposium Series; American Chemical Society: Washington, DC, 1979.

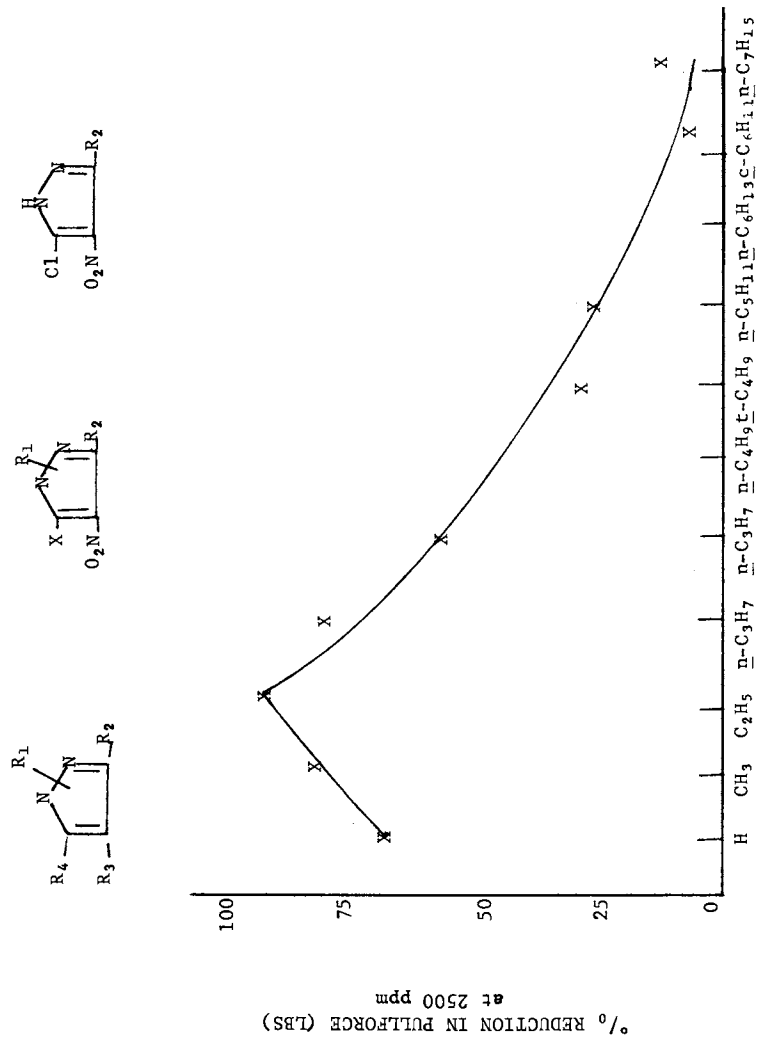


Figure 1. Structural activity of RELEASE

It is one thing to commit to a given discovery procedure once one feels comfortable with the ability to interpret or translate a response to possible field application. It is another matter entirely to "go all the way" in development stages to prove efficacy and utility; human and environmental safety; and acceptance by the farmer or grower. Many tests are required to have satisfaction in results obtained: efforts must include variation in rates, timing,spray, dilution, formulation or tank mix compatibilities, varietal responses, optimal environmental conditions, geographical dependencies and season long and carry-over observations.

The significance of laboratory or greenhouse screening data or difficulties in translation of these data can only be determined after field evaluations. Again, it must be realized that no amount of detailed research under field conditions can take into account all the variables encountered by a grower.

Generally there has been a good translation of results with RELEASE from initial limb tests to expanded whole tree experiments but still not all variables can be anticipated.

Thus in field testing of plant growth regulators, much needs to be learned but the investment of time and resources to develop a perfect product has been seriously questioned (8).

Concurrent Activities. While laboratory and field evaluations are in progress, several concurrent activities including patent preparation, chemical process development, pilot plant and manufacturing estimates are involved and must be seriously addressed (10).

The major activity or obstacle of concern in developing and registering a plant growth regulator, or any pesticide, involves the total toxicity and safety profile. Of primary concern is the impact of federal agency regulations; attention is generally focused on the EPA (14, 15, 16, 17, 18).

It is generally conceded (or assumed) that synthetics, rather than the naturally occurring plant growth regulators, will be developed for grower use. Therefore, clear understanding of the impact of government regulations becomes of prime importance. First it must be recognized, despite the sophisticated nature and functions of plant growth regulators, that they are in fact pesticides and, therefore, subject to the requirements of FIFRA originally enacted in 1947 and augmented in 1972 by the Federal Environmental Pesticide Control Act (FEPCA) (8). Deadlines for completion of registration guidelines have been progressively extended with seemingly perpetual new revisions. New provisions have included final implementation of FIFRA certification of applicators; re-registration procedures for all products; classification of toxicity categories relative to RPAR - Rebuttable Presumptions Against Registration.

In Plant Growth Substances; Mandava, N.;
ACS Symposium Series; American Chemical Society: Washington, DC, 1979.

Before the amendments of FIFRA, the concept of negligible residues was operative. Negligible residues were generally considered as any amount remaining which would result in a daily intake regarded as toxicologically insignificant on the basis of scientific judgment of adequate safety data. Ordinarily this would add an amount 1/2000 of the amount demonstrated to have no effect in feeding studies on the most sensitive animal studied as shown by at least two (2) 90-day feeding studies. Currently two-year chronic toxicity feeding studies are required for any pesticide requiring a tolerance or exemption from the requirement of tolerance. Safety and metabolism, carcinogenicity, teratogenicity, environmental and wildlife studies are required. In total, a series of 30 or more tests are necessary to meet the increasingly stringent requirements set out by the EPA.

The end result of these events include: (10, 11, 12, 13) (Fig. 2).

1. Cost per new product have nearly doubled since 1970:

   - 1967 - $ 3MM
   - 1970 - $ 5MM
   - 1978 - $10MM (Note: in the last year costs have reportedly increased $290,000 for registration alone).

2. Increase in length of time to product introduction from an average of 60 months in 1967 to 90-100 months.
3. Longer waiting periods, decreasing the length of useful patent life.
4. Cutbacks in R and D spending, with reduction of spending for innovative endeavors and increased emphasis on defensive or supportive research on existing products. Less interest results in special-use materials with priority assigned more to the major crop areas.

Perhaps one of the most politically sensitive areas is that of oncogenicity. Many arguments on the quantitation of carcinogenicity have included hip-shooting and emotional controversy. It is still hoped that evidence of carcinogenicity will not be the only step upon which regulations are based. Potency and risk of actual exposure from use patterns will hopefully be critically considered. If this can be accomplished, better delineation of impacts of the risk and benefits should result (19).

Economic Factors

Determination of opportunity for any specific end-use pesticide is complex and can be demonstrated by several models which illustrate the major areas critical in determining whether to cease or continue the pesticide or plant growth

In Plant Growth Substances; Mandava, N.;
ACS Symposium Series; American Chemical Society: Washington, DC, 1979.

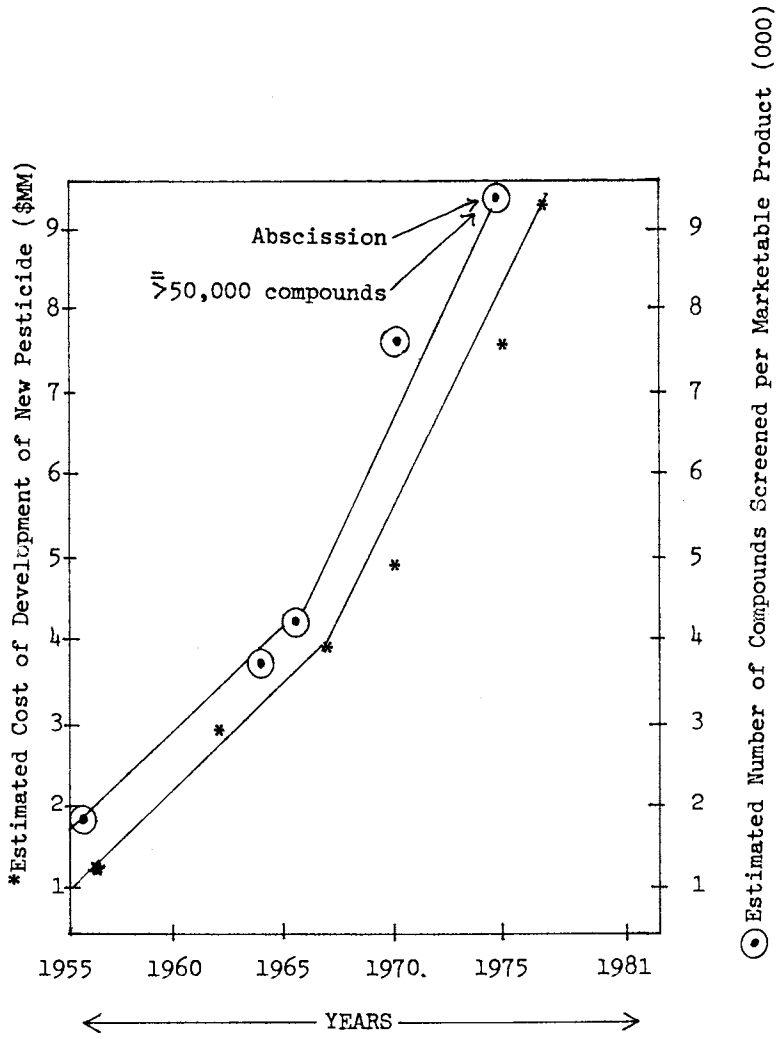


Figure 2. Cost of commercial agricultural chemical and number of compounds screened

In Plant Growth Substances; Mandava, N.;
ACS Symposium Series; American Chemical Society: Washington, DC, 1979.

regulator development.

Preliminary Marketing Profile. Several preliminary questions must be answered before a market group can recommend further capital investment. These include but are not limited to:

1. What is the end-use spectrum relative to market opportunity?
2. Is a total market projection possible? What "Fig.3" is the competitive situation?
3. What are the performance characteristics of the potential product?

4. What are the unit costs related to end-use "Fig.4" and production costs?
5. Does the company have expertise or capabilities to serve markets?

Early decisions based on quantification of these questions are necessary to determine alternative steps or a GO, NO-GO decision (Fig. 5). As a new product candidate, RELEASE has not scored well in appraisal of opportunity and investment demands to assure probability of success for potential marketing. As with any PGR material, constant assessment is necessary.

Pesticide Opportunity Profile. In looking at the total complex of R and D and marketing inputs, there are a number of points which must be understood to anticipate obstacles or roadblocks which can impede or eliminate further development (20). For example: (Fig. 6).

1. Underestimation of R and D and registration costs.
2. Lack of basic knowledge of toxicity and mechanism of action.
3. Product superiority may be overestimated and may not represent actual grower need. New practices or programs may require development for product acceptance by the grower (5). Success in the laboratory and the research plot is one thing - but unless someone buys the concept, a business venture can fail because estimates of cost-benefits are not valid.

*4. A multi-use product may be anticipated, for ease of development, but other uses do not materialize.
*5. Inadequate market potential ($<$\$5MM) (13).
*6. Length of R and D period is excessive ($>$5 years).
7. Fear of consumer complaints and litigation because of sophisticated product action or use pattern.

In Plant Growth Substances; Mandava, N.;
ACS Symposium Series; American Chemical Society: Washington, DC, 1979.

A. END-USE SPECTRUM

. LIMITED . RESTRICTED HIGHLY SPECIALIZED	. INTERMEDIATE	. MAJOR MARKET FIT . MANY END USES

B. PERFORMANCE (EFFICACY; SAFETY; EASE-OF-USE, ETC)

. COMPARABLE TO EXISTING PRODUCTS	. INTERMEDIATE	. SUPERIOR TO MARKET STANDARDS

Figure 3. Gross appraisal outline of candidate pesticide

C. MANUFACTURED COSTS OF EFFECTIVE RATE PER SEASON

COMPARABLE TO COMPETITIVE PRODUCTS	. INTERMEDIATE	. CHEAPER THAN MARKET STANDARDS

D. COMPANY FIT

. REQUIRES MAJOR ADDITIONAL RESOURCES	. INTERMEDIATE	. PERFECT MESH WITH COMPANY EXISTING AREAS OF COMPETENCE AND MARKETING STRENGTHS
. R AND D AREA		
. MARKETING AREA OF DOUBTFUL LONG-RANGE INTEREST		. FITS LONG-RANGE EXPANSION PLANS

Figure 4. Gross appraisal outline of candidate pesticide

NEW PRODUCT ASSESSMENT	ACTION
A. LOW	NO-GO - DROP (ALTERNATIVES).
B. INTERMEDIATE	RE-EVALUATE - CONSERVATIVE/REALISTIC CONTINUE R AND D FOR SPECIFIC NEEDS FOR BETTER DEFINITION. TARGET DECISION FOR GO/NO-GO.
C. HIGH	PROCEED WITH DETAILED FORCAST. IF GO - MOVE AHEAD.

Figure 5. Gross appraisal outline of candidate pesticide

In Plant Growth Substances; Mandava, N.;
ACS Symposium Series; American Chemical Society: Washington, DC, 1979.

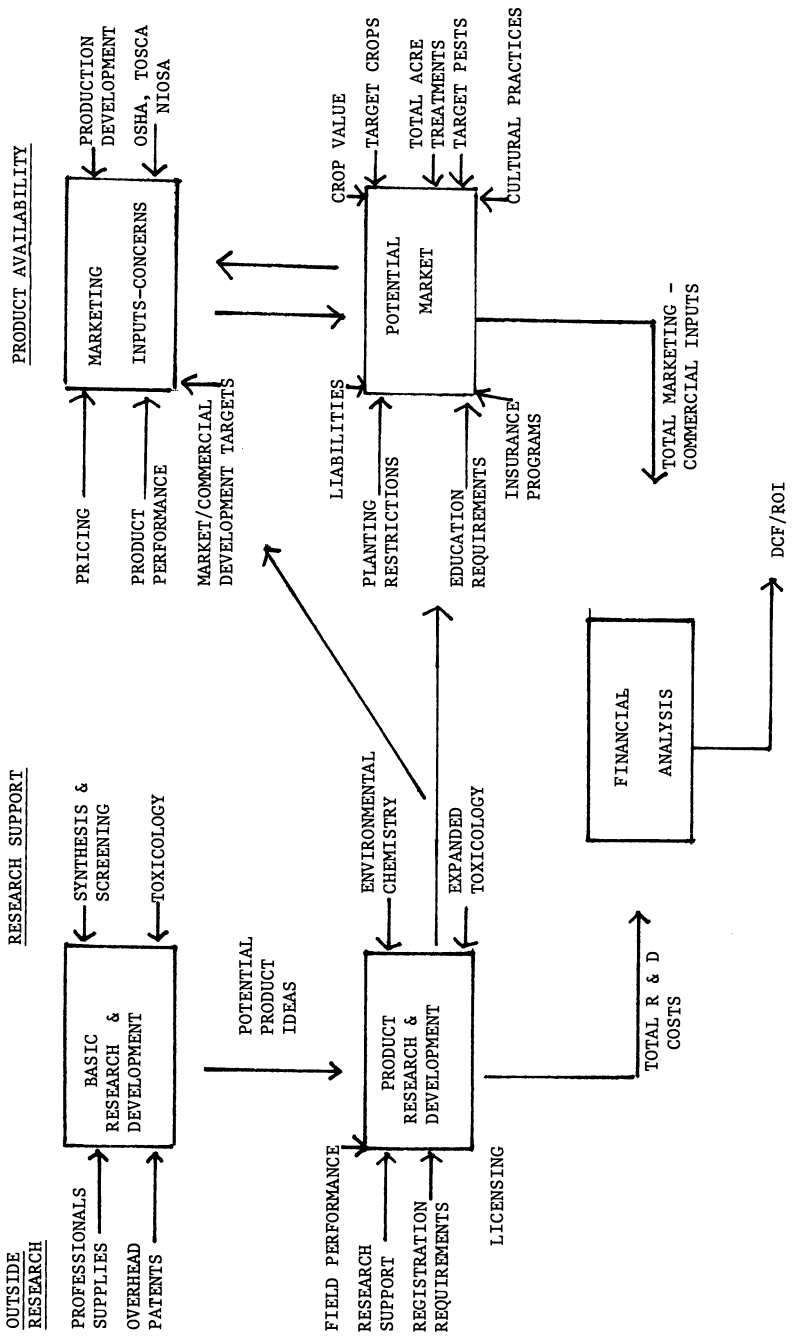


Figure 6. Pesticide opportunity profile

In Plant Growth Substances; Mandava, N.;
ACS Symposium Series; American Chemical Society: Washington, DC, 1979.

8. Lack of support from agricultural researchers in public and private sectors (i.e. product performance) (8).

*9. Overestimation of treatable acreage (5).
   - varietal use will restrict use of product
   - specific geographical areas limit product use

*10. Mechanical harvesting:
   - Levels of technology and availability of total systems are not sufficient to be adapted by grower industry to allow predictable penetration in a given crop.
   - Weather variables or requirements can eliminate or reduce marketing opportunities.
   - Ample labor supplies delay interest and demands for mechanical harvesting aids presented.

The above list of factors is not complete and is not presented to dilute the opportunities for specialty-use materials but does provide realistic check points of analysis.

* Factors Affecting Abscission Agents Such as RELEASE .

1. Limitation to a specific crop; e.g., citrus; difficulty in varietal response (Valencia). Failure to identify no multi-use pattern.

2. Limited market based on acreage expected effectively - minor uses. Potential demand is expected to mature to 300-400M acres "at some future date" which is difficult to define.

3. Mechanical harvesting development has fluctuated because of availability of sufficient hand labor. There is difficulty in establishing benefit ratios when need is not critical for the use of the plant growth regulator aid in mechanical harvesting.

Future Of Specialty-Use Compounds

The future of special-use plant growth regulators (PGR) can be summarized thus:

1. Plant growth regulators should be recognized as more than academic curiosities. They are not only interesting but profitable to use - to grower, distributor and manufacturer (21).

2. Stringent regulations for registration will continue to impact business ventures with PGR compounds.

3. Short-term opportunities will still be emphasized with attention turning to major field crops.

4. We may still be in the last phases of PGR history where products in the mill may still be ahead of their time in terms of today's applications and recognized needs (5).

5. Because demands for food and needs to increase land productivity will continue, hopes remain high for the practical use of existing and new PGR chemicals under development (4).

In Plant Growth Substances; Mandava, N.;
ACS Symposium Series; American Chemical Society: Washington, DC, 1979.

6. Industry will remain cautiously optimistic and will constantly reassess the size of investment demands and risk/benefit ratios by addressing a multitude of questions on product novelty, performance, safety, R and D, registration, production and marketing costs and the ultimate return on investment. It should be appreciated, that industry will not complain about investment requirements as long as a reasonable return on investment costs can be achieved within a reasonable length of time (8).

7. To maximize the chance of success, it must not be assumed that the chemical industry alone can afford to bear the total burden (22, 23). Industry, growers, grower companies, universities and extension must work together so that mutually supporting programs evolve. Together we must recognize the obligations necessary to introduce products commensurate with needs of the grower/farmer, his ability to understand and use the products correctly and the limits of commitment to assure registration of these products.

With such cooperation, mutual support and obviously some "luck" (23), plant growth regulators, including abscission agents, may still be expected to be developed (24, 25) not only to enhance quality and yields of major crops; e.g., cotton, corn, tobacco, but also important fruit crops; e.g., apples, oranges (citrus), grapes, cherries, etc.

REFERENCES

1. Wittwer S. H., Bull. Plant Growth Regulator, 1974, 2,5.

2. Chem. Wk., 1975, 116, (10), 41.

3. Farm Chem., 1975, 138, (3), 15-25.

4. Nickell L.G., Chem. and Eng. News, 1978, 56, 18.

5. Eggleton M.A., American Chemical Society Division of Chemical Economics and Marketing, National Meeting, 1977.

6. Leidner J., Prog. Farm., 1977, (4), 44.

7. Stanford Research Institute, 1973, Report 483.

8. Mitlehner A.W., Bull. Plant Growth Regulator, 1974, 2,8.

9. Wilson W.C. and Attaway J.A., Bull. Plant Growth Regulator, 1974, 2, 18.

10. Farm Chem. 1964, 127, (5), 28.

11. Chem. & Eng. News, 1969, 47 (24) 22.

In Plant Growth Substances; Mandava, N.;
ACS Symposium Series; American Chemical Society: Washington, DC, 1979.

12. NACA Study 1971 - July.

13. Haschen T.L., 1975. Florist and Nurseryman, September 5, pp. 10-12.

14. Schwartz I, 1974. Chem. Wk., 114, (8), 31-36.

15. Farm Chem. 1975, 138, (3), 16.

16. Ouellette Robert P. and King John 1976, Chem. Wk., 118, (25), 27-38.

17. Arthur D. Little Impact Study, 1976.

18. Chem. and Eng. News, 1978, 56 (17), 15.

19. Chem and Eng. News, 1978, 56, (13), 3.

20. Blue Thomas A. Chemical Marketing and Economic Division of the American Chemical Society 1977, P.313-344.

21. Upchurch R.P. Bull. Plant Growth Regulator 1974, 2, 20-22.

22. Holmsen T.W. Bull. Plant Growth Regulator 1976, 4, 2.

23. Upchurch R.P. 1976, ibid., 4, 4-5.

24. Tschabold Edward E., ibid. 1976, 4, 23.

25. Sharma M.P. Agriculture & Forestry Bulletin, 1977, Spring 27-32.

RECEIVED June 19, 1979.

INDEX

In Plant Growth Substances; Mandava, N.;
ACS Symposium Series; American Chemical Society: Washington, DC, 1979.

B

C

In Plant Growth Substances; Mandava, N.;
ACS Symposium Series; American Chemical Society: Washington, DC, 1979.

In Plant Growth Substances; Mandava, N.;
ACS Symposium Series; American Chemical Society: Washington, DC, 1979.

D

E

In Plant Growth Substances; Mandava, N.;
ACS Symposium Series; American Chemical Society: Washington, DC, 1979.

F

G

In Plant Growth Substances; Mandava, N.;
ACS Symposium Series; American Chemical Society: Washington, DC, 1979.

In Plant Growth Substances; Mandava, N.;
ACS Symposium Series; American Chemical Society: Washington, DC, 1979.

In Plant Growth Substances; Mandava, N.;
ACS Symposium Series; American Chemical Society: Washington, DC, 1979.

J

K

L

M

In Plant Growth Substances; Mandava, N.;
ACS Symposium Series; American Chemical Society: Washington, DC, 1979.

In Plant Growth Substances; Mandava, N.;
ACS Symposium Series; American Chemical Society: Washington, DC, 1979.

In Plant Growth Substances; Mandava, N.;
ACS Symposium Series; American Chemical Society: Washington, DC, 1979.

Q

R

In Plant Growth Substances; Mandava, N.;
ACS Symposium Series; American Chemical Society: Washington, DC, 1979.

S

In Plant Growth Substances; Mandava, N.;
ACS Symposium Series; American Chemical Society: Washington, DC, 1979.

T

U

V

W

X

In Plant Growth Substances; Mandava, N.;
ACS Symposium Series; American Chemical Society: Washington, DC, 1979.

Z

In Plant Growth Substances; Mandava, N.;
ACS Symposium Series; American Chemical Society: Washington, DC, 1979.