ISOLATION, IDENTIFICATION, AND RELATIONSHIP TO SEX EXPRESSION OF THE GIBBERELLINS OF CUCUMIS MELO AND CUCUMIS SATIVUS

Thesis for the Degree of Ph. D.

MICHIGAN STATE UNIVERSITY
DELBERT DEAN HEMPHILL Jr.

1971

This is to certify that the

thesis entitled

Isolation, Identification, and Relationship to Sex Expression of the Gibberellins of *Cucumis melo* and *Cucumis sativus*

presented by

DELBERT DEAN HEMPHILL, JR.

has been accepted towards fulfillment of the requirements for

Ph.D. degree in Biochemistry

Harold M. Sell

Major professor

Date 5/12/71

JUN 2 11 1973 156

ABSTRACT

ISOLATION, IDENTIFICATION, AND RELATIONSHIP TO SEX EXPRESSION OF THE GIBBERELLINS OF *CUCUMIS MELO* AND *CUCUMIS SATIVUS*

By

Delbert Dean Hemphill Jr.

Gibberellins and other phytohormones are known to alter sex expression in many plant species. Exogenously applied gibberellin preparations increase maleness in *C. sativus*, even inducing staminate flowers in homozygous gynoecious lines. In contrast, similar gibberellin treatments have no effect on sex expression in the closely related species *C. melo*. However, staminate flowers have been induced on gynoecious *C. melo* plants by grafting melon scions onto either rootstocks or interstocks of several other species.

Several lines of indirect evidence and a few experiments on endogenous gibberellin content have indicated that monoecious *C. sativus* seedlings contain more gibberellin activity than gynoecious seedlings at the same growth stage. This investigation was undertaken to determine the nature and levels of endogenous gibberellins in a wide variety of *C. sativus* and *C. melo* sex types. Gibberellin activity was determined in several bioassays after either aqueous buffer or organic solvent extraction of both free acidic

and enzyme-releasable gibberellins. Mature dry seeds, etiolated seedlings, and green plants of each sex type at a wide range of growth stages were used.

Monoecious and andromonoecious C. sativus seeds, etiolated seedlings, and green plants were found to contain higher levels of free acidic and total (free acidic plus enzyme-released) gibberellin than did the gynoecious at all growth stages tested. In monoecious and andromonoecious (but not gynoecious) green plants, free gibberellin reached a maximum at the growth stage corresponding to flower initiation at the cotyledonary and first leaf axils. Vernalization of gynoecious seed resulted in staminate flower induction in plants produced from this seed; extracts of one week old seedlings contained increased amounts of gibberellin relative to non-vernalized controls.

In contrast to C. sativus, seeds and green plants of monoecious and andromonoecious lines of C. melo contained far less gibberellin activity than those of gynoecious and hermaphroditic lines. This finding is consistent with the failure of exogenous gibberellins to induce staminate flowers on gynoecious C. melo plants. Seeds and leaves of two pumpkin varieties which, as rootstocks or interstocks, induce staminate flowers in gynoecious C. melo scions were found to contain less gibberellin than any C. melo sex type. Gibberellin is hypothesized to be the "male hormone" of C. sativus but to play no role in C. melo sex expression.

The predominant gibberellin of C. sativus was found to be A_1 by thin-layer and gas-liquid chromatography and mass spectrometry. Small quantities of gibberellins A_3, A_4, and A_7 are also present. The types and proportions of gibberellins did not vary with sex type. Gibberellins A_1, A_3, and A_5 were identified in C. melo.

A gibberellin A_1-β-D-glucoside was isolated from the n-butanol fraction of an extract of monoecious C. sativus seed. This compound may account for a portion of the enzyme-releasable gibberellin activity in both seeds and seedlings.

The n-propyl esters of gibberellins A_1 and A_3 were isolated from a neutral fraction of an extract of monoecious C. sativus seed. The compounds were characterized by thin-layer chromatography and mass spectrometry. The identity of the A_3 ester was confirmed through comparison of the isolated compound with an authentic sample: mass spectra, infrared spectra, melting points, and migrations in three solvent systems were identical. Growth promoting activity of the A_3 ester was very low in several bioassays.

ISOLATION, IDENTIFICATION, AND RELATIONSHIP TO SEX EXPRESSION OF THE GIBBERELLINS OF *CUCUMIS MELO* AND *CUCUMIS SATIVUS*

By

Delbert Dean Hemphill Jr.

A THESIS

Submitted to
Michigan State University
in partial fulfillment of the requirements
for the degree of

DOCTOR OF PHILOSOPHY

Department of Biochemistry

1971

ACKNOWLEDGMENTS

The author expresses appreciation to Professor Harold M. Sell for the suggestion of the problem and his advice and continued encouragement during the course of this work.

To Dr. L. R. Baker, my thanks for his advice, aid in preparation of this thesis, and provision of plant material used in this work.

Thanks are also due to my parents and my wife for their financial and moral support.

The financial support of the Herman Frasch Foundation and the Department of Biochemistry is gratefully acknowledged.

TABLE OF CONTENTS

LIST OF TABLES

Tabl

12.

13.

14.

15.

16.

17.

18.

19.

LIST OF FIGURES

LIST OF ABBREVIATIONS

CCC	(2-chloroethyl)-trimethylammonium chloride
IAA	β-indole-3-acetic acid
NAA	α-naphthaleneacetic acid
2,4-D	2,4-dichlorophenoxyacetic acid
TIBA	2,3,5-triiodobenzoic acid
Ethrel	2-chloroethylphosphonic acid, also Ethephon
ABA	abscisic acid
MH	maleic hydrazide
GA_1, GA_2, etc.	gibberellin A_1, gibberellin A_2 etc.
GA_3	gibberellin A_3 or gibberellic acid
Am	andromonoecious
Gyn	gynoecious
TLC	thin-layer chromatography
GLC	gas-liquid chromatography
IR	infrared
B-9	N,N-dimethylaminosuccinamic acid

INTRODUCTION

INTRODUCTION

Sex expression in the cucurbits, including cucumber and muskmelon, is a complex characteristic, subject to many genetic and environmental influences. Since there are three types of flowers found in cucurbits (perfect, male, and female) and any one plant may bear any combination of these three, theoretically there are a total of seven possible sex types. Cultivated varieties of muskmelon are most often andromonoecious, bearing male and perfect flowers on the same plant. There are several genes conditioning sex expression or flower sex type in both cucumber and muskmelon. In contrast to mammalian sex genes, they are not closely linked; each of the several genes segregates independently. Although the muskmelon and cucumber are closely related species of the same genus with a similar array of sex types, the genetics of sex expression is different for each species. This is exhibited in divergent inheritance of sex-linked characteristics and in different responses to plant growth regulators.

Several plant growth regulators, including some phytohormones, are known to affect plant sex expression when exogenously applied. Gibberellins, auxins, cytokinins, and ethylene are phytohormones which can alter plant sex expression, including that of the cucurbits.

The gibberellins exert myriad effects on plant metabolism and their occurrence in plants is probably ubiquitous. They appear to be involved in mitosis, cell enlargement, and cell differentiation. The gibberellins were first discovered as metabolites of the fungus Gibberella fujikuroi, the pathogen responsible for a foot-rot disease of rice characterized by excessive shoot elongation. The active substance isolated from the fungus, and later found to be a mixture of several structurally similar compounds, affected growth in many plants. Various gibberellins were subsequently identified as natural constituents of plants.

To date, a total of thirty-two gibberellins, all sharing a common gibbane skeleton and exhibiting some biological activity, have been isolated from a wide variety of sources. The structures and occurrence of each of the first twenty-nine gibberellins have recently been reviewed by Lang (1970) and will not be dealt with here. Gibberellins A_{30} and A_{31} (Murofushi et al., 1970) and A_{32} (Yamaguchi et al., 1970) were recently isolated from seeds of Calonyction aculeatum and peach, respectively. Many plants contain several of the gibberellins as well as gibberellin metabolites. Recently, naturally occurring gibberellin derivatives known as "bound" gibberellins have been isolated, including several gibberellin-glucose complexes. The structures of the thirty-two known gibberellins and the known "bound" gibberellins are presented in Figures 1 and 2.

Gibberellins often exert a masculinizing effect on plant sex expression. This is true for cucumber, but not for

muskmelon.

Heteroauxin (indoleacetic acid) was discovered as a promoter of cell elongation, but was soon found to affect cell division and cell differentiation as well. Auxins often feminize many plants including cucumber. The cytokinins were discovered as a factor occurring naturally in the vascular tissue of tobacco stems which promoted pith tissue cell division. Cytokinins have since been found in many species and are known to alter sex expression in several plants. Ethylene gas, considered by many physiologists to be a phytohormone, increases the numbers of female flowers in many species including cucumber and muskmelon. The structures of naturally occurring heteroauxin and cytokinin (zeatin) are seen in Figure 2.

The study of the biochemical control of plant sex expression is of commercial as well as theoretical interest. To control sex expression through genetic or chemical means would enable plant breeders to establish inbred lines for development of commercial F_1 hybrids. These inbred lines would also be invaluable for research into the genetics and biochemistry of sex expression and the advantages of hybrids in agriculture are well known. Cucumber and muskmelon present a unique opportunity for study of the biochemistry of sex expression due to the establishment of genetically pure lines of different sex types. In spite of the close relationship between these species, important differences have been found which imply differences in the mechanism of sex expression in the two species. In cucumber, but not muskmelon,

Figure 1

Structures of the thirty-two known free acidic gibberellins. [Gibbane skeleton numbering system]

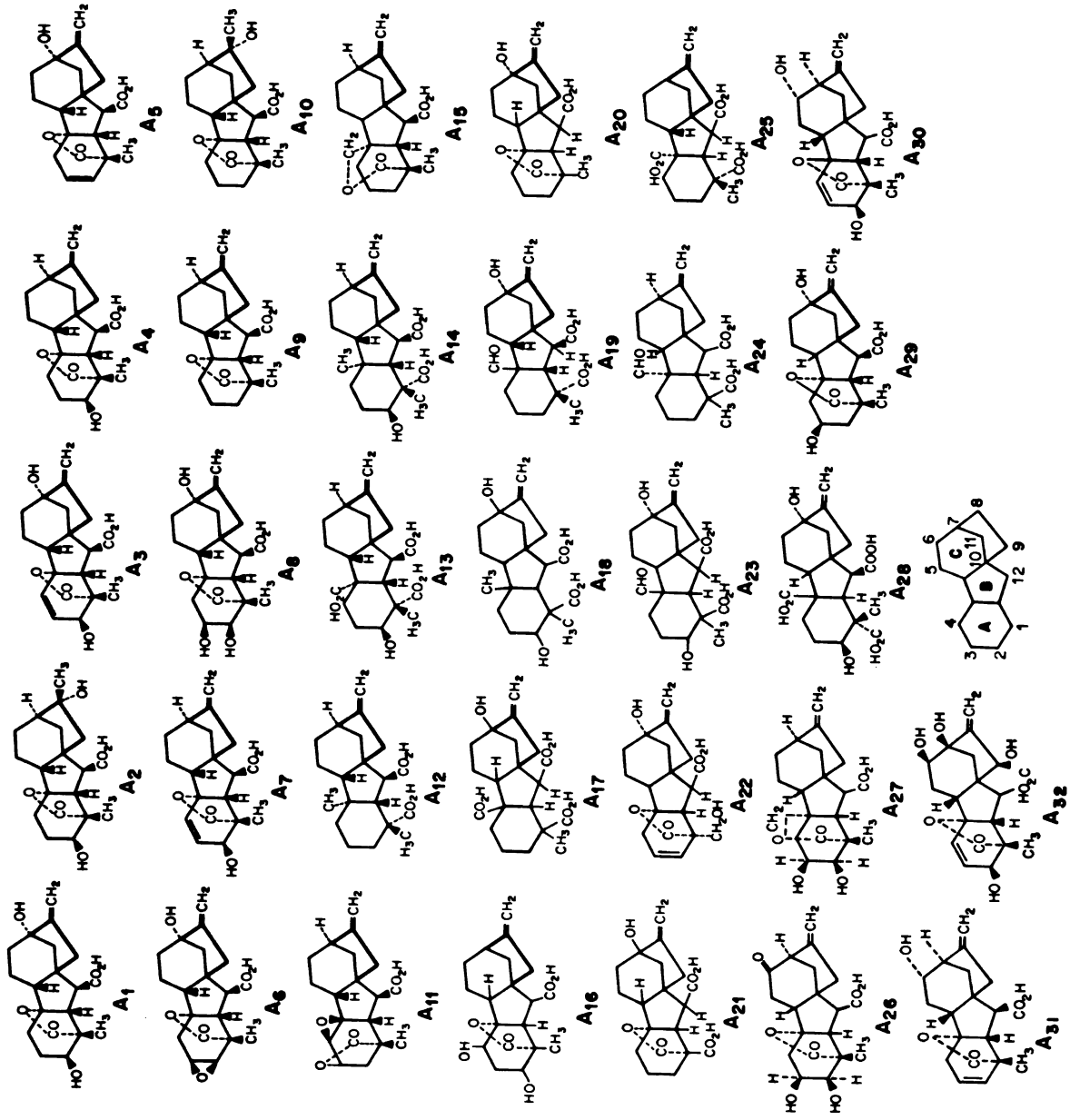
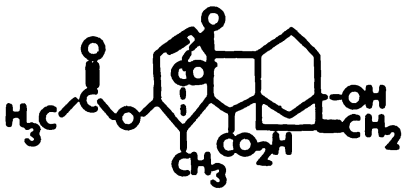

A1
A2
A3
A4
A5
A6
A7
A8
A9
A10
A11
A12
A13
A14
A15
A16
A17
A18
A19
A20
A21
A22
A23
A24
A25
A26
A27
A28
A29
A30
A31
A32

Figure 1

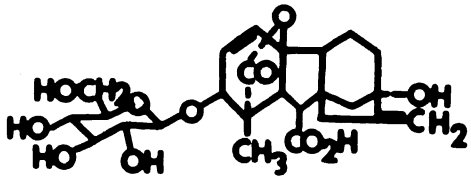
Figure 2

Structures of known conjugated gibberellins and representative examples of other phytohormones and growth regulators.

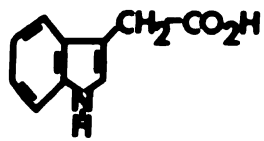
Figure 2



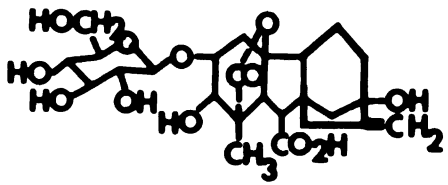
O-2-acetyl-GA_3



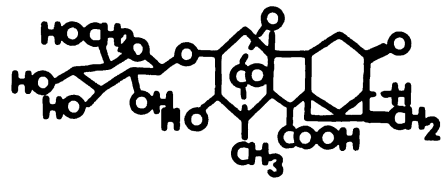
O-2-β-D-glucopyranosyl-GA_3



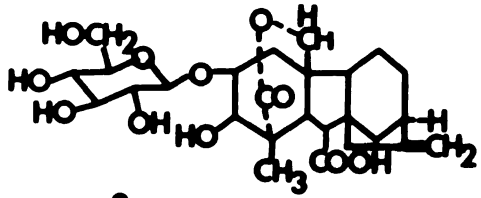
indole-3-acetic acid



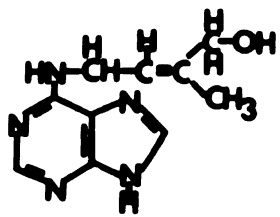
O-3-β-D-glucopyranosyl-GA_8



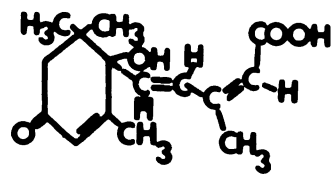
O-3-β-D-glucopyranosyl-GA_{26}



O-3-β-D-glucopyranosyl-GA_{27}



zeatin



abscisic acid

gibberellins have been used to induce male flowers in female plants. Grafting of muskmelon scions onto pumpkin rootstocks can induce male flowers in female muskmelon, but this does not hold for female cucumber scions. Thus, it is of interest to determine the relationship, if any, between endogenous gibberellin activity and sex expression in the two species.

The objectives of the research presented here were to quantitatively assay and identify the gibberellins occurring in a wide range of muskmelon and cucumber sex types at various growth stages and to correlate the quantity or types of gibberellins with the sex expression and genetic makeup of the plants. The principles guiding this research and the reasons for postulating that endogenous gibberellins are involved in male sex expression in cucumber are further elucidated in the following literature review.

LITERATURE REVIEW

A. Cucumber Genetics and Morphology

Sex expression in the cucumber (Cucumis sativus L.) is marked by a wide variety of sex types. Monoecious[1], andromonoecious, gynomonoecious, trimonoecious, androecious, gynoecious, and hermaphroditic lines either occur in the wild or have been established in breeding programs. However, Atsmon and Galun (1960) have established that pistillate, staminate, and perfect flowers all pass through a bisexual stage. Divergence is then accomplished by inhibition of development of pistil or stigma. The genetic control of these sex types is complex and the environment may also affect the sex type. Atsmon and Galun (1962) noted that when the female tendency of the cucumber is strong, floral buds develop adjacent to young leaves, the reverse being true for more male plants. They postulated that environmental and genetic factors which affect sex tendency change the leaf age-flower bud relationship and that this may be related to the hormonal balance in the vicinity of the developing floral primordium.

---

[1]Monoecious: staminate and pistillate flowers occurring on the same plant; andromonoecious: staminate and perfect flowers on the same plant; gynomonoecious: pistillate and perfect on the same plant; trimonoecious: pistillate, perfect, and staminate on the same plant; androecious: staminate flowers only; gynoecious: pistillate flowers only; hermaphroditic: perfect (hermaphroditic) flowers only.

Several genes are known to condition cucumber sex types. The first of these, denoted M by Rosa (1928), conditions the potentiality for pistillate flowers. When M is substituted by its homozygous recessive allele mm, bisexual flowers replace pistillate flowers. Galun (1961) concluded that allele m also increases the number of staminate flowers. Kubicki (1969d) concluded that M must act by inhibiting stamen production while m conditions simultaneous development of pistils and stamens.

Another set of genes governs the intensity of femaleness in monoecious and gynoecious cucumber plants. The alleles of this locus have been designated f and F (Tkaczenko, 1935), or st and st^+ (Galun, 1961), or acr and Acr (Shifriss, 1961a). Kubicki (1969a) discovered other alleles of this locus. The allele conditioning monoecism without a continuous pistillate stage was designated acr^+, monoecism with continuous pistillate stage acr^1, while gynoecious lines contain allele acr^F. Kubicki concluded that the physiological action of the several alleles of locus acr is connected with the quantity or activity of an ovary-producing hormone.

Another gene conditioning femaleness which segregates independently of acr is designated F (Kubicki, 1969b). The dominant allele F conditions formation of a high number of pistillate nodes, while f governs formation of relatively fewer pistillate nodes in monoecious and gynoecious sex types.

Kubicki (1969c) assumed androecism to be governed by a single recessive gene a which is independent of acr. Androecious plants would then represent the genotype acr^+acr^+aa.

Kubicki (1969e) has also established that gene Tr, which conditions trimonoecism, acts through the control of superior ovary development. Bisexual flowers in trimonoecious plants differ from those occurring in hermaphroditic plants in both origin and structure. Trimonoecious perfect flowers are derived from modification of staminate flowers rather than replacing female flowers.

These results permit the assumption that sex determination in cucumber is influenced by two different groups of genes. One group, including loci acr, F, and A, controls the ratio of ovary-bearing flowers to staminate flowers. The second group, loci M and Tr, controls floral structure, giving rise to epigynous (inferior ovary) and hypogynous (superior ovary) bisexual flowers, respectively.

B. Muskmelon Genetics and Morphology

The muskmelon (Cucumis melo L.) appears to have the potential for the same array of sex types as cucumber. However, androecious and trimonoecious lines have not been reported. Perfect flowers are usually born singly on small side branches arising from the main stem or major branches. Staminate flowers usually occur in groups on main stem or major branch nodes (McGlasson and Pratt, 1963).

Rosa (1928) found that monoecism in muskmelon is dominant over andromonoecism and this difference is conditioned by a single allelic pair. Poole and Grimball (1939) discovered hermaphroditic forms and proposed a two gene explanation of muskmelon sex expression: AG is monoecious, Ag gynomonoecious,

aG andromonoecious, ag hermaphroditic. However, these authors included trimonoecious and gynoecious plants under the heading gynomonoecious. Kubicki (1969f) explored this problem and concluded that at least four sets of genes were involved. M (A) conditions formation of pistillate flowers in monoecious plants, while recessive allele m (a) is associated with perfect flowers. Gene G leads to formation of staminate flowers in monoecious plants, while lines containing its homozygous recessive allele gg would have perfect flowers. Complementary genes Trl and Tr2 condition formation of bisexual flowers with superior ovaries. The dominant Tr2 greatly strengthens the effect of recessive trl in stimulating ovary development.

Muskmelon is the closest relative to cucumber among the cultivated cucurbits and the similarity of sex types in these species is an example of this relationship. The gene pair Mm governs floral structure in both species as well as in watermelon. However, similarities in sex types need not indicate similar biological mechanisms. Gynoecism in cucumber is a partially dominant property, but gynoecism in muskmelon is totally recessive. This is presumably due to a difference in the dominance of sex genes in the two species. This individuality in genetics of sex expression is also supported by differences in the effects of environmental factors and exogenously applied phytohormones on sex differentiation in cucumber and muskmelon.

C. Environmental Effects on Plant Sex Expression

Environmental factors, including day-length, temperature, moisture, and amounts of certain elements, are known to enhance or modify sex expression in a wide range of plants. The Heslop-Harrisons (1957) found that carbon monoxide induced pistillate and perfect flowers in androecious plants. Czao (1957) reported that carbon monoxide treatment of cucumber seedlings increased the pistillate/staminate flower ratio while phytohormones, fertilizer, and high soil moisture also increased female flower development.

Janick and Stevenson (1955) reported that high temperatures caused increased maleness in monoecious and some gynoecious spinach plants, whereas Heslop-Harrison (1957) concluded that, in general, low temperature favors pistillate flower formation. Ito and Saito (1957a) reported that high night temperature favors staminate flower formation in Japanese cucumber, while Kooistra (1967) induced staminate flowers on predominately female cucumbers with high night temperatures. Bukovac and Wittwer (1961) found that low temperatures accelerated formation of pistillate flowers in pickling cucumber.

Nitrogen levels also affect sex expression. Tibeau (1936) found that high nitrogen levels favor pistillate flowers in hemp and Thompson (1955) reported similar results with spinach. Tiedjens (1928) and Hall (1949), working with cucumber and gherkin, respectively, found that high nitrogen levels promoted pistillate flowers and low

nitrogen levels promoted staminate flower production. Brantley and Warren (1960) concluded that high levels of nitrogen resulted in an increased proportion of perfect flowers in andromonoecious muskmelon. In contrast, Kooistra (1967) reported that high nitrogen favored formation of staminate flowers in predominately female cucumbers.

Another important environmental factor in sex expression is photoperiod. Many plants specifically require long- or short-day conditions to induce flowering and pistillate/staminate ratios are often affected. Schaffner (1921, 1923, 1925) found that hemp plants underwent sex reversion under short-day (winter) conditions and that several other species including Humulus, Myrica and Plantago also experienced sex reversions under the influence of an altered photoperiod. Thompson (1955) promoted pistillate flower formation in spinach under a long-day regime and andromonoecious muskmelons produced an increased proportion of perfect flowers under long-days (Brantley and Warren, 1960).

In contrast, Vergeley et al. (1967) promoted femaleness in hemp with short days and Nitsch et al. (1952) reported that long days promoted male sex expression in gherkin and squash, while short days favored femaleness.

Working with cucumber, Tiedjens (1928) and Ito and Saito (1957a) found that long days favor staminate flower production. Atsmon et al. (1967) found that continuous light inhibited pistillate flower formation in gynoecious

cucumber while staminate flowers in monoecious cucumber were inhibited by a twelve-hour light regime. Comparing these results with those produced by exogenous application of gibberellins, the authors suggest that continuous light inhibits femaleness through an effect on endogenous gibberellins. At least in cucumber, it is clear that short days, low temperatures, and high soil moisture favor femaleness while conditions involving greater stress such as long days, high temperatures, and drought reduce female tendency.

D. Effects of Gibberellins and Anti-Gibberellins on Sex Expression

The effects of gibberellins on flowering often resemble effects of photoperiod, and there may be a relationship between long- and short-day regimes and levels of endogenous gibberellins.

1. Gibberellin-induced sex modifications in plants.

Reports of sex modification in many plants under the influence of gibberellins have been widespread during the last decade. Interest particularly focused on the application of gibberellins to hemp and cucumber, and in the latter case, gibberellin-induced staminate flowers have been used to self-pollinate gynoecious plants. Atal (1959) first reported a sex-modifying effect of gibberellins on hemp, noting that seedlings of female plants treated with 100 ppm of gibberellin produced staminate flowers. This result was confirmed by Vergeley et al. (1967). Davidyan (1967) found that

gibberellin treatment of 25 to 35 day old gynoecious hemp plants caused formation of monoecious plants. In the case of one variety, this change was transmitted to the progeny. Kutuzova (1968) also confirmed sex conversion in hemp, but a concentration of 0.03% gibberellic acid was needed for optimal results in inducing male flowers. On the other hand, Khryanin (1969) found that two applications of only 0.0025% gibberellin converted gynoecious hemp to monoecious. He concluded that gibberellin derepressed certain genes participating in flower formation.

A scattering of results have also been reported for other species. Splittstoesser (1970) found that in monoecious Cucurbita moschata L., gibberellin increased the proportion of staminate flowers and delayed fruit-set. The masculinizing effect of gibberellin may be exerted in processes subsequent to flower initiation. Phatak et al. (1966) reached this conclusion after finding that addition of 25 ppm of the potassium salt of gibberellic acid to a root culture of a stamenless tomato mutant caused development of anthers and viable pollen. Glushchenko (1970) found that gibberellins applied to five hyacinth varieties resulted in increased growth and branching of pollen tubes.

In contrast to these reports, Herich (1960) noted that application of gibberellin to hemp caused a slight increase in female tendency. In another case (Shifriss, 1961b) the pistillate/staminate flower ratio of monoecious castor beans was increased by a gibberellic acid spray. In addition,

Bose and Nitsch (1970) reported that GA_3 inhibited male flower formation and weakly promoted female flower formation in Luffa acutangula.

The effects of various gibberellins on sex expression of cucumber have been widely investigated. Most of this research has focused on commercial interest in the application of gibberellins to development of inbred gynoecious lines as hybrid parents. The earliest report on the effect of gibberellins was by Galun (1959a) who found that GA_3 applied to young cucumber leaves altered sex tendency toward maleness in gynoecious and monoecious plants. For monoecious plants this effect was attributed to an increase in number of nodes preceding the first flower, thus shifting flowering to a normally staminate stage. Peterson and Anhder (1960) induced staminate flowers on the gynoecious line MSU 713-5 with GA_3. Pykhtina (1968) found that spraying cucumber seedlings with 0.2% gibberellin increased both the total number and the staminate/pistillate ratio of flowers. However, the high level of gibberellin used in this experiment appears to rule out an interpretation of the results as indicative of an endogenous role for gibberellin in staminate flower formation. Hayase and Tanaka (1966) reported that for the gynoecious line MSU 713-5, the younger the seedling and higher the gibberellin dosage, the larger the number of staminate flowers induced. Stambera and Zeman (1969) reported similar results on staminate flower production and also inhibition of pistillate flower initiation.

Differences in the activity of various gibberellins in inducing staminate flowers in cucumber have been noted by several authors. Bukovac and Wittwer (1961) found that GA_4 was about ten-fold more active than GA_3 in induction of staminate flowers on monoecious and gynoecious plants. Wittwer and Bukovac (1962) then investigated the effects of all the then-known gibberellins on staminate flower formation in the gynoecious line MSU 713-5. They found that GA_7 was the most active followed by A_4, A_2, and A_9. GA_8 was least active. These most active gibberellins all lack a C-7[2] hydroxyl group and all gibberellins possessing a C-7 hydroxyl group are significantly less active. Pike and Peterson (1969) also found that a gibberellin A_4/A_7 mixture at 50 ppm was more effective in staminate flower induction than GA_3 at 1000 ppm and did not cause the excessive stem elongation associated with GA_3. Clark and Kenney (1969) found that GA_{13}, which also lacks a C-7 hydroxyl, produced staminate flowers on a gynoecious cucumber but was less effective than gibberellins A_4 and A_7.

In contrast to the masculinizing effect of gibberellins on cucumber, Peterson (1963) was unable to induce staminate flowers on the closely related species, gynoecious muskmelon, with GA_3. Kubicki (1966a) also failed to obtain male flower induction with GA_3 in muskmelon and hypothesized (Kubicki, 1969g) that this failure is understandable since the

---

[2] Gibbane skeleton numbering system

differentiation of staminate flowers in muskmelon is governed by a gene other than that in cucumber.

In summary, research with cucumber, hemp, and other species has demonstrated that exogenous application of gibberellins often, but not always, has a masculinizing effect on the treated plants. However, this is only the most indirect evidence for a role, directly or indirectly, of gibberellins as the "male hormone" of these species. Other indirect evidence is found in studies which appear to correlate high gibberellin levels, long internodes, and increasing male tendency (Atsmon et al., 1967).

2. Anti-gibberellins and sex expression in plants.

Other evidence for a role of gibberellins in sex expression comes from experiments with growth retardants which are either known or presumed inhibitors of gibberellin biosynthesis or action. Ota (1962) found that (2-bromoethyl)-trimethylammonium bromide increased femaleness in cucumber while Mitchell and Wittwer (1962) noted that allyltrimethylammonium bromide induced increased formation of pistillate flowers in monoecious cucumber plants. Hayase and Tanaka (1967) found that (2-chloroethyl)-trimethylammonium chloride (CCC), a specific inhibitor of gibberellin biosynthesis, did not affect gibberellin-induced staminate flower induction in cucumber. Tanaka et al. (1970) reported that CCC and N,N-dimethylaminosuccinamic acid (B-9) shifted cucumber sex expression toward femaleness, while Mishra and Pradhan (1970) found that CCC decreased staminate flower but increased pistillate

flower formation in cucumber.

Other species have exhibited similar responses. CCC inhibits staminate flower production in squash (Abdel-Gawad and Ketellapper, 1968), while Phatak et al. (1966) determined that CCC induces abnormal pollen growth in tomato. In contrast to the failure of exogenous gibberellins to induce staminate flowers on gynoecious or other sex types of muskmelon, Halevy and Rudich (1967) found that B-9 induced a female shift in andromonoecious muskmelon. Subsequently, Rudich et al. (1970) reported that B-9 applied to young muskmelon seedlings inhibited staminate flower production for the first two to three weeks of the flowering period. These results, contrasted with the results of gibberellin application, indicate that the role, if any, of gibberellin in muskmelon sex expression is indirect.

3. Endogenous gibberellins and sex expression.

A more direct approach to the role of gibberellins in sex expression is to relate the endogenous content to sex type and study any differences in gibberellin metabolism in various sex types. Kamienska (1966) determined that the endogenous gibberellin-like activity of male poplar plants is greater than that of female poplars.

Of direct interest here are the two studies made on levels of gibberellins in gynoecious and monoecious cucumber. Atsmon et al. (1968) used shoot diffusates from four day old seedlings and root exudates from six week old plants. Although different parts of the plant were assayed at the

two growth stages and there was no attempt to identify the endogenous gibberellins, the gibberellin levels in the monoecious line were significantly higher at both growth stages than in the gynoecious line. Recovery of applied ^{3}H-GA_1 from monoecious plants was also much greater than the recovery from gynoecious ones, indicating a relative gibberellin destruction or inactivation in the gynoecious line.

Hayashi et al. (1967, 1971) harvested etiolated cucumber seedlings of closely related monoecious and gynoecious lines at several growth stages over a period of zero to eighteen days. The gibberellin content of whole seedlings was determined in three bioassays. The monoecious line contained significantly higher gibberellin levels at all growth stages than the gynoecious. GA_1 was identified as the primary active component, although trace amounts of gibberellins A_3, A_4, and A_7 were also present. Thus, the gibberellins most active in inducing male tendency when exogenously applied do not make up the bulk of the endogenous gibberellin activity.

E. Effects of Auxins on Sex Expression

1. Sex modification by exogenous auxins.

The phytohormone β-indole-3-acetic acid (IAA) or heteroauxin, like gibberellins, affects many phases of plant life. There have been numerous reports of effects of heteroauxin on flowering. Those dealing particularly with sex expression in cucumber and other plants are reviewed here. Several synthetic auxins which have not been shown to occur in plants, but which induce auxin-like responses in bioassays, have

also been shown to affect plant sex expression. These include α-naphthaleneacetic acid (NAA) and 2,4-dichlorophenoxyacetic acid (2,4-D).

Wittwer and Hillyer (1954) found that spraying young seedlings of the curcurbit 'Table Queen' squash with NAA increased the pistillate/staminate flower ratio. Heslop-Harrison (1956) noted that application of NAA to the third node of young male hemp plants caused them to bear female flowers up to the thirteenth node. He postulated that flower sex type may be regulated by the auxin level at the time of flower primordia differentiation. The same author (1957) stated that, in general, exogenous auxins accelerate appearance of female flowers and increase the numbers of female flowers in plants.

Saito (1957) found that NAA at 10 ppm induced a female sex transition in normally male strobili of Japanese red pine and black pine. Mallik et al. (1959) determined that 100 ppm of NAA applied to mango just previous to onset of flower budding caused a doubling in the number of female flowers. NAA, 2,4-D, and p-chlorophenoxyacetic acid have been used to increase the pistillate/staminate ratio and total fruit set in the ribbed gourd (Satyanarayana and Rangaswami, 1959). Vergeley et al. (1967) found that both IAA and NAA caused increased femaleness in hemp, reproducing the effect of short days. Bose and Nitsch (1970) reported that NAA promoted femaleness in Luffa acutangula, while Saeed and Akbar (1970) reported that IAA induced female flowers in watermelon.

In contrast to these reports of a feminizing effect of auxins, Champault (1969) used IAA and NAA to produce staminate flowers on newly formed female inflorescences of Mercurialis aunnua. These staminate flowers were morphologically identical to genetically male flowers.

As with gibberellins, much of the early work with auxins focused on application of the hormone to development of commercial breeding lines of cucumber. Laibach and Kribben (1950a,b) found that application of a 0.1% paste of either NAA, IAA, or 2,4-D to the leaf or petiole stump of young cucumbers caused increased numbers of pistillate flowers. Wittwer and Hillyer (1954) also found that spraying young cucumber seedlings with 100 ppm of NAA greatly increased the pistillate/staminate ratio of two cucumber varieties. Galun (1956) treated monoecious cucumber seeds with 10^{-3} to 10^{-1} ppm of NAA, causing a reduction in the number of nodes preceding the first pistillate flower. Vernalization of the seed had a similar effect.

Ito and Saito (1956a,b) found that application of 2,4-D, NAA, or IAA to cucumber seedlings following cotyledon expansion caused retardation of staminate flower formation and acceleration of pistillate flower formation. The same authors (1957b) also reported that 10 ppm of NAA increased pistillate flowers even under environmental conditions favoring staminate flowers. Galun (1959b) also reported increased femaleness of cucumber due to NAA and found that

monoecious and gynoecious plants responded differently to removal of young leaves, whereas mature leaves of monoecious plants contain a larger content of auxin inhibitor than mature leaves of gynoecious plants. Choudhury and Phatak (1960) also found that IAA and NAA at 100 ppm increased the number of female flowers in cucumber, but that at lower concentrations, IAA increased production of staminate flowers.

Galun et al. (1962,1963) cultured young monoecious floral buds at a morphologically bisexual stage and supplied either IAA or GA_3 in the medium. Ovaries developed only in IAA-supplemented media; GA_3 reduced the IAA effect. In experiments with field-grown cucumbers, Kubicki (1966b) sprayed monoecious plants with NAA or gibberellin. NAA checked development of male flowers and stimulated that of female flowers. Gibberellin was found to inhibit pistillate flower development, and counteracted the NAA effect. Alvim and Quinones (1967) confirmed that NAA caused increased pistillate flowers in cucumber, but found no influence of NAA on flower morphology and development.

The effects of auxins on cucumber discussed thus far have been on monoecious or gynoecious plants. However, Kubicki (1969c) induced a few pistillate flowers in an androecious line with IAA and induced perfect flowers at the expense of staminate flowers in a trimonoecious line using NAA (Kubicki, 1969e).

In muskmelon, the results of auxin application on sex expression are not as clear. Brantley and Warren (1960)

fou

dec

in

flo

flo

tha

mus

lit

sid

clo

org

2.

hav

pec

Kru

sex

the

inc

(19

fem

hes

sex

FEM

a ne

ffe

bole

found that under masculinizing long-day conditions, NAA decreased the number of both staminate and perfect flowers in andromonoecious muskmelon without inducing pistillate flowers and that NAA increased both staminate and perfect flowers under a short-day regime. Kubicki (1969f) noted that NAA induced more pistillate flowers in a monoecious muskmelon and more perfect flowers in an andromonoecious line, but these effects were attributed to NAA promotion of side shoots where these flowers occur. The author concluded that NAA had no specific influence per se on sex organ differentiation in muskmelon.

2. Anti-auxins and sex expression in plants.

Presumed inhibitors of auxin biosynthesis or action have also been found to affect flower sex expression, especially 2,3,5-triiodobenzoic acid (TIBA). Laibach and Kribben (1950a) reported that TIBA had no effect on cucumber sex expression, while Wittwer and Hillyer (1954) found that 25 ppm of TIBA sprayed on cucumber seedlings greatly increased the pistillate/staminate ratio. Ito and Saito (1956a), on the other hand, found that TIBA slightly retarded female flower formation in cucumber. Heslop-Harrison and Heslop-Harrison (1957) reported no effect of TIBA on hemp sex expression while Choudhury and Babel (1969) found that TIBA greatly increased the number of pistillate flowers in a monoecious line of the cucurbit bottle gourd. Thus, TIBA effects are not consistent and shed little light on the role of auxins in sex expression.

3. Endogenous auxin and sex expression.

As with gibberellins, there have been few studies of endogenous auxin content of female versus male lines of the same species. Galun (1959b) found no significant difference in auxin content between monoecious and gynoecious cucumber lines, although an auxin inhibitor appeared to occur in higher concentrations in the monoecious line. Hashizume (1960) reported that the content of auxin-like substances of Cryptomeris japonica decreased in flower-bearing portions of the plant during flower initiation, but increased in flowers during growth. He found a higher auxin content in female than in male flower-bearing portions during the sex differentiation period. Conrad and Mothes (1961) found approximately thirty-fold more auxin in female than in male hemp plants. Galun et al. (1965) found about twice as much IAA in a hermaphroditic cucumber compared to a closely-related andromonoecious line. Thus, some evidence exists for a role for endogenous auxin in female sex expression in these plants, but the evidence is not as strong as for the role of gibberellin in male sex expression.

F. Effects of Ethylene on Sex Expression

1. Ethrel and sex expression.

The effects of ethylene on plant growth have been widely noted and the compound has been recognized as a phytohormone, perhaps arising in vivo from enzymatic oxidation of methional (Yang, 1967). Effects of ethylene on plant sex expression have been widely investigated, usually by means

of exogenous application of the presumed ethylene-generating compound 2-chloroethylphosphonic acid (Ethrel, CEPHA, Ethephon).

Several reports of ethylene evolution from Ethrel lend support to attributing its effects on plant growth to ethylene. Cooke and Randall (1968) found that basic aqueous media cause slow ethylene evolution from Ethrel at room temperature and rapid, quantitative release at 75°C. Ethylene is also evolved when Ethrel is added to extracts of etiolated pea seedlings (Warner and Leopold, 1969). Yang (1969) reviewed some proposed mechanisms for water or base catalyzed Ethrel decomposition. Shannon and De la Guardia (1969) applied Ethrel to cucumber plants and found that increasing amounts of ethylene were given off with increasing Ethrel dosage.

Ethrel effects on sex expression have been observed in several species. Rudich et al. (1969a) found that application of Ethrel to monoecious squash (Cucurbita pepo L.) and andromonoecious muskmelon increased the number of pistillate flowers (squash) and perfect flowers (melon) and decreased staminate flower production in both. Pistillate flowers occurred at normally staminate lower nodes in squash and perfect flowers occurred on the main axis of the muskmelon where, normally, only staminate flowers are found. The same authors (1970) also found that treatment of squash and muskmelon seedlings with Ethrel completely inhibited staminate flower production for the first three weeks of the growing period. Karchi (1970) determined that Ethrel, unlike auxin, was effective in promoting female sex expression in

muskmelon. In andromonoecious, monoecious, and hermaphroditic lines Ethrel promoted pistillate and inhibited staminate flowers. Pistillate flowers were produced in the andromonoecious and hermaphroditic lines and perfect flowers were initiated in the monoecious line.

Coyne (1970) and Splittstoesser (1970) reported that application of Ethrel to Cucurbita moschata varieties led to an increased production of pistillate flowers; Splittstoesser also found that Ethrel counteracted the masculinizing effect of gibberellin. Robinson et al. (1970) found that four cultivated Cucurbita species, C. pepo L., C. maxima Duch., C. moschata Duch. ex Poir, and C. mixta Pang., responded to Ethrel treatment by developing only pistillate flowers for extended periods. Ethrel treatment was not successful in inducing pistillate flowers in vegetative plants of several wild Cucurbita species. The only wild species responding to Ethrel were those which flowered at an early stage of development when untreated. This indicates that Ethrel does not influence floral initiation in cucurbits, but rather influences development after initiation. Ram et al. (1970) found that Ethrel sprayed on male hemp plants induced over ninety percent female or bisexual flowers. The plants reverted to staminate flowering after six weeks.

Interest in alteration of cucumber sex expression with Ethrel has produced several reports in the last two years. McMurray and Miller (1968, 1969) found that 120 ppm of Ethrel applied to monoecious plants resulted in as many as

nineteen continuous pistillate nodes. Rudich et al. (1969, 1970) reported that Ethrel application to monoecious plants at the second leaf stage inhibited staminate flower production for the first three weeks of the flowering period and stimulated pistillate flower formation on normally staminate nodes. Similarly, Robinson et al. (1969) found that cucumbers sprayed at the first or third leaf stage produced only pistillate flowers. Stamen formation was inhibited and the authors postulated an anti-gibberellin role for Ethrel. Lower and Miller (1969) reported similar results for monoecious cucumber and other cucurbits at the first leaf stage. Iwahori et al. (1969, 1970) also noted that 50 ppm Ethrel applied at the first leaf stage was effective in producing earlier and larger numbers of female flowers in cucumber.

2. Effects of free ethylene.

Ethylene itself has occasionally been used to alter flowering. The earliest report was by Rodriguez (1932) who found that ethylene gas induced flowering in pineapple under otherwise non-inductive conditions. Iwahori et al. (1970) found that 50 ppm of ethylene induced female flowers in monoecious cucumber.

These reports provide evidence for a feminizing effect of ethylene on plants and indicate that endogenous ethylene may also play a role as a plant sex hormone. Studies on endogenous ethylene levels versus sex expression are not available at this time.

The question of the role of auxins and ethylene in sex

expression is complicated by indications that auxin application gives rise to ethylene production in plants. Thus, effects attributed to auxin may, in fact, be due to ethylene. Zimmerman and Wilcoxon (1935) felt that auxin application might stimulate plants to evolve ethylene. Abeles and Rubinstein (1964) found that rates of ethylene evolution were closely allied with auxin levels in nine species, including pea, bean, maize, and tobacco, and hypothesized that acceleration of abscission by NAA or IAA might be due to evolved ethylene. However, in apple and pear trees with ripening fruit, NAA was found to decrease ethylene evolution. Burg and Burg (1966) noted that IAA induced ethylene evolution in a variety of stem sections. Ethylene gas was found to produce effects on the sections which had previously been attributed to auxin. Fuchs and Lieberman (1968) found that IAA enhanced ethylene evolution from Alaska pea seedlings, but gibberellin had no effect. Shannon and De la Guardia (1969) demonstrated that application of either IAA or NAA to young cucumber seedlings led to increased ethylene evolution with increased auxin dosage.

G. Cytokinins and Sex Expression

Sex expression in some plants has been altered by exogenous application of cytokinins, but in all reports to date, synthetic cytokinins, rather than the naturally occurring phytohormones, have been used. The first report of sex modification was by Catarino (1964) who demonstrated that 500 ppm of kinetin applied to inflorescences of

Kalanchoe crenata stimulated development of the calyx and gynoecium while inhibiting development of corolla and androecium. Durand (1966) found that daily spraying of Mercurialis annua with one ppm of kinetin transformed staminate flowers into pistillate flowers. The inflorescence structure was not modified. IAA and gibberellin abolished the kinetin effect but had no positive effect of their own. Negi and Olmo (1966) and Gargiulo (1968) found that the synthetic cytokinin 6-(benzylamino)-9-(tetrahydropyran-2-yl)-9H-purine converted male flowers of a grape vine to perfect flowers. Negi and Olmo also found that indole-butyrate, TIBA, CCC, β-naphthoxyacetate, IAA, NAA, and GA_3 had no effect on the flowers. Bose and Nitsch (1970) found that N^6-benzyladenine and 1-(m-chlorophenyl)-3-phenylurea strongly stimulated formation of female flowers in L. acutangula even under long days.

In contrast to these reports of a feminizing effect of cytokinins, Abdel-Gawad and Ketellapper (1968) reported inhibition of pistillate flowers in squash with N^6-benzyladenine. This is the only known report of cytokinin-induced sex alteration in cucurbits. However, a role for cytokinins in cucurbit sex expression appears possible since endogenous cytokinins have been identified in seeds of two species. Gupta and Maheshwari (1970) found that extracts of pumpkin (Cucurbita pepo L.) seeds contain three cytokinins, one of which may be zeatin. Extracts of immature watermelon seeds also contain three cytokinins,

possibly including zeatin and zeatin ribotide (Prakash and Maheshwari, 1970).

As with auxins, cytokinin effects on plants may be involved with ethylene production. Abeles et al. (1967) found that high concentrations of either kinetin or N^6-benzyladenine applied to bean explants doubled ethylene production. Fuchs and Lieberman (1968) reported that 10^{-8} to 10^{-4} M kinetin also stimulated ethylene production in Alaska pea seedlings.

H. Effects of Various Other Chemicals on Sex Expression

Abscisic acid (ABA), the newest phytohormone, has not been reported to alter sex expression in plants. However, ABA often counteracts the growth-promoting effects of gibberellins, auxins, and cytokinins, and ABA treatment enhanced production of ethylene by Citrus leaves (Cooper et al., 1968) and bean explants (Abeles, 1967). In view of these effects, ABA may also affect sex expression.

Several other compounds are also known to affect sex expression in plants. Some of these effects are probably "pharmacological" and not related to hormone levels; effects of other compounds such as maleic hydrazide (MH) may be due to a role as hormonal antagonists. Naugoljnyh (1955) reported that seeds treated with methylthionine chloride produced far more pistillate flowers than did the controls. Mehanik (1958a,b) found that acetylene gas greatly stimulated pistillate flower formation in young cucumber plants. Weston (1960) observed induction of

staminate flowers in genetically gynoecious hop (Humulus lupulis L.) plants following treatment with α-(2-chlorophenylthio)propionic acid. Lange (1961) used 2,3-dichloroisobutyrate and 2,2-dichloropropionate to induce pistillate flowers on two hermaphroditic papaya lines. Colchicine has been reported to feminize hemp plants (Wichert-Kolus, 1969). Bose and Nitsch (1970) found that morphactin completely inhibited pistillate and strongly increased staminate flower formation in Luffa acutangula.

Hillyer and Wittwer (1959) reported complete suppression of staminate flowers in acorn squash with MH. Choudhury and Phatak (1959) and Prasad et al. (1966) obtained increased numbers of pistillate flowers on monoecious cucumber with MH, while Prasad and Tyagi (1963) obtained the same effect of MH on bitter gourds.

I. Effects of Grafting on Sex Expression

Grafting of a scion of one sex type onto a stock of another sex type has been found to induce sex reversal in the scion and/or stock. Limberk (1954) induced sex reversal of staminate hop stocks with pistillate scions while Mockaitis and Kivilaan (1964) induced staminate flowers in gynoecious muskmelon scions with andromonoecious muskmelon and monoecious pumpkin rootstocks. Rowe (1969) induced small numbers of staminate flowers in gynoecious muskmelon scions with pumpkin, squash, monoecious and gynoecious cucumber, and andromonoecious and hermaphroditic muskmelon rootstocks. The induced staminate flowers were

not normal, containing a rudimentary stigma. The effect of pumpkin rootstocks was duplicated by use of pumpkin interstocks, indicating that the stimulus for staminate flower formation came from the pumpkin leaves rather than the roots. The sex reversal effects of grafting are presumably due to transport of a plant growth regulator, perhaps one of the phytohormones, from stock to affected scion.

J. The Occurrence of "Bound" or "Conjugated" Hormones

Findings that endogenous phytohormones are often associated with carbohydrates, amino acids, or other compounds has led to a new class of phytohormones, the "bound" or "conjugated" hormones. Gibberellins, in particular, have been found over the last decade to occur in 'aqueous-soluble' fractions of plant extracts, indicating complexing with polar compounds, or in neutral fractions, indicating conjugation of the gibberellin carboxyl group. More recently, some of these "bound" gibberellins have been isolated and identified.

Hayashi et al. (1962a,b) found that the neutral fraction of methanol extracts of potato tubers contained gibberellin-like compounds but these were not isolated or identified. Ogawa (1966a) and Hashimoto and Rappaport (1966a) reported neutral gibberellin-like substances in Sechium edule seeds and bean seeds. Pegg (1966) reported gibberellin-like substances with basic and neutral properties in tomato seeds and seedlings.

Water- or n-butanol-soluble gibberellin-like substances have been found in developing seeds of Sechium edule

(Ogawa, 1966a), in seeds of Japanese morning glory (Pharbitis nil), lupine, and peach (Ogawa, 1966b), in Pharbitis purpurea (Reinhard and Sacher, 1967a,b), and in developing bean seeds (Hashimoto and Rappaport, 1966a). Murakami (1962) found water-soluble gibberellins, including possibly a GA_3 glucoside, in seeds of Japanese morning glory and Japanese wisteria. Sembdner et al. (1964) found several free acidic gibberellins and five other gibberellin-like substances in Phaseolus coccineus. One of the latter, on acid hydrolysis, yielded gibberellic acid and carbohydrates as well as ninhydrin-positive compounds. Sembdner and Schreiber (1965) found three polar gibberellins in shoot apices and flower buds of tobacco. On acid hydrolysis, one of these yielded gibberic acid (GA_3 breakdown product), sugars, and various amino acids including the carboxyl-reactive serine and methionine.

Evidence for the presence of conjugated gibberellins has also come from enzymatic release of gibberellin from plant tissues and extracts. The earliest report was by McComb (1961) who found about six times as much gibberellin activity in ficin-hydrolyzed runner bean extracts than in non-ficin treated extracts. Jones (1964) obtained release of increased amounts of gibberellins from seeds of Zea mays and Phaseolus multiflorus with ficin while Pegg (1966) used ficin to liberate unique gibberellins and increased total gibberellin from tomato seed and seedling proteins. Reinhard and Sacher (1967c) used emulsin and ficin to release

gibberellins A_8, A_5, and A_3 from seeds and fruit walls of Pharbitis purpurea, while Jones (1968) obtained release of gibberellins from pea tissue with ficin. Hayashi et al. (1971) obtained increased amounts of gibberellin after hydrolysis of cucumber extracts with ficin and β-glucosidase.

The occurrence of conjugated gibberellins has also been studied by following incorporation of exogenously applied gibberellins into other gibberellin-like substances. Murakami (1961) found that applied GA_3 was incorporated and converted by young cucumber leaf disks into a butanol-soluble compound which produced GA_3 and glucose upon hydrolysis with β-glucosidase. The same phenomenon was observed with tissue sections from several other plants including peanut, sweet potato, and pear. Hashimoto and Rappaport (1966b) found that applied GA_1 induced a large increase in the neutral gibberellin-like fraction of bean seeds. Kende (1967) found that physiological concentrations of ^3H-GA_1 applied to dwarf pea induced label into an acidic gibberellin-like compound and several neutral compounds. No lasting attachment of the GA_1 to macromolecules was observed. Barendse et al.(1968) injected ^3H-GA_1 into excised fruit of pea and morning glory and followed the label during development of the seeds. A large part of the label became associated with the aqueous fraction, with two new acidic compounds, and with neutral compounds which gave labelled gibberellin A_1 on mild acidic hydrolysis.

The structures of relatively few conjugated gibberellins have been elucidated. The source materials have often been

seeds, where concentrations of these novel gibberellins are often particularly high. Schreiber et al. (1966) isolated 2-O-acetyl-GA_3[3] from the mold Gibberella fujikuroi. 3-O-β-D-glucopyranosyl-GA_8 was isolated from both immature and mature seeds of Phaseolus coccineus (Sembdner et al., 1968; Schreiber et al., 1967; Sembdner, 1970) and from immature seeds of Pharbitis nil (Yokota et al., 1969a,b). A GA_8 glucoside has also been isolated from shoot apices of Althaea rosea (Harada and Yokota, 1970). 2-O-β-D-glucopyranosyl-GA_3 was also found in immature seeds of Pharbitis nil (Tamura et al., 1968; Yokota et al., 1969a; Yokota et al., 1969b), as well as the 3-O-β-D-glucopyranosides of GA_{26} and GA_{27} (Yokota et al., 1969a,b).

The role of conjugated gibberellins in plant metabolism remains unclear but some evidence exists for a storage function in the case of seeds. Radley (1958) reported that free gibberellins in bean seeds fall drastically with increasing maturity. Hashimoto and Rappaport (1966a) reported an increase in butanol-soluble and neutral fraction gibberellins during bean seed development. Sembdner et al. (1968) found that GA_8 glucoside increased during bean seed development while free gibberellins decreased. Only the glucoside was present in dry mature seeds. They also noted that maturing bean seeds incorporated labelled gibberellins A_3 and A_6 into A_8 and A_3 glucosides. Reinhard and Sacher (1967a) reported an increased amount of bound gibberellin

---

[3] gibbane numbering system used for conjugated gibberellins

in both seed and fruit wall of Pharbitis purpurea during development.

There have also been reports that conjugated gibberellins were converted into free gibberellins during seed germination in tomato (Pegg, 1966), pea and morning glory (Barendse et al., 1968), Althaea rosea (Harada and Yokota, 1970), and bean (Sembdner et al., 1968). Aung et al. (1969) found that transfer of tulip bulbs from 13° to 18°C, favorable for shoot and root growth, resulted in a rapid decrease in the level of bound gibberellins together with an increase in free gibberellins.

Among the phytohormones the occurrence of conjugated forms is not unique to the gibberellins. Andreae and Good (1955) obtained incorporation of exogenous IAA into IAA-aspartate in pea seedlings and Klämbt (1960) isolated indole-3-acetylaspartate from three species of plants. Klämbt (1961) and Zenk (1962) obtained incorporation of labelled IAA into indoleacetylglucosides in wheat coleoptiles and Colchicum neapolitanum, respectively, and Varga and Bito (1968) found IAA-glucose conjugates in bean hypocotyl tissue. Indoleacetylinositol esters have been isolated from Zea mays (Bandurski et al., 1969; Nicholls, 1967; Labarca et al., 1965). Winter and Thimann (1966) found that labelled IAA became bound to a protein in Avena coleoptiles. Koshimizu et al. (1968) isolated a β-D-glucopyranoside of abscisic acid from immature seeds of Lupinus luteus. The roles of these IAA and ABA conjugates are unclear although storage and membrane transport functions have been postulated.

MATERIALS AND METHODS

Background of Plant Material

1. Cucumber (*Cucumis sativus* L.)

Gynoecious, monoecious, and andromonoecious lines were used. The gynoecious line MSU 713-5, developed by Peterson (1960), is homozygous for gynoecious sex expression under normal field and greenhouse conditions. It was developed from a cross between a gynoecious segregate of the Korean variety 'Shogoin' (PI 220860) and the monoecious pickling variety 'Wisconsin SMR 18'. Two generations of inbreeding were achieved by self-pollinating predominately female segregates. Three additional self-pollinated generations were obtained by inducing staminate flowers in strictly gynoecious plants with gibberellin (Peterson and Anhder, 1960).

The monoecious line used, MSU 736, is predominately staminate-flower bearing and was chosen for high male intensity. Only staminate flowers are produced on the first flower-bearing nodes under greenhouse conditions. The andromonoecious variety 'Lemon' was obtained from Dessert Seed Co.

2. Muskmelon (*Cucumis melo* L.)

Gynoecious, andromonoecious, monoecious, and hermaphroditic lines were used. All lines were obtained by Dr. L. R. Baker, Dept. of Horticulture, M. S. U. or from commercial sources.

The inbred gynoecious line MSU 1G was derived from an F_2 gynoecious segregate of a monoecious x hermaphroditic cross and was increased by self-pollination after induction of staminate flowers by grafting onto pumpkin (Rowe, 1969).

The original source for the hermaphroditic line, MSU 3897, was received from Dr. B. Kubicki, Institute of Plant Genetics, Poland. The monoecious line, MSU 3898, was derived from a cross between the variety 'Iroquois' and a monoecious strain received from the Plant Introduction Service of the U. S. D. A. The cross was made by Dr. H. M. Munger, Cornell University. The andromonoecious line used was the commercial variety 'Rocky Ford'.

3. Pumpkin (Cucurbita pepo L.)

The two pumpkin varieties tested for gibberellin content were 'Small Sugar', W. Atlee Burpee Co., and 'Small Sugar Pie', Vaughn Seed Co. Both varieties successfully induce staminate flowers on gynoecious C. melo (MSU 1G) scions when used as either rootstock or interstock in grafts.

Growth, Care, and Harvesting of Plant Materials

1. Cultural conditions, etiolated C. sativus seedlings.

For many gibberellin extraction experiments, etiolated (dark-grown) seedlings at various growth stages were utilized. This prevented interference with gibberellin extraction and assay procedures by excessive amounts of chlorophyll and other pigments.

Generally, 30 g of seed (ca. 1100 seeds) were placed in a plastic pot, covered with cheesecloth, and soaked in running

tap water at room temperature. When necessary (MSU 736 seed) fungicide-treated seeds were washed for five seconds in 75% ethanol to remove the fungicide (Captan) which interfered with bioassays. These seeds were then washed with water and placed in pots as above. After soaking overnight, the water was drained off; the seeds transferred to glass crystallization dishes (approximately 7.5 g/dish), and again covered with cheesecloth. Five ml distilled water was added to each dish; the dishes were placed in plastic pans containing one cm of water into which the cheesecloth extended to maintain high humidity. Water was added as needed to maintain growth. The germinated seeds were grown in absolute darkness at a constant temperature of 27°C and harvested at growth stages from one to twenty-two days.

For gibberellin extraction of ungerminated seeds, dry seeds were used, after washing (if necessary) with 75% ethanol. Seeds soaked overnight were considered to be germinated.

C. melo seeds were treated in the same fashion. Dark-grown C. melo seedlings were not used due to limited material.

2. Cultural conditions, greenhouse-grown plants.

Germinated C. sativus seeds were placed in six-inch diameter clay pots filled with sterilized sandy soil enriched with peat moss and black top soil. Several seeds were planted per pot and the seedlings thinned as necessary to three plants per pot. The plants were watered daily. Banks of flourescent light were used to provide at least twelve

hours of light per day; temperature was maintained at or above 25°C. The vines were trellised about sticks placed in each pot. Plants were harvested at various growth stages by uprooting the entire plant and washing it free of soil. Entire plants were then used for gibberellin extracts.

Only one growth stage of C. melo plants was extracted due to limited amounts of seed of the gynoecious and hermaphroditic lines. This plant material was grown under conditions similar to the above, except that raised soil beds were used (ca. 1.5 sq. ft. per plant) rather than clay pots.

3. Cultural conditions, vernalized seeds.

In one set of experiments, monoecious and gynoecious C. sativus seeds were vernalized and then planted according to the procedure for greenhouse-grown plants. These plants were studied for any change in sex expression and gibberellin activity was measured at three growth stages.

Germinated seeds were placed between several layers of cheesecloth; the cheesecloth was placed between one inch layers of sterilized, acid-washed, white sand in plastic pans. The sand was moistened and the pans were covered, sprayed with mercuric chloride, and placed in the cold room. Temperature was maintained at 4°C for one month, after which seeds were recovered and planted in the greenhouse.

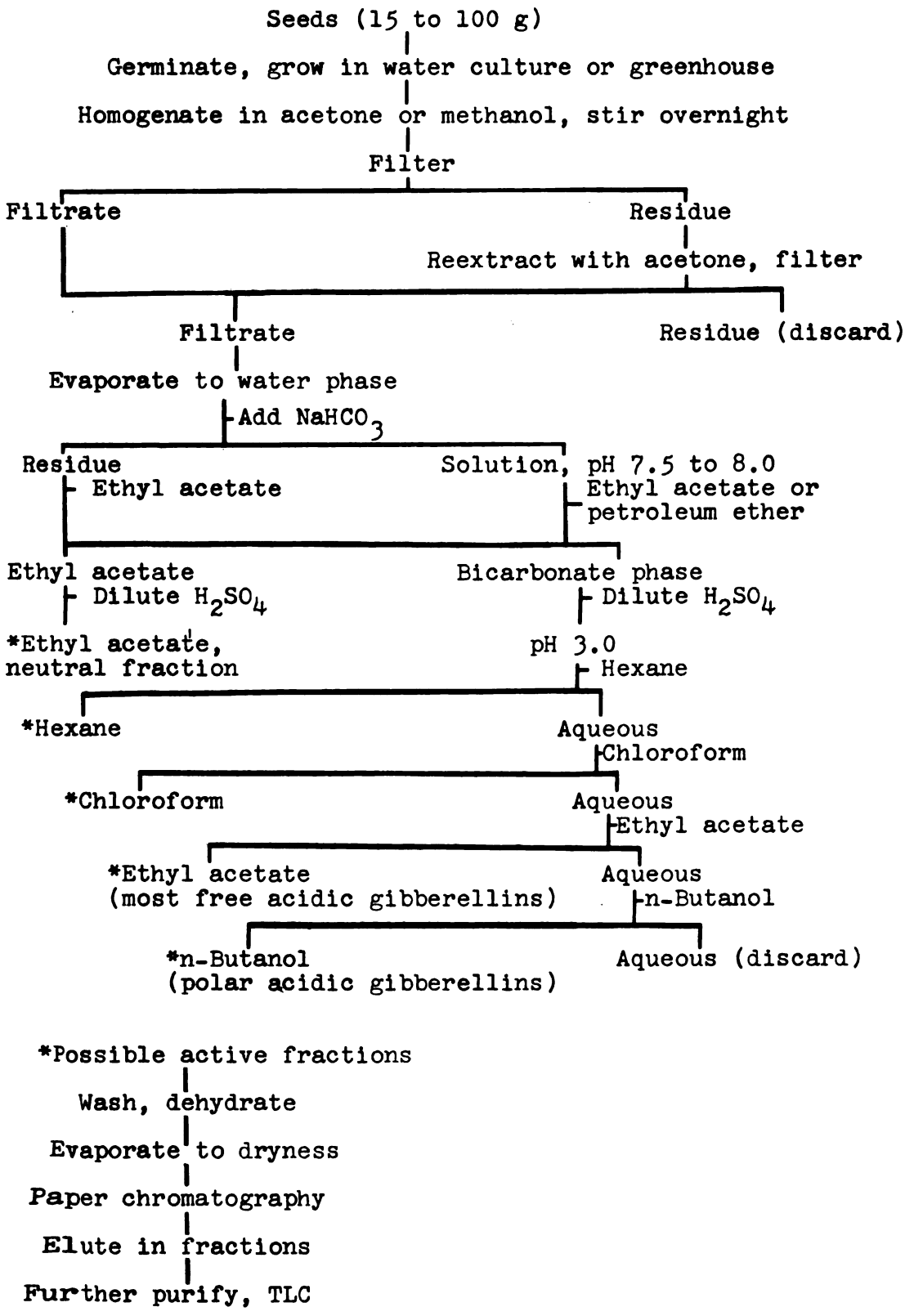
Gibberellin Extraction Procedures

1. Organic solvent extractions.

Seeds, etiolated seedlings, or entire green plants were homogenized for three minutes at 4°C with 90% aqueous acetone or 90% aqueous methanol in a Waring Blendor. The homogenate was stirred for twenty-four hours at 4°C, then filtered under vacuum. The residue was reextracted for twenty-four hours with acetone or methanol and again filtered. The extracts were combined, evaporated to dryness, and 5% sodium bicarbonate was added. After repeated attempts to solubilize the material, any residue was dissolved in ethyl acetate. The sodium bicarbonate solution was extracted with ethyl acetate to remove oils, pigments, neutral gibberellins and other neutral and basic compounds. This fraction was combined with the ethyl acetate-soluble residue left after sodium bicarbonate extraction. The aqueous layer, containing acidic gibberellins, was adjusted to pH 3.0 with dilute sulfuric acid and extracted with hexane. This hexane layer was washed with distilled water, dried over anhydrous sodium sulfate, and evaporated to dryness. The aqueous layer was further partitioned against chloroform, ethyl acetate, and n-butanol. Each organic layer was treated similarly to the hexane layer. The gummy residue of each fraction was further purified by chromatography. All solvents used in these extractions were redistilled prior to use and the residue left after evaporation of each solvent was bioassayed with negative results. Figure 3 is a flow sheet of the complete extraction procedure.

Figure 3

Flow sheet for organic solvent extraction of gibberellins from C. sativus and C. melo seeds and vegetative tissue.


Seeds (15 to 100 g)
Germinate, grow in water culture or greenhouse
Homogenate in acetone or methanol, stir overnight
Filter
Filtrate
Residue
Reextract with acetone, filter
Filtrate
Residue (discard)
Evaporate to water phase
Add NaHCO3
Residue
Ethyl acetate
Solution, pH 7.5 to 8.0
Ethyl acetate or petroleum ether
Ethyl acetate
Dilute H2SO4
Bicarbonate phase
Dilute H2SO4
*Ethyl acetate, neutral fraction
pH 3.0
Hexane
*Hexane
Aqueous
Chloroform
*Chloroform
Aqueous
Ethyl acetate
*Ethyl acetate (most free acidic gibberellins)
Aqueous
n-Butanol
*n-Butanol (polar acidic gibberellins)
Aqueous (discard)
*Possible active fractions
Wash, dehydrate
Evaporate to dryness
Paper chromatography
Elute in fractions
Further purify, TLC

2. Phosphate buffer extractions.

Organic solvent extractions proved unsatisfactory for green plants due to large amounts of chlorophyll which were difficult to completely remove. In partitioning, some gibberellin activity moved with the chlorophyll, and small residues interfered with bioassays. Phosphate buffers were used to extract acidic gibberellins from green plant material, avoiding solubilization of chlorophyll and plant lipids. Gibberellin activity recovered from phosphate buffer extracts of etiolated material or seeds did not vary significantly from the total amount recoverable from organic extracts.

Fresh plant material was homogenized for three minutes in a Waring Blendor at $4^{o}C$ with 0.1 M phosphate buffer, pH 6.0. The homogenate was stirred for twenty-four hours in the cold and then filtered through cheesecloth. The filtrate was centrifuged at 15,000 x g for thirty minutes. The pH of the buffered solution was adjusted to 3.0 with dilute sulfuric acid and the solution was extracted three times with equal volumes of ethyl acetate. The aqueous phase was discarded, unless bioassayed for polar gibberellin activity. The combined ethyl acetate-rich layers were reduced *in vacuo* to an aqueous residue of thirty to forty ml. This phase was readjusted to pH 3.0 and again extracted with three equal volumes of ethyl acetate. The combined ethyl acetate phases were reduced in volume, dried over anhydrous sodium sulfate, and evaporated to dryness. The

resi

cont

). I

bere

ml ph

tease

from

Incub

after

ml et

to dr

Part

1. P

resi

proce

were

or n

dilut

devel

(Dril

of th

evapo

thin-

2. Py

residue was further purified by chromatography. Figure 4 contains a flow sheet of the buffer extraction process.

3. Enzymatic release of "bound" gibberellins.

In order to free protein- or carbohydrate-bound gibberellins, seeds or plants were harvested as above in 150 ml phosphate buffer, pH 6.0. Twenty mg of either the protease ficin (Pierce Chemical Co.) or β-glucosidase (emulsin from Sigma Chemical Co.) were added to the buffered solution. Incubation was continued at 25°C for twenty-four hours after which enzyme activity was stopped by addition of 450 ml ethanol. The solutions were then filtered and evaporated to dryness or treated as above for phosphate buffer extracts.

Purification of Gibberellins from Crude Extracts

1. Paper chromatography.

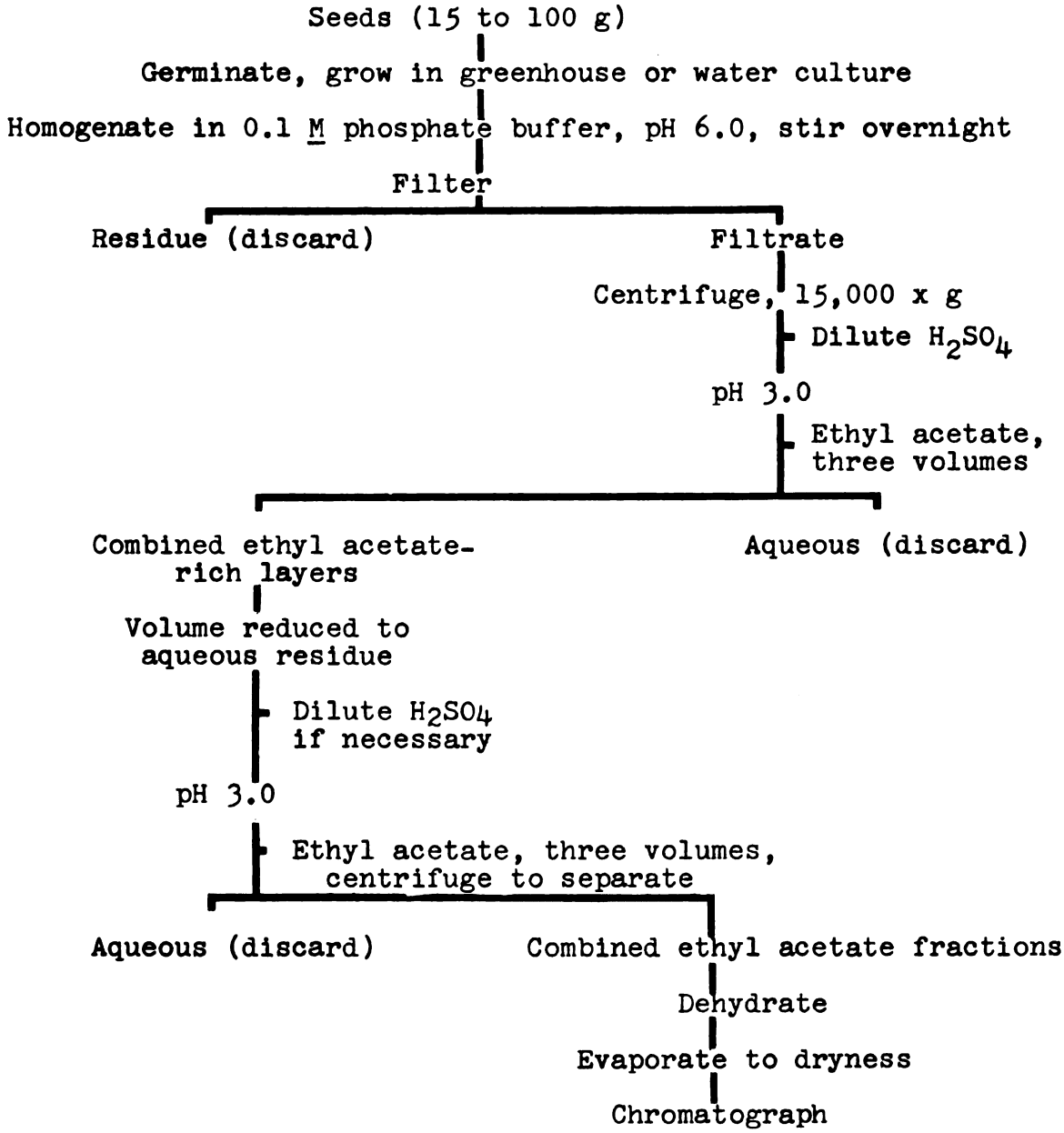
The first step in purification of all crude extract residues, whether from buffer or organic solvent extraction procedures, was usually paper chromatography. The residues were dissolved in a minimal amount of either ethyl acetate or n-butanol and applied as a thin streak to ethanol-washed Whatman 3 MM paper. The descending chromatograms were developed with isopropanol-ammonium hydroxide-water (10:1:1, v/v). The active compounds were eluted from strips of the chromatograms with 90% ethanol. The eluates were evaporated to dryness, bioassayed, and further purified by thin-layer chromatography.

2. Preparative thin-layer chromatography.

Generally, for identification purposes, individual

Figure 4

Flow sheet for phosphate buffer extraction of gibberellins from C. sativus and C. melo seeds and vegetative tissue.


Seeds (15 to 100 g)
Germinate, grow in greenhouse or water culture
Homogenate in 0.1 M phosphate buffer, pH 6.0, stir overnight
Filter
Residue (discard)
Filtrate
Centrifuge, 15,000 x g
Dilute H_2SO_4
pH 3.0
Ethyl acetate, three volumes
Combined ethyl acetate-rich layers
Aqueous (discard)
Volume reduced to aqueous residue
Dilute H_2SO_4 if necessary
pH 3.0
Ethyl acetate, three volumes, centrifuge to separate
Aqueous (discard)
Combined ethyl acetate fractions
Dehydrate
Evaporate to dryness
Chromatograph

extracts were further purified by thin-layer chromatography (TLC). A wide assortment of TLC solvent systems was used. Silica Gel F_{254} and Silica Gel G pre-coated preparative plates were obtained from Brinkmann Instruments Inc. and, in addition, plates were prepared using a DESAGA-Brinkmann spread-coater. A typical purification for each of the three main types of gibberellin fractions is as follows.

a. TLC purification of free acidic gibberellins. Dried eluates from paper chromatograms were dissolved in small amounts of ethyl acetate or ethanol and a thin streak was made across the TLC plate. The chromatogram was developed with carbon tetrachloride-acetic acid-water (8:3:5, v/v), non-aqueous phase plus ten percent ethyl acetate. Gibberellins A_1, A_2, A_3, A_6, and A_8 do not migrate in this system, but much interfering material is removed. The residue at the origin and at R_f 0.5 to 0.7 (gibberellins A_4, A_7, and A_9) was eluted with ethanol and rechromatographed using benzene-n-butanol-acetic acid (70:25:5, v/v) as the solvent system. This chromatogram was eluted in fractions and the eluates evaporated. The gibberellin residues were further purified by recrystallization from ethyl acetate-petroleum ether.

When identifying gibberellins by TLC, the unknown and gibberellin standards were spotted on Silica Gel F_{254} plates having a thickness of 0.25 mm. Several different solvent systems were employed to separate different gibberellins. Developed plates were dried, sprayed with five

percent alcoholic sulfuric acid, heated at 110°C for ten minutes, and viewed under ultraviolet light.

b. TLC purification of polar gibberellins. n-Butanol fractions of seeds were not purified by paper chromatography, but were applied directly to TLC plates. These were developed with chloroform-acetic acid-ethyl acetate (5:1:4, v/v) to remove interfering materials and then rechromatographed with isopropanol-5 N NH_3 (5:1, v/v) as solvent. The zones were eluted with n-butanol or ethanol and evaporated to dryness. One fraction at R_f 0.2 was active in bioassay. It was hydrolyzed with either 1 N sulfuric acid at 100°C for two hours or emulsin; the hydrolysate was applied to thin-layer chromatograms for indentification of the gibberellin conjugate. Isopropanol-acetic acid-water (3:1:1, v/v) and n-butanol-acetic acid-water (4:1:2, v/v) were used to determine the sugar residue of the conjugate. Chromatograms were sprayed with Folin-Ciocalteau reagent to reveal gibberellin spots and periodate-benzidine or α-naphthol-sulfuric acid to reveal sugar spots.

c. TLC purification of neutral gibberellins. TLC of neutral fraction gibberellins followed paper chromatography. The TLC solvents and techniques employed were the same as for the acidic gibberellins. Neutral gibberellins were crystallized from ethyl acetate-petroleum ether. Several solvent systems were used to compare the neutral gibberellins with acidic gibberellins: benzene-n-butanol-acetic acid (70:25:5, v/v) and chloroform-ethyl acetate-acetic acid (60:35:5, v/v) were particularly useful.

Bioassays

Bioassays were used to determine the gibberellin-like activity of various fractions of plant material. The dwarf pea assay was used as the routine screening assay while dwarf corn, cucumber hypocotyl, and barley half-seed amylase assays were also utilized to determine the amounts and types of gibberellins present.

1. Dwarf pea bioassay.

The dwarf pea technique is slow, requiring several days per assay, but sensitivity is high with 0.0001 μg gibberellin measurable. The assay is based on the method of Hayashi et al. (1962a) and was modified for use with cucumber and muskmelon extracts.

One hundred grams of dwarf pea seeds (Pisum sativum L., 'Morse's Progress No. 9', Ferry Morse Seed Co.) were soaked overnight in running tap water, planted between layers of moist vermiculite, and grown in the dark at 27°C for five days. Seedlings of approximately 2.5 cm height were then transplanted to water culture and irradiated with a bank of red lights (2000 ergs/in^2) placed two feet above the seedlings. After twenty-four hours a ten μl sample was applied to the apex of each seedling. Generally, ten seedlings were treated with each sample or standard. The treated seedlings were kept under red light at 25°C for five days. Response to treatment was determined by measuring the distance from cotyledonary node to highest visible node; average heights for standards, samples, and controls were then compared.

Samples were prepared for bioassay by dissolving each in one ml of 0.1% aqueous Tween-80 and one ml absolute ethanol. Further serial dilutions in a 50% alcoholic, 0.5% Tween-80 solution were made if necessary. Control plants were treated with 10 µl of the 50% alcoholic, 0.5% Tween-80 solution. Standard gibberellin samples were similarly treated. Figure 5 is a typical standard curve for this assay.

2. Dwarf corn bioassay.

This bioassay is also time consuming but is excellent for detecting gibberellins A_1, A_3, A_4, A_5, and A_7. The method is based on that of Phinney (1956) and the d_1 and d_5 dwarf corn used was obtained from Phinney. Both of these mutants have a single recessive dwarfing gene and the seedlings segregate 3 tall: 1 dwarf. Several times as many seeds as required for the bioassay were soaked overnight in running tap water and planted in autoclaved vermiculite in the greenhouse. Seeds were planted one-half inch deep in rows two inches apart. The temperature was kept near 30°C and the seedlings were watered daily. The day before treatment all tall plants were cut out and dwarfs were thinned to two cm apart if necessary. The plants were treated when about 3 cm tall (about six days after planting). Ten µl of a sample, prepared as for the dwarf pea bioassay, were applied to the primary leaf. Standards used were .001, .002, .02, .2, and 2 µg GA_3 per plant. Seven days after treatment the plants were uprooted

Figure 5

Standard curve for the dwarf pea bioassay. [GA_3 standard solutions of 10^{-4} to 10^{-1} mg/ml, 10 µl/plant; response measured as stem elongation relative to control]

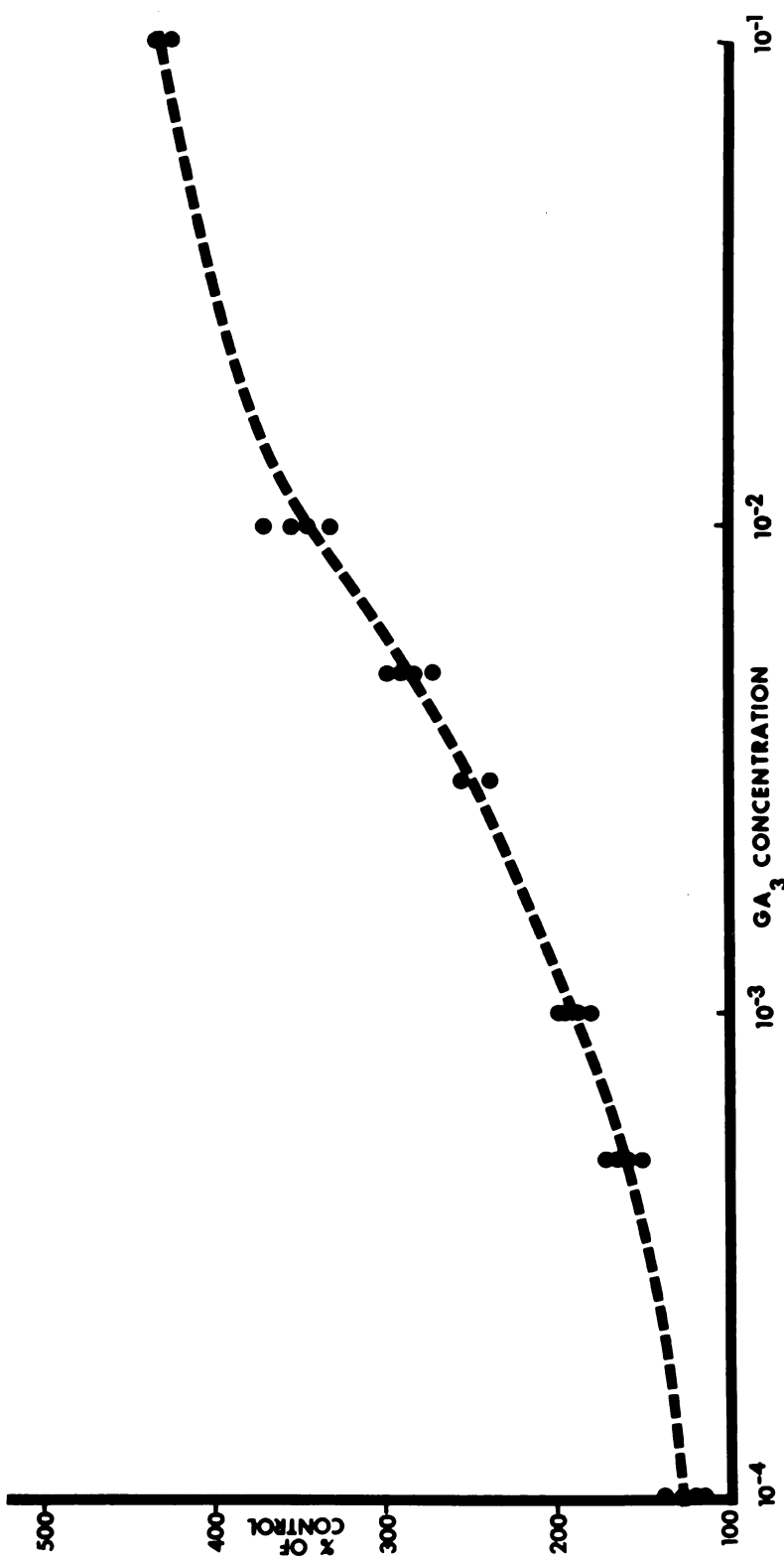

500
400
300
200
100
% OF CONTROL
10^{-4}
10^{-3}
10^{-2}
10^{-1}
GA_3 CONCENTRATION mg/ml

Figure 5

Figure 6

Standard curves for the dwarf corn bioassay. [gibberellin A_3 standard solutions of 10^{-4} to 10^{-1} mg/ml, 10 µl/plant; response measured as leaf sheath elongation relative to control]

broken line: d_1 corn

solid line: d_5 corn

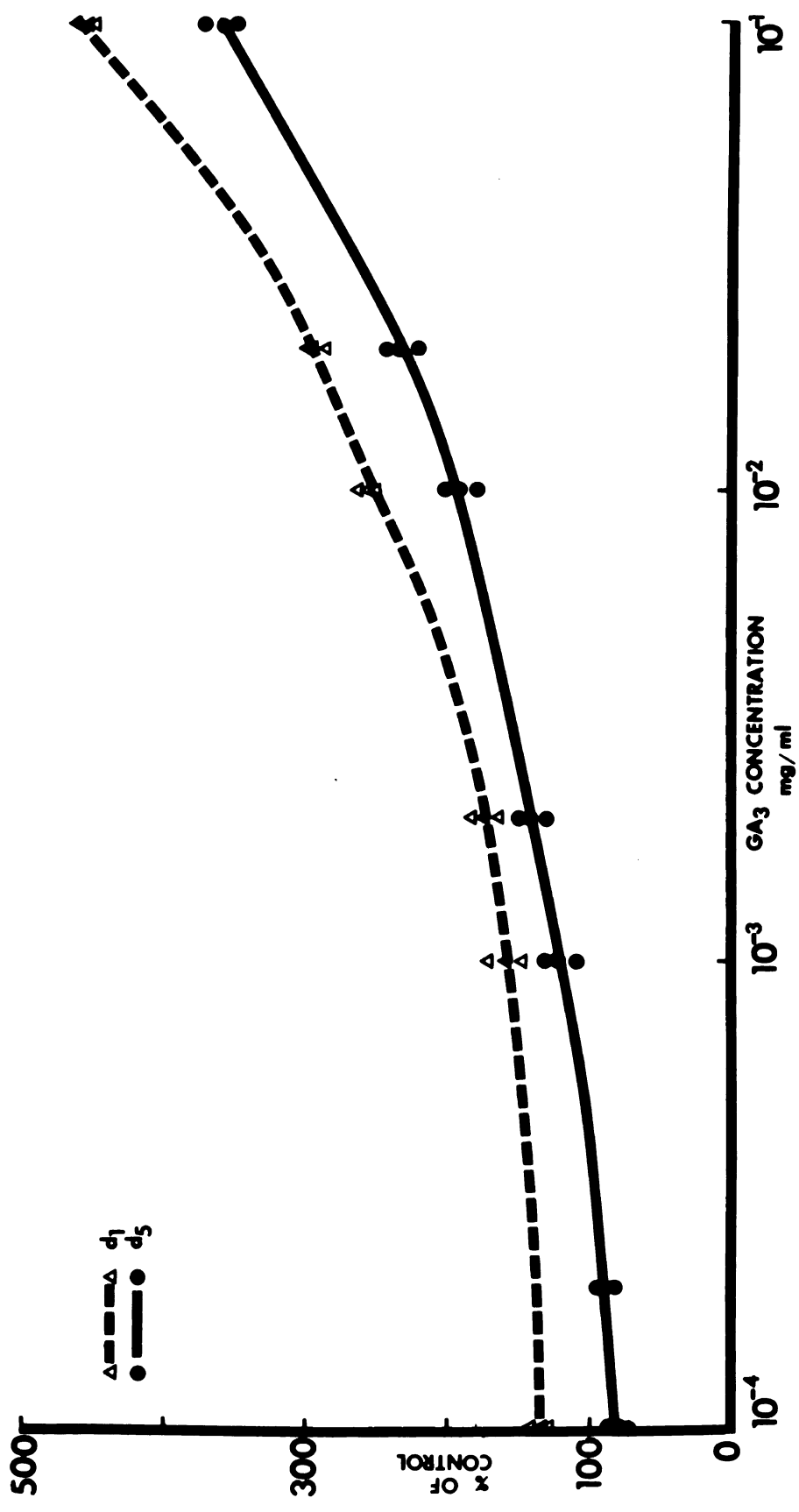

500
300
100
0
% OF CONTROL
d_1
d_5
10^{-4}
10^{-3}
10^{-2}
10^{-1}
GA_3 CONCENTRATION mg/ml

Figure 6

and the lengths of the first and second leaf sheaths measured. Figure 6 contains a typical standard curve for this assay.

3. Cucumber hypocotyl bioassay.

This assay employed monoecious C. sativus seedlings (line MSU 736) to detect gibberellins A_4, A_7, A_9, and A_{13}, the most active gibberellins in promoting cucumber hypocotyl elongation. The seeds were soaked overnight in running tap water, planted in vermiculite, and grown in growth chambers for five days at 25°C. Ten µl samples were applied to the apex. Biological activity was measured by hypocotyl elongation eight days after treatment. Figure 7 contains a bar graph of GA_3 and GA_7 standards in this assay.

4. Barley amylase assay.

The procedure of Jones and Varner (1967) was followed without modification. The assay is comparatively rapid and yields good quantitative results. Figure 8 contains a bar graph for GA_3 standards in this assay.

Instrumental Techniques

1. Preparation of samples for gas-liquid chromatography (GLC).

For GLC analysis, acidic gibberellins were first derivatized to increase their volatility. This was usually accomplished by methylation with diazomethane, although trimethylsilyl ethers were also used. Diazomethane was generated in small amounts as needed by trapping the gas generated from warm alcoholic KOH breakdown of Diazald (N-methyl-N-nitroso-p-toluenesulfonamide, Aldrich Chemical

Figure 7

Response to gibberellin standards in the cucumber hypocotyl bioassay. [10^{-3} to 1 μg gibberellin/ seedling; response measured as hypocotyl elongation relative to control]

solid line: GA_7

broken line: GA_3

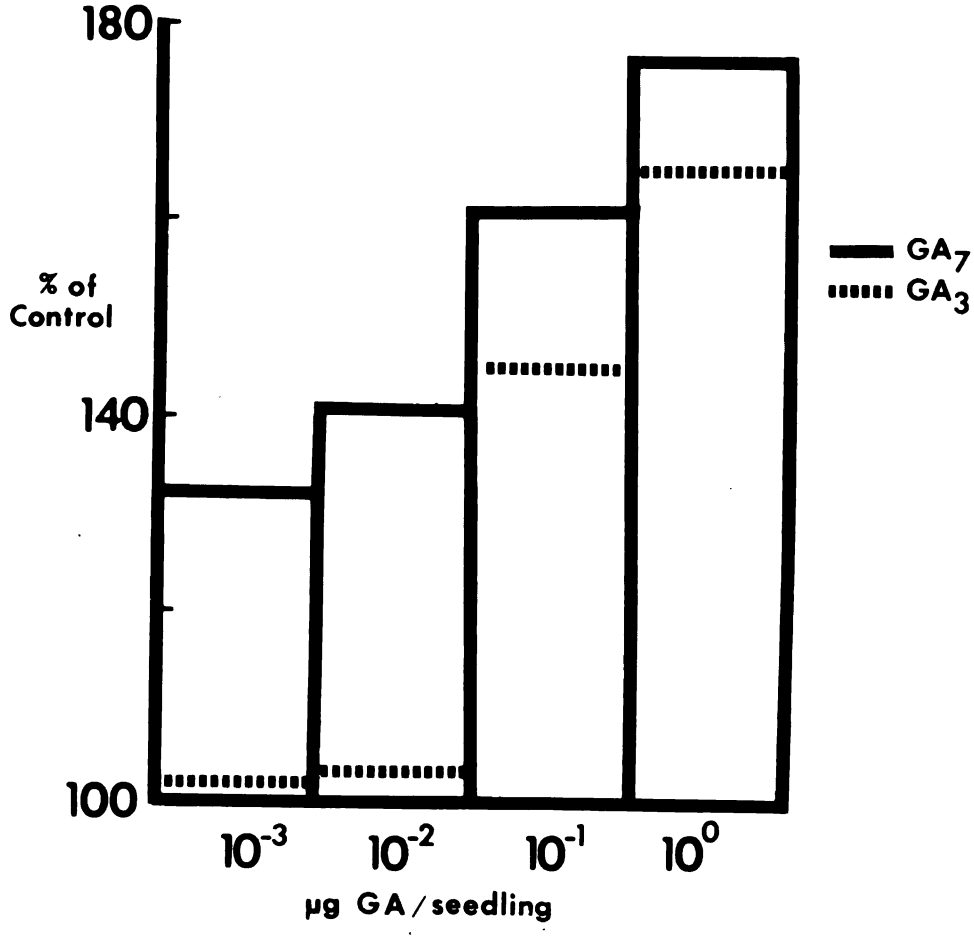


Figure 7

Figure 8

Response to GA_3 standards in the barley aleurone amylase bioassay. [10^{-3} to 1 μg GA_3 per sample]

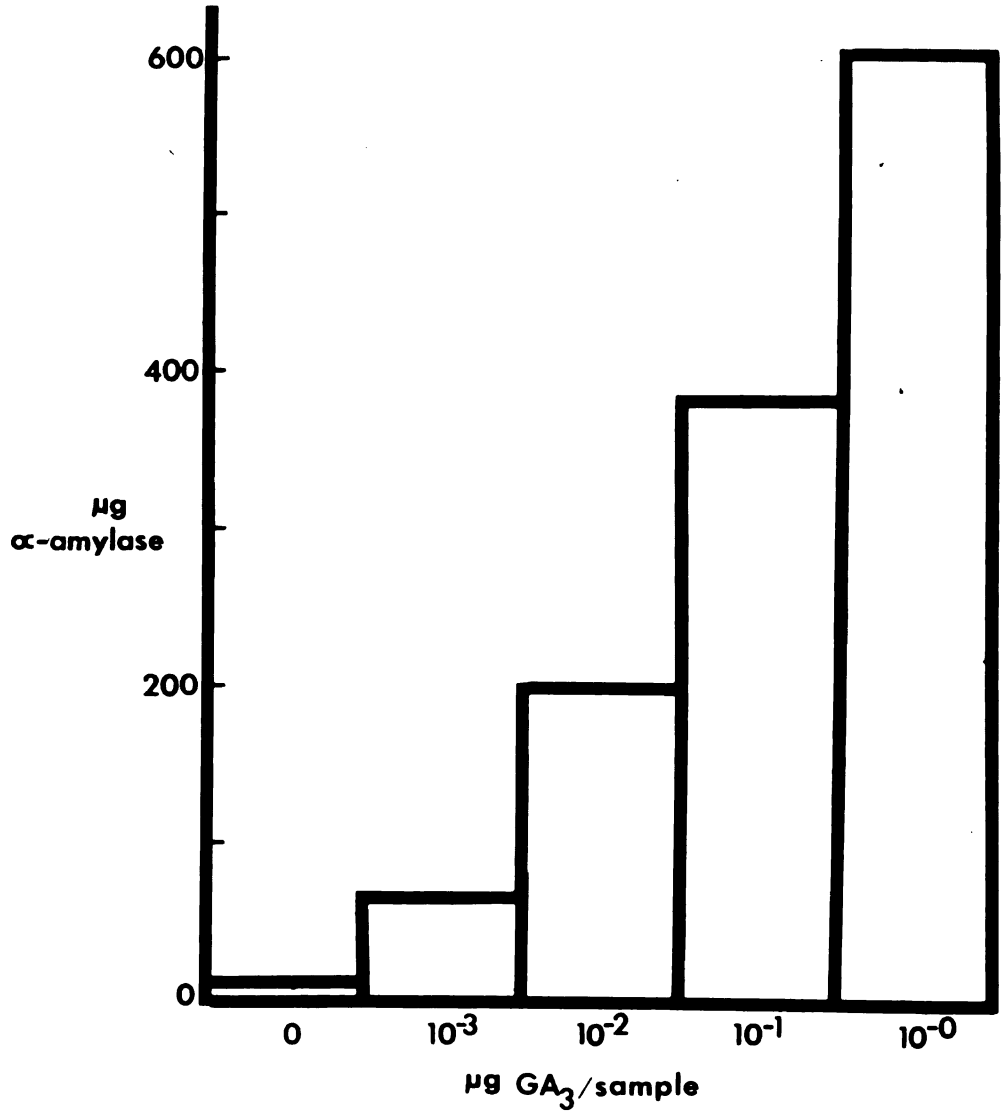


Figure 8

Co.) in cold ether. The purified gibberellin sample was added to the diazomethane-ether solution and stored overnight in the cold. The solution was then evaporated to dryness and dissolved in ethanol.

2. GLC conditions.

Gibberellin methyl esters were determined on an F and M Model 400 Gas-Liquid Chromatograph equipped with hydrogen flame ionization detector and pyrex glass U column one meter long and 4.5 mm in diameter. The column packings were either 3.0% OV-1 (methyl silicone polymer, Applied Science Laboratory) or 1.5% QF-1 (fluorinated alkyl silicone polymer, D. C.) on 70-80 mesh chromasorb (Analabs, Inc.). Column temperature was 210°C or 180° to 225° programmed at 2° per minute. Retention times for the gibberellin esters were 5 to 30 minutes.

3. Preparation of samples for mass spectrometry.

Partially purified samples, particularly of neutral fraction gibberellins, were prepared for GLC-mas spectrometry by silylation to form trimethylsilyl ester-ethers. Samples were dissolved in anhydrous pyridine and an excess of bis-trimethylsilyltrifluoroacetamide (Applied Science Laboratory) was added. Samples were allowed to stand for 15 minutes before introduction to the GLC column. Highly purified samples were not derivatized and direct probe mass spectrometry was used.

4. Mass spectrometry conditions.

Mass spectra were obtained with the assistance of

Dr. Ray Hammond and Mr. Jack Harten, Dept. of Biochemistry, using an LKB 9000 Gas Chromatograph-Mass Spectrometer (LKB Produktor, Stockholm). The LKB 9000 consists of a gas chromatograph and single-focusing 60° magnetic sector mass spectrometer directly coupled to Becker-Ryhage type molecular separators. For direct probe analysis, the instrument was operated at 3500 volts, 70 eV electron beam energy, 60 μA electron current, and an ion source temperature of 190°C.

5. Infrared Analyses.

The infrared spectra of certain purified gibberellins from both C. melo and C. sativus were obtained on a Perkin-Elmer 337 Grating Infrared Spectrophotometer. The compounds were analyzed in the form of potassium bromide pellets made from either 250 mg potassium bromide and 2 mg of sample in 12 mm dies or, for small amounts of material, 20 mg potassium bromide and 0.5 mg sample in 5 mm dies. Each sample of dried infrared-grade potassium bromide and gibberellin was thoroughly mixed in a dental amalgamator ('Wig-l-bug', Crescent Dental Manufacturing Co.) using glass beads as the muller. The sample was then placed in a Beckman pellet press and subjected to a force of 20,000 lb. in vacuo with a Carver hydraulic press. All samples were run against potassium bromide pellet blanks.

RESULTS

I. Gibberellin Activity in C. sativus: Seeds and Etiolated Seedlings

Hayashi et al. (1971) determined the free acidic gibberellin activity as well as ficin- and emulsin-releasable gibberellin activity in etiolated monoecious and gynoecious C. sativus seedlings. The first section of results presented below consists of an extension of Hayashi's experiments with etiolated monoecious and gynoecious seedlings to include ungerminated seeds and further growth stages of etiolated seedlings. In addition, sufficient amounts of andromonoecious seed became available during this research to permit similar gibberellin extraction experiments with these seeds and etiolated andromonoecious seedlings.

Table 1 is a summary of the gibberellin-like activity in phosphate buffer extracts of dry andromonoecious, monoecious, and gynoecious C. sativus seeds as determined by dwarf pea bioassay. The results of assays on three separate extractions are presented. Averages are from the three separate determinations. Free acidic gibberellins as well as ficin- and β-glucosidase-releasable gibberellins were measured. Table 1 also contains results of cucumber hypocotyl and barley amylase bioassays of one extract of each sex type, intended as a check on the dwarf pea

Table 1

Gibberellin activity in phosphate buffer extracts of andromonoecious, monoecious, and gynoecious *C. sativus* seed (dry). [dwarf pea, cucumber hypocotyl[1], and barley amylase bioassays; 200 g seed per experiment; expressed in $\mu g\ GA_3/kg$ seed]

Sex type and form of GA measured	Dwarf pea expt. no. 1	2	3	mean	Cucumber hypocotyl	Barley amylase
Andromonoecious						
Free acidic GA	2.1	2.4	1.6	2.0	0.2	2.5
Enzyme-releasable-GA[2]	6.8	7.0	7.1	7.0	-	7.0
Total GA	8.9	9.4	8.7	9.0	-	9.5
Monoecious						
Free acidic GA	2.0	2.4	1.9	2.1	0.2	2.5
Enzyme-releasable-GA	8.0	7.9	8.0	8.0	-	8.4
Total GA	10.0	10.3	9.9	10.1	-	10.9
Gynoecious						
Free acidic GA	1.5	1.6	1.5	1.5	0.1	1.6
Enzyme-releasable-GA	5.0	4.8	5.2	5.0	-	5.3
Total GA	6.5	6.4	6.7	6.5	-	6.9

[1]Measure of $GA_{4/7}$ activity in GA_7 equivalents

[2]Released after 24 hour incubation with ficin and emulsin

bioassay and an aid in identifying the types of gibberellins present. Only free gibberellins were measured in the cucumber hypocotyl assay.

Activity of the extracts was measured relative to GA_3 standards and represents activity in GA_3 equivalents. The response of dwarf pea to gibberellin standards is as follows: $A_3>A_7>A_1>A_5>A_4>A_6=A_9>A_8$. Most other gibberellins are relatively inactive. The cucumber hypocotyl assay permits detection of those gibberellins which lack a C-7 hydroxyl (A_4, A_7, A_9, and A_{13}, referred to below as less-polar gibberellins) independently of A_1 or A_3. At a concentration of 10^{-2} μg per cucumber seedling, A_4, A_7, A_9, and A_{13} are active whereas A_1 and A_3 do not induce a response (Figure 7). Relative activity of the gibberellins in the barley amylase assay is as follows: $A_1=A_3>A_2>A_7>A_4>A_5>A_6>A_9>A_8$. Hence, activity in this assay is mainly attributable to A_1 and A_3. These bioassays can give a strong indication of the class of gibberellins present in an extract. Thus, the cucumber hypocotyl assay (Table 1) indicates that the less-polar gibberellins are present in cucumber seed extracts to a limited extent. However, proof of their presence requires isolation and identification by other means.

Table 2 contains the results of dwarf pea bioassay of phosphate buffer extracts of etiolated andromonoecious C. sativus seedlings from zero to fourteen days old. Free and ficin- and β-glucosidase-releasable gibberellins

Table 2

Gibberellin activity in phosphate buffer extracts of etiolated andromonoecious C. sativus seedlings. [dwarf pea bioassay, zero to fourteen days old; cucumber hypocotyl and barley amylase bioassays of six day old seedlings; expressed in μg GA_3/kg seedling fresh weight]

Days after germination	Fresh weight (g)	Free acidic GA			Enzyme-released GA[1]			Total GA (mean)
		1	2	mean	1	2	mean	
0	30.0	5.9	5.8	5.9	4.1	3.9	4.0	9.9
1	30.2	5.9	5.6	5.8	9.0	7.3	8.1	13.9
2	38.6	8.4	9.0	8.7	14.8	12.7	13.8	22.5
4	41.5	21.2	22.0	21.6	19.0	18.7	18.9	40.5
6	60.4	40.6	41.6	41.1	19.6	14.3	17.0	58.1
10	69.7	34.1	33.2	33.7	35.7	39.1	37.4	71.1
14	73.5	33.0	30.9	32.0	36.9	39.6	38.3	70.3
6a[2]	60.4	2.9	-	-	-	-	-	-
6b[3]	60.4	42.5	-	-	18.2	-	-	60.5

[1]Released after 24 hour incubation with ficin and emulsin

[2]Cucumber hypocotyl assay, measure of $GA_{4/7}$ activity

[3]Barley amylase assay

Table 3

Gibberellin activity in phosphate buffer extracts of 22 day old etiolated andromonoecious, monoecious, and gynoecious C. sativus seedlings. [dwarf pea bioassay; expressed in μg GA_3/kg seedling fresh weight[1]]

Seedling sex type	Free acidic GA			Enzyme-released GA[2]			Total GA
	1	2	mean	1	2	mean	
Andromonoecious	29.4	29.7	29.6	42.8	41.5	42.2	71.7
Monoecious	28.1	30.2	29.2	43.8	42.3	43.1	72.3
Gynoecious	22.5	23.4	23.0	33.1	30.4	31.8	54.7

[1]Fresh weights: andromonoecious, 45.1 g; monoecious, 45.0 g; gynoecious, 41.1 g

[2]Released after 24 hour incubation with ficin and emulsin

were

amyl

day

free

also

mono

urge

CA_1

also

of c

twer

thus

prac

ext

C.

May

to

lin

see

ber

see

fic

fre

mon

fro

were measured. Table 2 also contains the results of barley amylase and cucumber hypocotyl assay of extracts of six day old etiolated andromonoecious seedlings. A peak of free gibberellin activity occurs here; this growth stage also corresponds to the maximum free gibberellin level in monoecious seedlings (Hayashi et al., 1971). As in ungerminated seeds most activity appears to be due to GA_1 and/or GA_3, but traces of the less-polar gibberellins also appear to be present. Table 3 contains the results of dwarf pea bioassay of phosphate buffer extracts of twenty-two day old etiolated seedlings of each sex type, thus extending experiments on etiolated seedlings to the practical limit for growth under these conditions.

Figure 9 compiles results for dwarf pea bioassay of extracts of seeds and etiolated seedlings of three C. sativus sex types. This Figure combines the data of Hayashi et al. (1971) with that of Tables 1, 2, and 3 to indicate the relationship among free and total gibberellin activity, growth stage, and sex type.

Andromonoecious and monoecious seeds and etiolated seedlings contained more free gibberellin and total gibberellin at all growth stages than gynoecious seeds and seedlings. Dry monoecious seeds contained about 8.0 μg/kg ficin- and emulsin-releasable gibberellin and 2.1 μg/kg free acidic gibberellin (GA_3 equivalents). In germinated monoecious seeds the amount of bound gibberellin decreased from 8.0 to 3.8 μg/kg with a corresponding increase in

Figure 9

Free acidic and total (free acidic plus enzyme-released) gibberellin activity in etiolated monoecious, andromonoecious, and gynoecious C. sativus seedlings at several growth stages. [dwarf pea bioassay, results expressed as μg GA_3/kg seedling fresh weight]


monoecious free


monoecious total


andromonoecious free


andromonoecious total


gynoecious free

gynoecious total

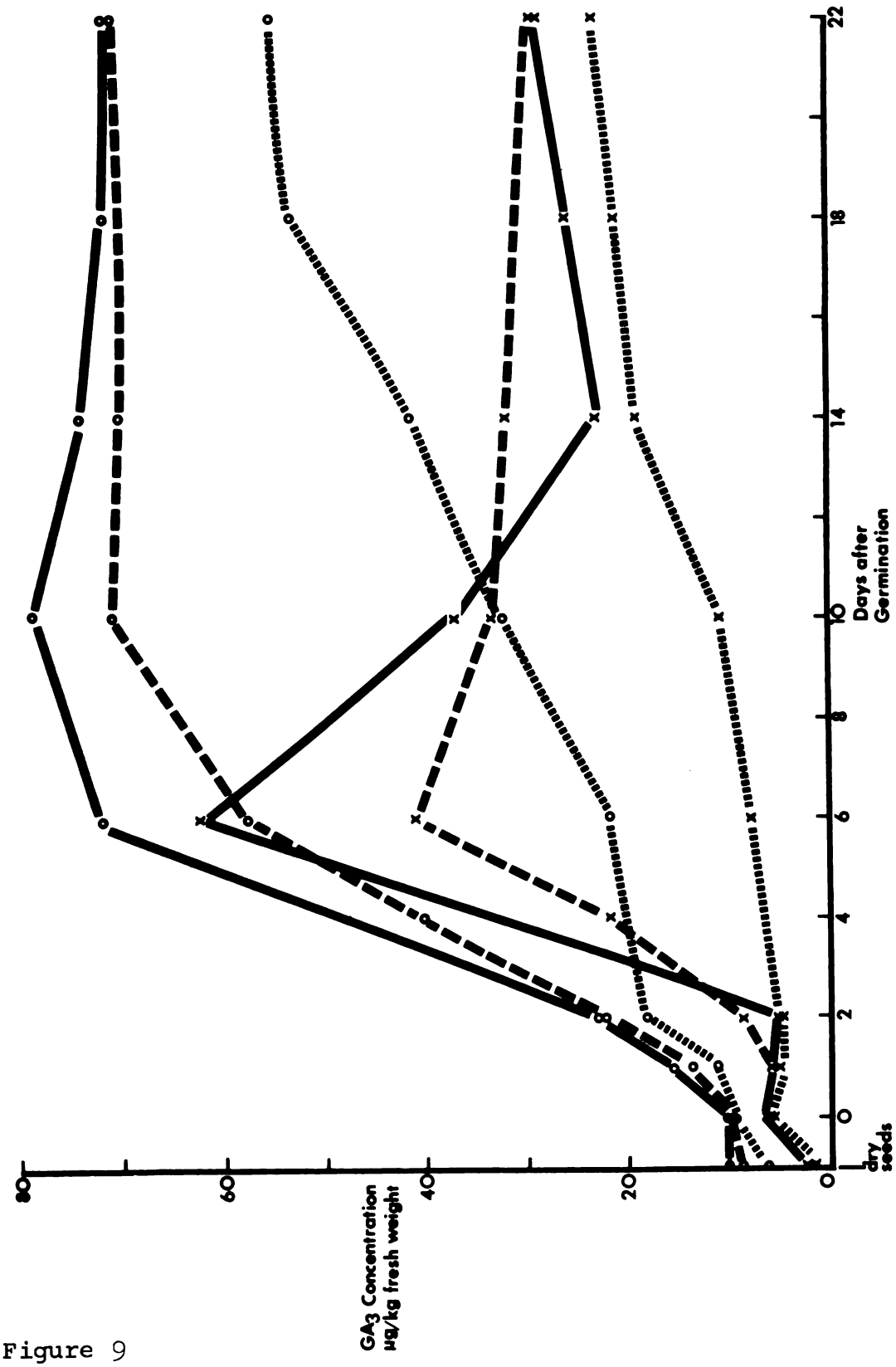

80
60
40
20
0
GA3 Concentration
µg/kg fresh weight
dry seeds
0
2
4
6
8
10
14
18
22
Days after Germination

Figure 9

free gibberellin. During seedling growth free gibberellin increased rapidly, reached a maximum after six days (63 μg/kg), then decreased and leveled off in older seedlings (25 to 30 μg/kg). The amount of ficin- and emulsin-releasable gibberellin slowly increased during seedling growth. The total gibberellin content increased rapidly to 72.7 μg/kg after six days and then stabilized.

The curves for gibberellin activity in andromonoecious seedlings are similar to those for monoecious seedlings although the levels were somewhat lower. Free acidic gibberellin also reached a maximum after six days.

In contrast, gibberellin in gynoecious seedlings was no more than half the amount in the more male sex types at corresponding growth stages. Free and enzyme-releasable gibberellin exhibited no peak of activity but continued to increase slowly during growth. At the six day stage, free acidic gibberellin activity in andromonoecious and monoecious seedlings was five and eight times, respectively, that in gynoecious seedlings. If the dwarf pea bioassay results had been expressed as μg GA per seed of starting material rather than μg GA per kg fresh weight, the differences would have been greater since the seedling fresh weight of the more male sex types increased more rapidly than did that of the gynoecious. Also, the relative order of gibberellin activity in ungerminated seeds, i.e. monoecious $\approx$ andromonoecious$>$gynoecious, was maintained throughout seedling growth (0 to 22 days).

II. Gibberellin Activity in C. sativus: Greenhouse-grown Plants

Extraction of gibberellins from dark grown seedlings of three C. sativus sex types strongly suggested that the gynoecious line contained considerably less free and total gibberellin activity than the monoecious and andromonoecious lines. Etiolated seedlings were primarily used to facilitate extraction and quantitation, but beyond the four to six day stage they may not represent an accurate picture of the gibberellin levels at corresponding growth stages in green plants (See Discussion). In order to avoid extrapolating results from etiolated seedlings to green plants, gibberellin activity in greenhouse-grown plants was measured. Since free acidic gibberellins proved to be a reliable indicator of total gibberellin content (Figure 9) in seeds and etiolated seedlings, enzyme-releasable gibberellins were not measured.

Table 4 and Figure 10 contain results of phosphate buffer extraction of gibberellins from one, two, four, and eight week old plants of each sex type. Figure 10 also incorporates the results for dry and germinated seeds. The gibberellin content (in μg/kg fresh weight) in one and two week old greenhouse-grown plants was only slightly higher than in etiolated seedlings at similar growth stages (Table 4). However, total free acidic gibberellin per plant was much higher since the fresh weight of light-grown plants far exceeds that of etiolated seedlings at the

Figure 10

Free acidic gibberellin content of greenhouse-grown monoecious, andromonoecious, and gynoecious C. sativus plants at several growth stages. [activity measured relative to GA_3 standards in dwarf pea bioassay; expressed as μg GA_3/kg fresh weight of seed or entire plant]

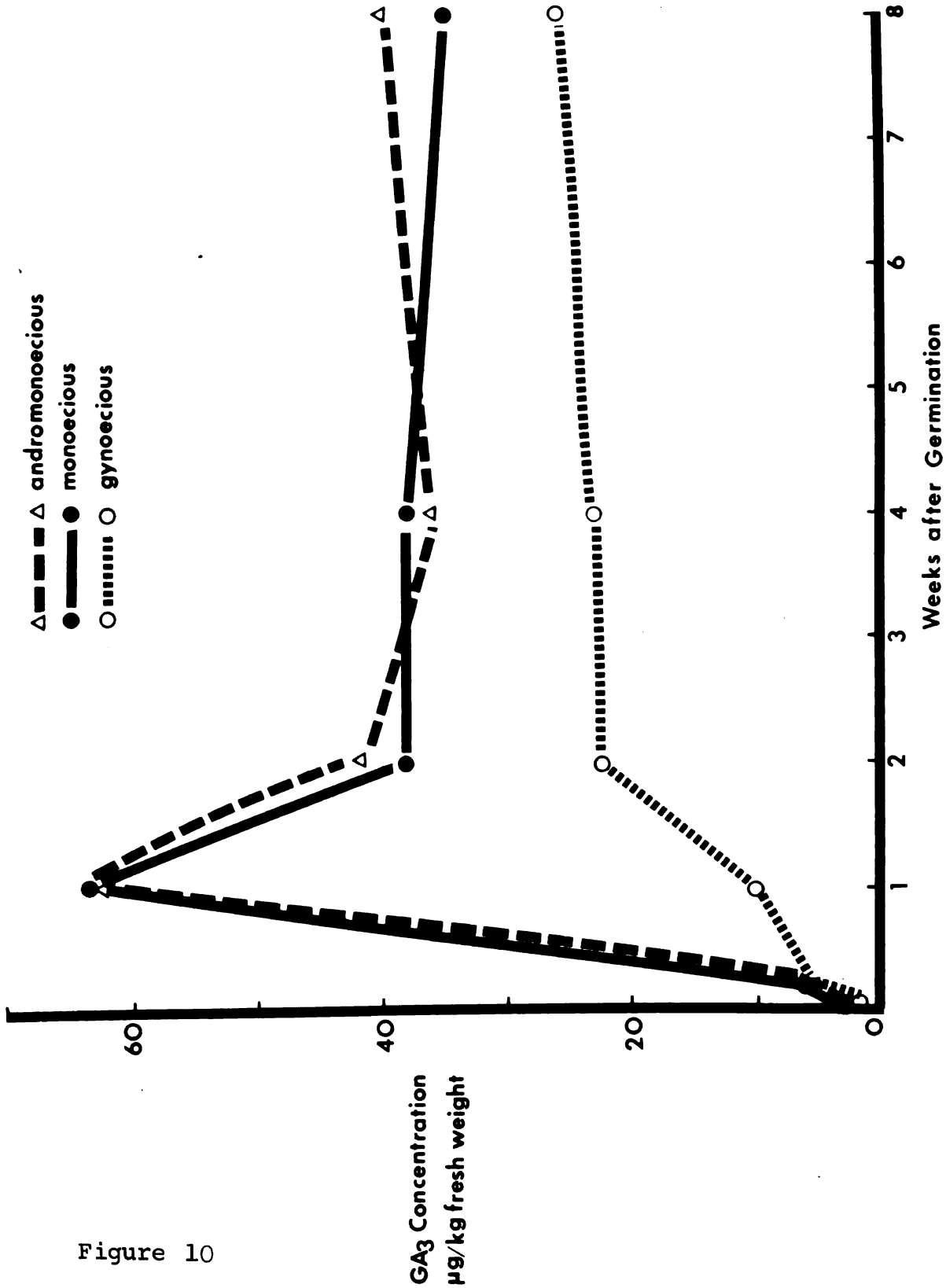

Δ andromonoecious
● monoecious
O gynoecious
60
40
20
0
GA3 Concentration
µg/kg fresh weight
1
2
3
4
5
6
7
8
Weeks after Germination

Figure 10

same stage. Gibberellin activity in monoecious and andromonoecious plants (Figure 10) increased rapidly to a peak at one week and then descended to a relatively stationary level from two to eight weeks. In contrast, the gynoecious plants showed no corresponding early peak in gibberellin activity and levels from two to eight weeks were only fifty to sixty percent of those in the more male plants. At one week the gynoecious plants contained only 16% as much gibberellin activity as the monoecious and andromonoecious plants. At this stage a rudimentary male flower bud is often observed on the cotyledonary node.

The results with greenhouse-grown plants were in all respects consistent with those for etiolated seedlings. Furthermore, the relative gibberellin levels in dry seeds were maintained throughout the test period for both greenhouse plants and etiolated seedlings, i.e. the gynoecious type contained lower levels of gibberellin activity compared to other sex types for seeds, germinated seeds, etiolated seedlings, and greenhouse-grown plants at all growth stages up to eight weeks. Thus, the gibberellin level in mature, dry seeds of a certain sex type may be a strong indicator of relative gibberellin levels in growing plants of that sex type.

Table 4.

Free acidic gibberellin activity in phosphate buffer extracts of greenhouse-grown monoecious, andromonoecious, and gynoecious C. sativus plants. [dwarf pea and dwarf corn bioassays; expressed in μg GA_3/kg fresh weight; 500 to 2000 g material extracted]

Sex type	Growth stage (wk)	Dwarf pea expt. no. 1	2	mean	Dwarf corn[1] d_1	d_5
Andromonoecious	1	64	61	63	62	63
	2	40	42	41	45	43
	4	38	33	36	33	39
	8	39	40	40	39	43
Monoecious	1	57	68	63	61	72
	2	39	37	38	41	40
	4	41	35	38	33	37
	8	38	32	35	34	37
Gynoecious	1	10	9	10	10	11
	2	21	22	22	22	25
	4	25	21	23	21	22
	8	26	26	26	28	29

[1]Same extract as for Dwarf pea expt. 2

III. Gibberellin Activity and Sex Expression of Chilled C. sativus Seeds

Greenhouse-grown gynoecious (MSU 713-5) plants produced from chilled, germinated seeds were observed to see if cold-treatment of seeds would affect sex expression. Table 5 lists the total number of flowers of each sex type, including aborted staminate flowers, for control and chilled plants at the eight week stage. Monoecious plants produced from chilled seed exhibited little change in either the total number of flowers or sex ratio compared to monoecious controls. In contrast, gynoecious plants produced from chilled seed produced fewer flowers, but an increased

proportion of normal and aborted staminate flowers relative to gynoecious controls. All staminate flowers occurred at early nodes; after six weeks no staminate flowers were produced. The gynoecious controls produced about three percent (of total flowers produced) aborted staminate flowers and no normal staminates, while the gynoecious plants from chilled seeds produced about 18% normal and aborted staminate flowers. At the end of six weeks nearly one-third of the flowers produced by the chilled gynoecious plants were staminate.

Table 5

Flower sex types in chilled and control C. sativus plants, monoecious and gynoecious lines. [m = staminate, f = pistillate, m_a = aborted staminate]

Plant no.	Monoecious		Gynoecious	
	control	chilled	control	chilled
1	7m	7m	$1m_a$, 6f	6f
2	14m	11m, 1f	5f	1m, 2f
3	13m	7m	8f	$3m_a$, 3f
4	9m	8m	$1m_a$, 8f	8f
5	12m	6m	8f	6f
6	7m	8m	5f	$3m_a$, 7f
7	7m	8m, 1f	4f	$1m_a$, 9f
8	6m	9m	11f	6f
9	8m	16m	$1m_a$, 10f	1m, 0f
10	6m, 2f	15m	10f	2m, 1f
11	12m	11m	12f	$1m_a$, 10f
12	7m	6m	10f	2m, 5f
Total:	108m, 2f	112m, 2f	$3m_a$, 97f	$8m_a$, 6m, 63f

Phosphate buffer extracts of chilled and non-chilled seeds and plants indicated that for the monoecious line, gibberellin activity in seeds and plants was unchanged (Table 6). However, chilling increased gibberellin activity slightly in gynoecious seeds. At the one week stage, a very significant three-fold increase in activity was noted. At the eight week stage, there was very little difference in gibberellin activity between chilled and non-chilled gynoecious plants.

Table 6

Effects of chilling of seeds on free acidic gibberellin activity in phosphate buffer extracts of monoecious and gynoecious lines of C. sativus. [dwarf pea bioassay, expressed as μg GA_3/kg fresh weight]

Sex type	Gibberellin Activity		
	germinated seeds	1 week plants	8 week plants
Monoecious control	7	65	35
Monoecious chilled	7	64	37
Gynoecious control	6	9	25
Gynoecious chilled	7	27	27

IV. Gibberellin Activity in C. melo

Seeds of four sex types of C. melo were available in sufficient quantity to measure gibberellin activity. However, only the andromonoecious type was available in sufficient quantity for experiments at several growth stages. Only one to two ounces of the monoecious and hermaphroditic types were available, so gibberellin activity was measured only in dry seeds and four week old greenhouse-grown plants. Dwarf pea and barley amylase assays were employed to

quantit

was em

D

and ne

-g/Ag

pea bi

these

monoec

gibber

C. sa

defici

G

gynoec

monoec

differ

C. me

old,

if an

of bo

With

sex t

may r

in th

V. G

Seed

2.9 μ

quantitate the gibberellins. The cucumber hypocotyl assay was employed to detect less-polar gibberellins.

Dry seeds of the relatively more female gynoecious and hermaphroditic lines were found to contain 43 and 40 μg/kg free acidic gibberellin, respectively, in the dwarf pea bioassay; the barley amylase assay results support these data (Table 7). In contrast, the more male andromonoecious and monoecious lines contained only about 7 μg/kg gibberellin. These results are the converse of those for C. sativus where the gynoecious line was gibberellin deficient.

Gibberellin activity in extracts of four week old gynoecious and hermaphroditic plants was higher than in monoecious and andromonoecious plants (Table 8), but the differences were not as pronounced as for the seeds. C. melo plants normally abort all flowers until five weeks old, but flower buds were developing at the four week stage.

Cucumber hypocotyl bioassays indicated very little, if any, activity due to less-polar gibberellins in extracts of both seeds and green plants. The small response seen with seeds and plants of the gynoecious and hermaphroditic sex types, although calculated relative to a GA_7 standard, may represent the slight activity of other gibberellins in this bioassay.

V. Gibberellin Activity in Pumpkin Seeds and Leaves

Methanol extracts of two different varieties of pumpkin seed and leaves of one variety contained 1.0, 1.1, and 2.9 μg/kg free acidic gibberellin, respectively (Table 9).

| Free
dry
typo
GA3/ |
| --- |
| Sex |
| Andr
Mono
Gyno
Herm |
| 1San
2Wea |
| Free
of f
type
bioa |
| Sex |
| Andr
Mono
Gyno
Herm |
| 1San
2Wea |
| Free
seed
one
in 4 |
| Vari |
| 1Smal
1Smal
1Smal |
| 1Five |

Table 7

Free acidic gibberellin activity in methanol extracts of dry seed of four C. melo sex types. [dwarf pea, cucumber hypocotyl, and barley amylase bioassays; expressed in μg GA_3/kg seed]

Sex type	Gibberellin Activity						
	Dwarf pea expt. no.					Barley amylase[1]	Cucumber hypocotyl[2]
	1	2	3	4	mean		
Andromonoecious	6.7	5.7	6.7	9.3	7.1	7.5	none
Monoecious	6.1	7.3	-	-	6.7	6.9	none
Gynoecious	47	33	46	45	43	40	0.1
Hermaphroditic	40	39	-	-	40	42	0.1

[1]Same extract as dwarf pea expt. 1
[2]Measure of $GA_4/_7$ activity (GA_7 equivalents)

Table 8

Free acidic gibberellin activity in phosphate buffer extracts of four week old greenhouse-grown C. melo plants of four sex types. [dwarf pea, barley amylase, and cucumber hypocotyl bioassays; expressed in μg GA_3/kg fresh weight]

Sex type	Gibberellin Activity				
	Dwarf pea expt. no.			Barley amylase[1]	Cucumber hypocotyl[2]
	1	2	mean		
Andromonoecious	9.0	13.2	11.1	12.3	none
Monoecious	14.1	12.5	13.3	14.0	none
Gynoecious	31.0	28.0	29.5	34.6	0.1
Hermaphroditic	20.2	21.7	21.0	22.2	0.1

[1]Same extract as dwarf pea expt. 1
[2]Measure of $GA_4/_7$ activity (GA_7 equivalents)

Table 9

Free acidic gibberellin activity in methanol extracts of seeds of two monoecious varieties of pumpkin and leaves of one variety. [dwarf pea and dwarf corn bioassays; expressed in μg GA_3/kg seed]

Variety	Gibberellin Activity			
	Dwarf pea expt. no.			Dwarf corn d_1
	1	2	mean	
'Small Sugar' seed	0.9	1.0	1.0	1.2
'Small Sugar Pie' seed	1.2	0.9	1.1	1.1
'Small Sugar' leaves[1]	3.2	2.5	2.9	-

[1]Five hundred and four g leaves from four week old plants

Thes

the

VI.

and

laye

spec

mcro

of g

A.

lin

gibb

bere

this

alco

gibb

prod

0.69

corr

spot

corr

B.

mixt

este

andro

These levels of gibberellin activity are far lower than the gibberellin activity in any muskmelon sex type.

VI. Identification of Free Acidic Gibberellins in C. sativus

Free acidic gibberellins in andromonoecious, monoecious, and gynoecious C. sativus seeds were determined by thin-layer chromatography, gas-liquid chromatography, and mass spectrometry. Figures are presented for extracts of andromonoecious seed, but the same types and relative proportions of gibberellins were found in the other two sex types.

A. Thin-layer chromatography.

A TLC plate comparing a purified free acidic gibberellin extract of andromonoecious C. sativus seeds with several gibberellin standards is represented in Figure 11. Gibberellins having a C-7 hydroxyl group (A_1, A_3, and A_5 in this Figure) do not develop dark spots when sprayed with alcoholic H_2SO_4; therefore, the dim yellow spots for these gibberellins were outlined. The andromonoecious extract produced spots at R_f values 0.69, 0.49, and 0.20. The R_f 0.69 spot corresponds closely with GA_4; the R_f 0.49 spot corresponds with GA_1 and/or GA_3. The very faint R_f 0.20 spot, evidently due to a highly polar compound, did not correspond with any of the standards.

B. Gas-liquid chromatography.

Figure 12 represents a gas-liquid chromatogram of a mixture of equal parts of GA_1, GA_3, GA_4, and GA_7 methyl esters and a methylated acidic gibberellin extract from andromonoecious seeds. Peaks with retention times of

Figure 11

Thin-layer chromatogram of gibberellin extract from 100 g of andromonoecious C. sativus seed. [plate: Brinkmann Pre-coated Silica Gel F_{254}, 0.25 mm thickness, sprayed with alcoholic H_2SO_4; authentic samples of gibberellins A_1, A_2, A_3, A_4, A_5, A_7, A_9 spotted for comparison; solvent system: benzene:n-butanol:acetic acid (70:25:5, v/v)]

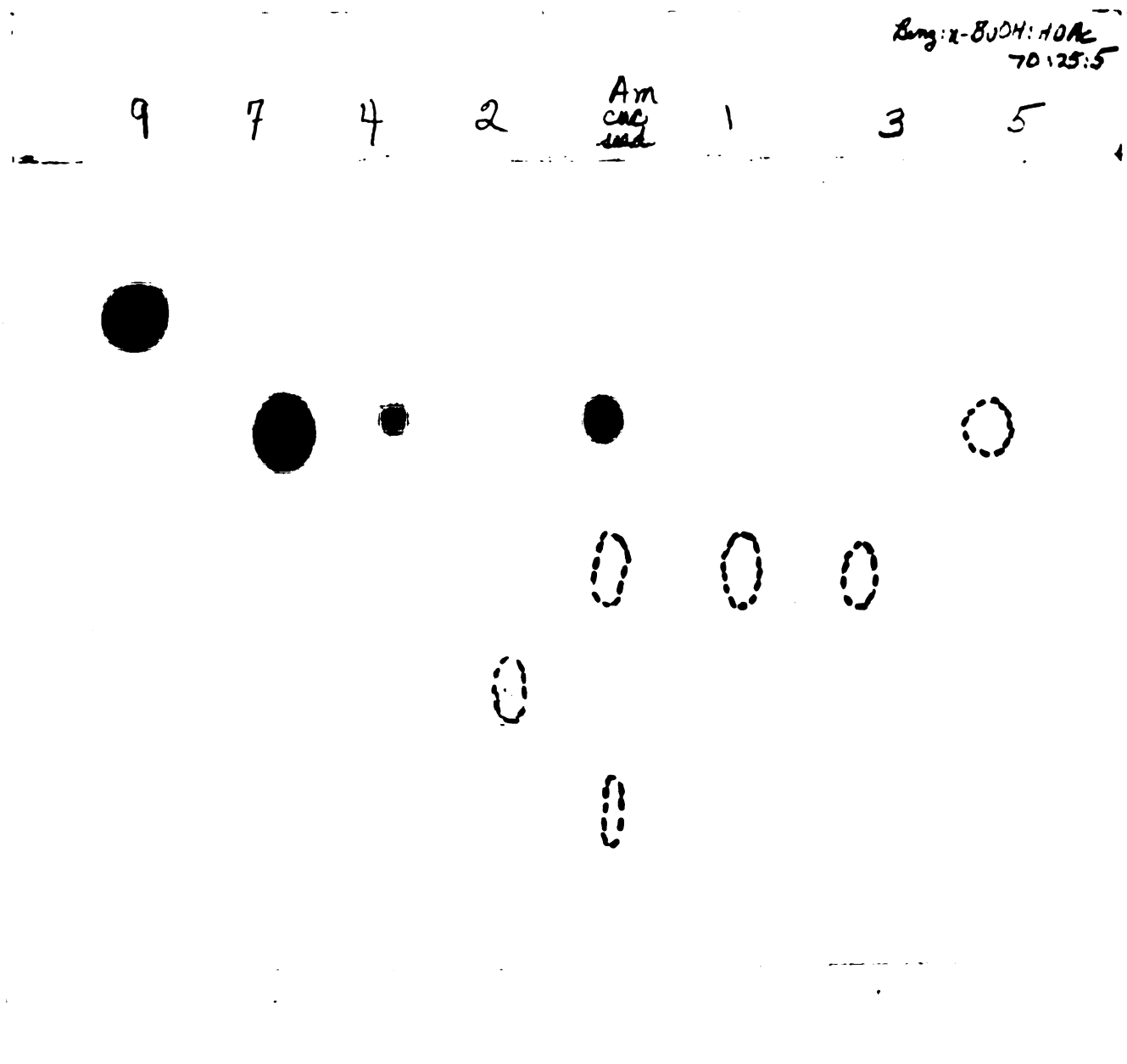


Figure 11

ca. 6, 7, 15, and 17.5 minutes correspond well with gibberellin A_4, A_7, A_1, and A_3 standards, respectively. The peak corresponding with GA_1 is the predominant compound present in the extract. Small peaks at 4 and 9.5 minutes do not correspond to any of the standards used.

C. Mass spectrometry.

Three highly purified eluates (labelled C_1, C_2, and C_3) from thin-layer chromatography of an andromonoecious extract, suspected to contain gibberellins A_1, A_3, A_4 and/or A_7, were crystallized and subjected to direct probe mass spectrometry. Figures 13, 14, and 15 contain the mass spectra of these samples. Table 10 contains an analysis of the major peaks in Figure 13 (C_1). The molecular ion mass (m/e 348) is identical to the molecular weight of GA_1. Published spectra of the free acidic gibberellin series are not available but the mass spectra of the methyl esters of gibberellins A_1 through A_{24} have been reported (Binks et al., 1969). The spectrum of Figure 13 corresponds very closely to that for authentic methyl A_1 if the necessary correction of 14 mass units is made: the spectra of Figure 13 and methyl A_1 both have prominent peaks at m/e 284, 261, 243, 237, 213, 201, 199, 185, 163, 135, 121, 109, 108, 107, 105, 95, 69, 67, 55, 43, and 41. Thus, the presence of GA_1 in the extract is confirmed.

Table 11 contains an analysis of the spectrum of Figure 14 (C_2). The molecular ion mass (m/e 346) is identical to the molecular weight of GA_3. This spectrum

Figure 12

Gas-liquid chromatogram of a methylated gibberellin extract of andromonoecious C. sativus seed (lower graph) and a mixture of methylated gibberellin standards (upper graph). [column: QF-1, 200°C; two μl of a 1% alcoholic solution of the extract and standards were injected]

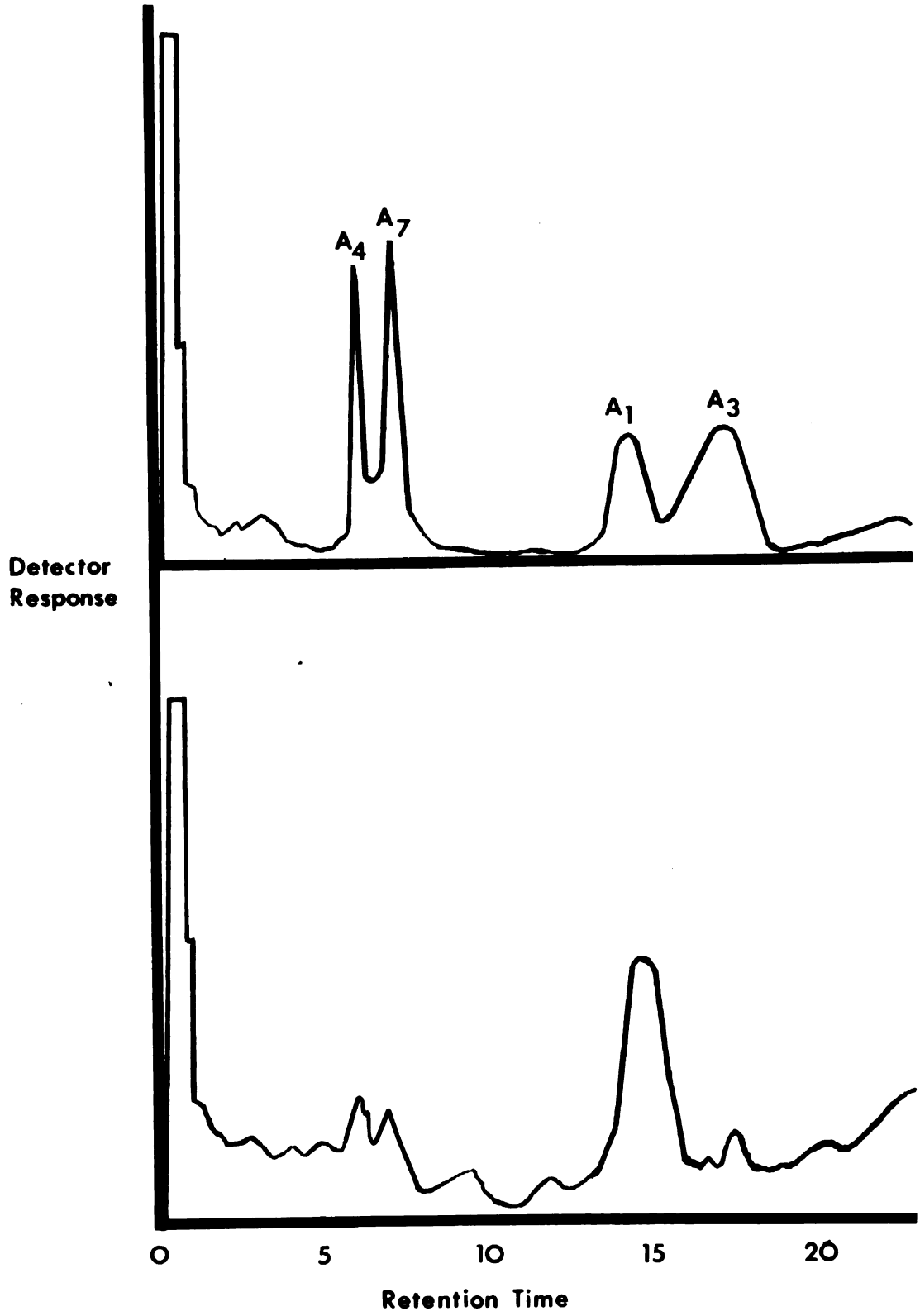


Figure 12

Figure 12

Gas-liquid chromatogram of a methylated gibberellin extract of andromonoecious C. sativus seed (lower graph) and a mixture of methylated gibberellin standards (upper graph). [column: QF-1, 200°C; two μl of a 1% alcoholic solution of the extract and standards were injected]

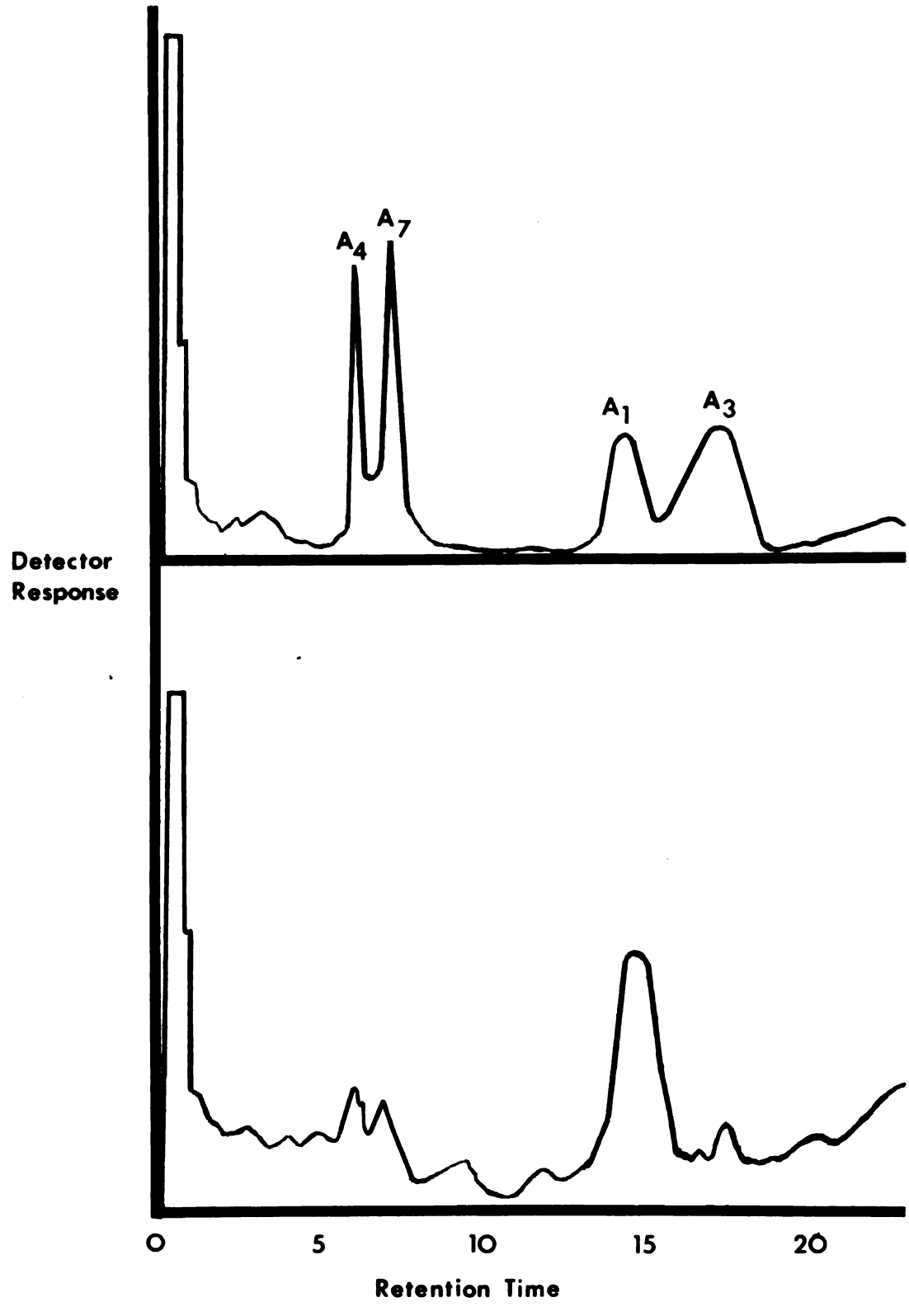


Figure 12

corresponds closely to that of methyl A_3: prominent common peaks are m/e 255, 237, 236, 227, 225, 223, 209, 195, 181, 179, 155, 136, 121, 105, 91, 77, 69, 65, 55, 44, 43, and 41. The prominent peak at m/e 136 has been assigned to cleavage of rings C and D from B (refer to Figure 1) by Wulfson et al. (1965) for methyl A_3 and the same ion would be expected for GA_3. The peak at M^+-63 (m/e 283) has been attributed to loss of H_2O, CO_2, and H from ring A to give a tropyllium ion. Peaks at M^+-63 are characteristic of gibberellins having a double bond at the 3,4 position of ring A, e.g. GA_3 and GA_7 (Binks et al., 1969).

Table 12 contains an analysis of the spectrum of Figure 15 (C_3). The molecular ion (m/e 332) has a mass identical to that of GA_4. The spectrum corresponds closely to that of methyl A_4: prominent common peaks include m/e 258, 255, 242, 241, 224, 199, 197, 183, 181, 169, 157, 155, 143, 129, 128, 119, 117, 115, 105, 92, 69, 67, 65, 55, and 41. The peak at m/e 136 is attributed to loss of rings A and B (as above). The M^+-63 peak associated with A_3 and A_7 is not very prominent. Thus, GA_4 is present in andromonoecious seed extracts.

Table 10

Analysis of mass spectrum in Figure 13.

m/e	M^+-m/e	assignment (loss of fregment)
348	0	molecular ion
330	18	H_2O
312	36	2 x H_2O
302	46	CO_2, H_2 (HCOOH)
284	64	H_2O, HCOOH

Figure 13

Mass spectrum of C_1, a gibberellin-like compound isolated from andromonoecious C. sativus seed.

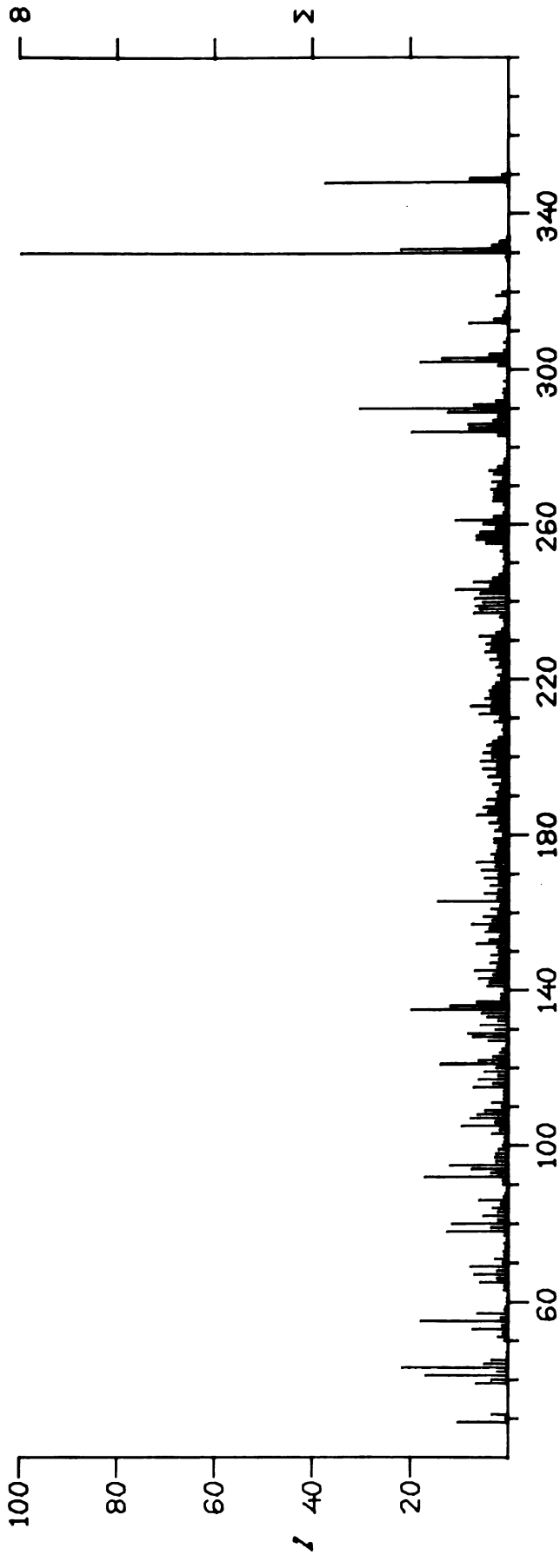


Figure 13

Figure 14

Mass spectrum of C_2, a gibberellin-like compound isolated from andromonoecious C. sativus seed.

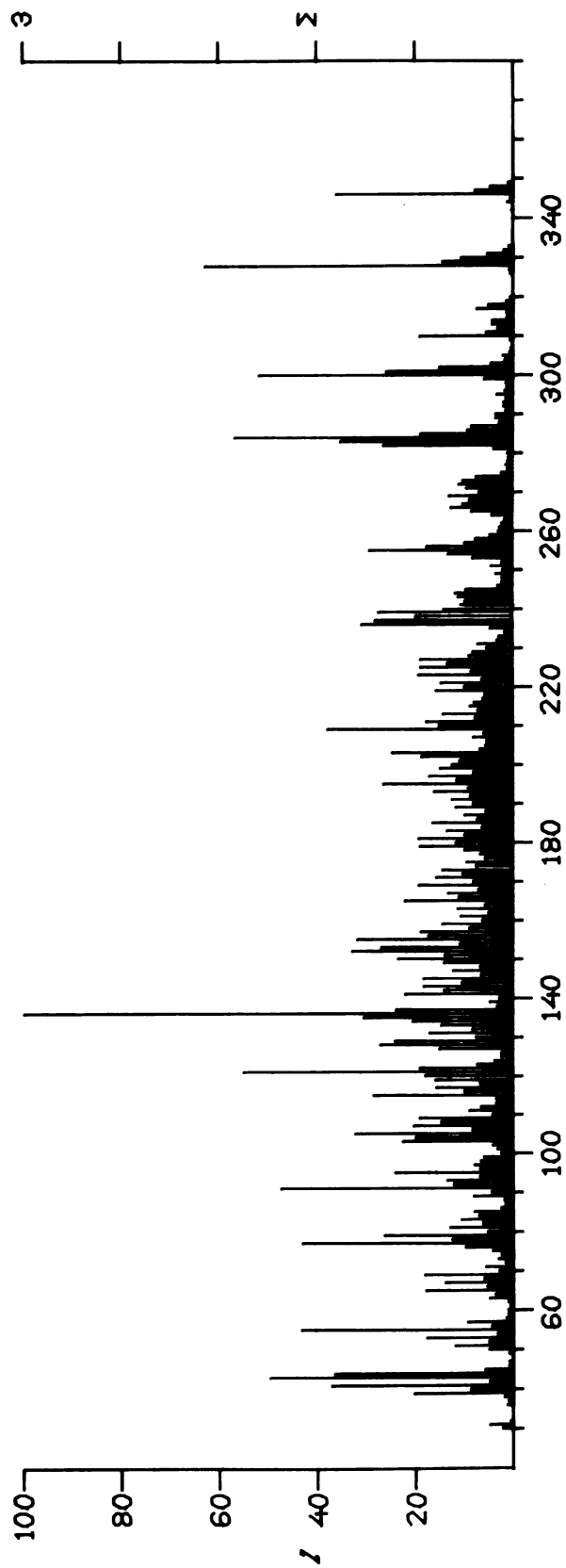


Figure 14

Figure 15

Mass spectrum of C_3, a gibberellin-like compound isolated from andromonoecious C. sativus seed.

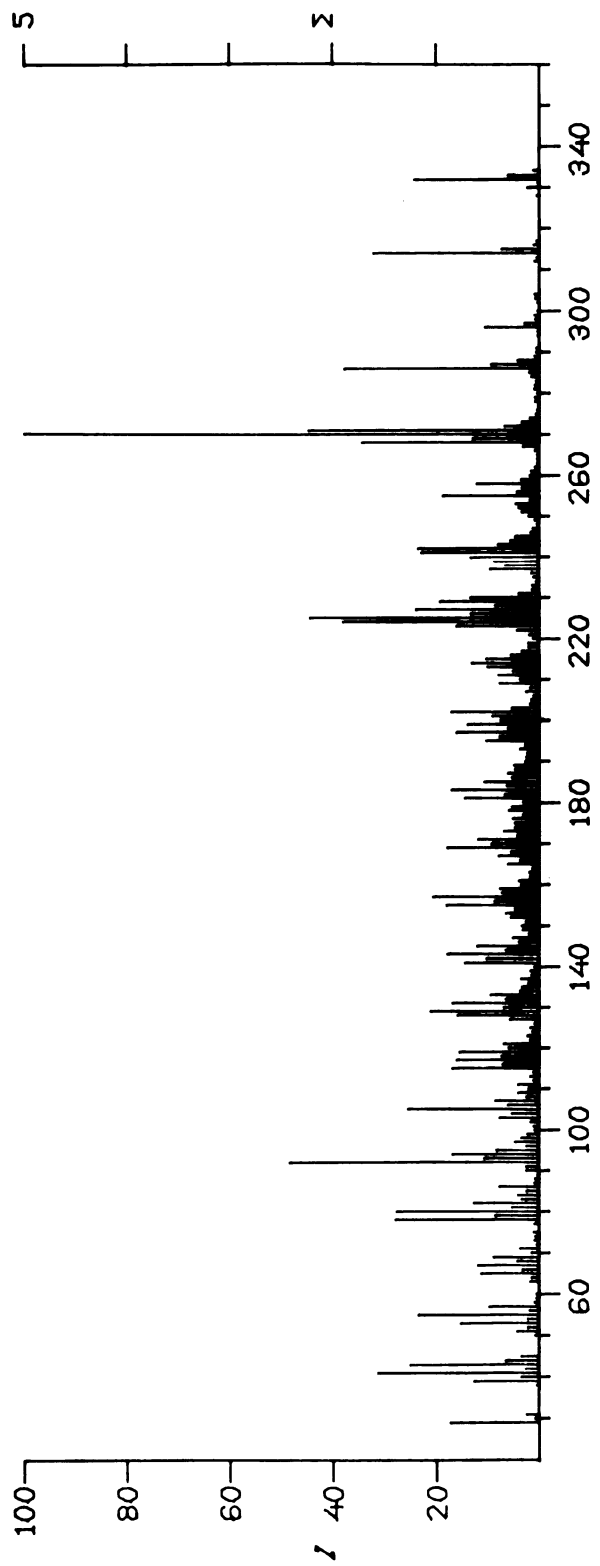


Figure 15

Table 11

Analysis of mass spectrum in Figure 14.

m/e	M^+-m/e	assignment (loss of fragment)
346	0	molecular ion
328	18	H_2O
310	36	2 x H_2O
300	46	CO_2, H_2 (HCOOH)
284	62	H_2O, CO_2
283	63	H_2O, CO_2, H
282	64	H_2O, CO_2, H_2
238	108	H_2O, CO_2, HCOOH
136	210	rings A and B

Table 12

Analysis of mass spectrum in Figure 15.

m/e	M^+-m/e	assignment (loss of fragment)
332	0	molecular ion
330	2	H_2 (from 2-hydroxyl)
314	18	H_2O
286	46	CO_2, H_2 (HCOOH)
270	62	H_2O, CO_2
268	64	H_2O, CO_2, H_2
136	196	rings A and B

VII. Identification of Free Acidic Gibberellins in *C. melo*

The free acidic gibberellins in gynoecious, monoecious, andromonoecious, and hermaphroditic *C. melo* seed were determined by thin-layer chromatography, infrared analysis, and mass spectrometry. The figures presented are for extracts of gynoecious seed, but the same types and relative proportions of gibberellins were found in all sex types.

A. Thin-layer chromatography.

A purified extract of gynoecious seed was compared with several gibberellin standards on a TLC plate (Figure 16). The only visible spot corresponds well with GA_1 and/or GA_3.

Figure 16

Thin-layer chromatogram of gibberellin extract of 100 g of gynoecious C. melo seed. [plate: Brinkmann Precoated Silica Gel F_{254}, 0.25 mm thickness, sprayed with alcoholic H_2SO_4; authentic samples of gibberellins A_1, A_2, A_3, A_4, A_5, A_7, A_9 spotted for comparison; solvent system: benzene:n-butanol:acetic acid (70:25:5, v/v)]

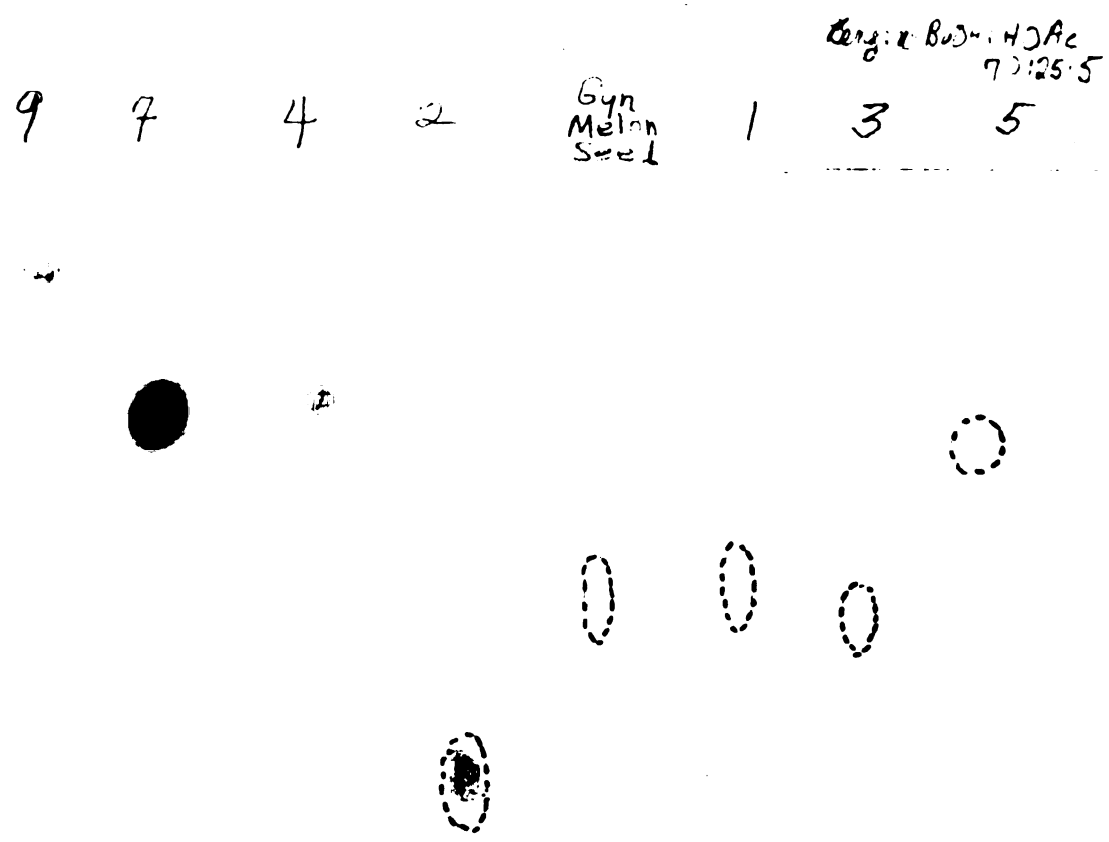


Figure 16

B. Infrared analysis.

Infrared (IR) spectra (Figures 17 and 18) were obtained for two fractions (M_1 and M_2) crystallized from the gynoecious extract suspected to contain GA_1 and/or GA_3. Results with gibberellin standards have shown that IR cannot be used for definitive structure determination with impure samples, but certain differences in the spectra of GA_1 and GA_3 are useful for identifying an unknown compound suspected to be one of these gibberellins. The spectra of Figures 17 (M_1) and 18 (M_2) each have major peaks at 3450 (2^o or 3^o O-H stretching), 2920 and 2950 (COOH vibration), 1750 ($C=CH_2$ vibration), 1740 (COOH vibration), 1170 (3^o OH vibration), 1100 (2^o OH vibration) and *ca.* 900 cm^{-1} ($C=CH_2$ vibration). However, the latter spectrum contains major peaks at 1020 and 1030 cm^{-1} which are lacking in Figure 17 and also peaks at 1260 (cis disubstituted C=C), 970 (cis C=C), and 770 cm^{-1} (cis C=C). Each of these peaks is characteristic of GA_3. Thus, the TLC spot (Figure 16) corresponding to GA_1 and/or GA_3 appears to be due to both of these gibberellins. Another fraction (M_3) was also purified, but in insufficient quantity for IR analysis.

C. Mass spectrometry.

Three highly purified fractions (M_1, M_2, M_3) of an extract of gynoecious seed were subjected to direct probe mass spectrometry. Two fractions (M_1 and M_2) produced spectra (Figure 19) which are almost identical to those of Figures 13 and 14 which were attributed (above) to GA_1

Figure 17

Infrared spectrum of M_1, a gibberellin-like compound isolated from gynoecious C. melo seed.

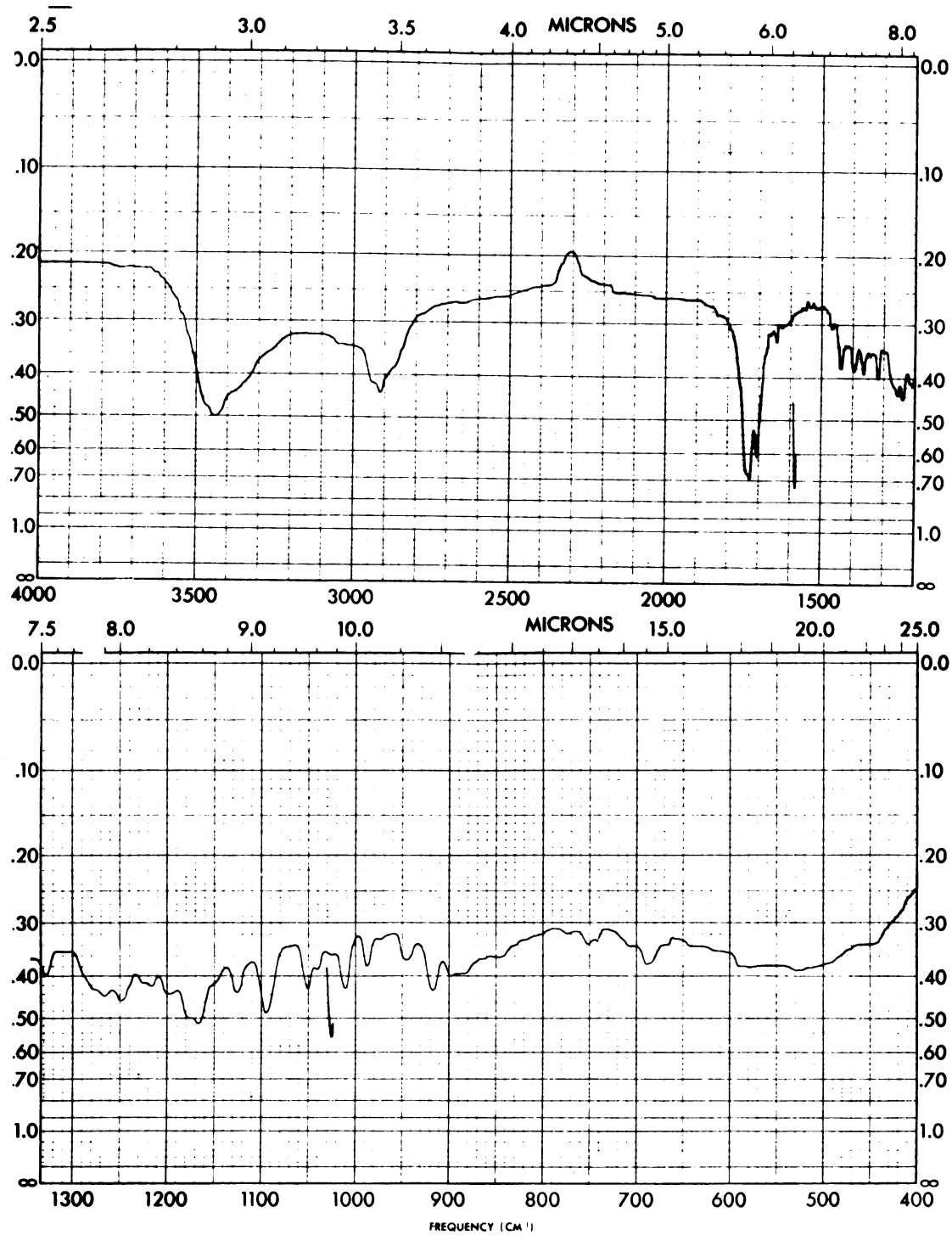

2.5
3.0
3.5
4.0
MICRONS
5.0
6.0
8.0
0.0
.10
.20
.30
.40
.50
.60
.70
1.0
∞
4000
3500
3000
2500
2000
1500
7.5
8.0
9.0
10.0
MICRONS
15.0
20.0
25.0
1300
1200
1100
1000
900
800
700
600
500
400
FREQUENCY (CM⁻¹)

Figure 17

Figure 18

Infrared spectrum of M_2, a gibberellin-like compound isolated from gynoecious C. melo seed.

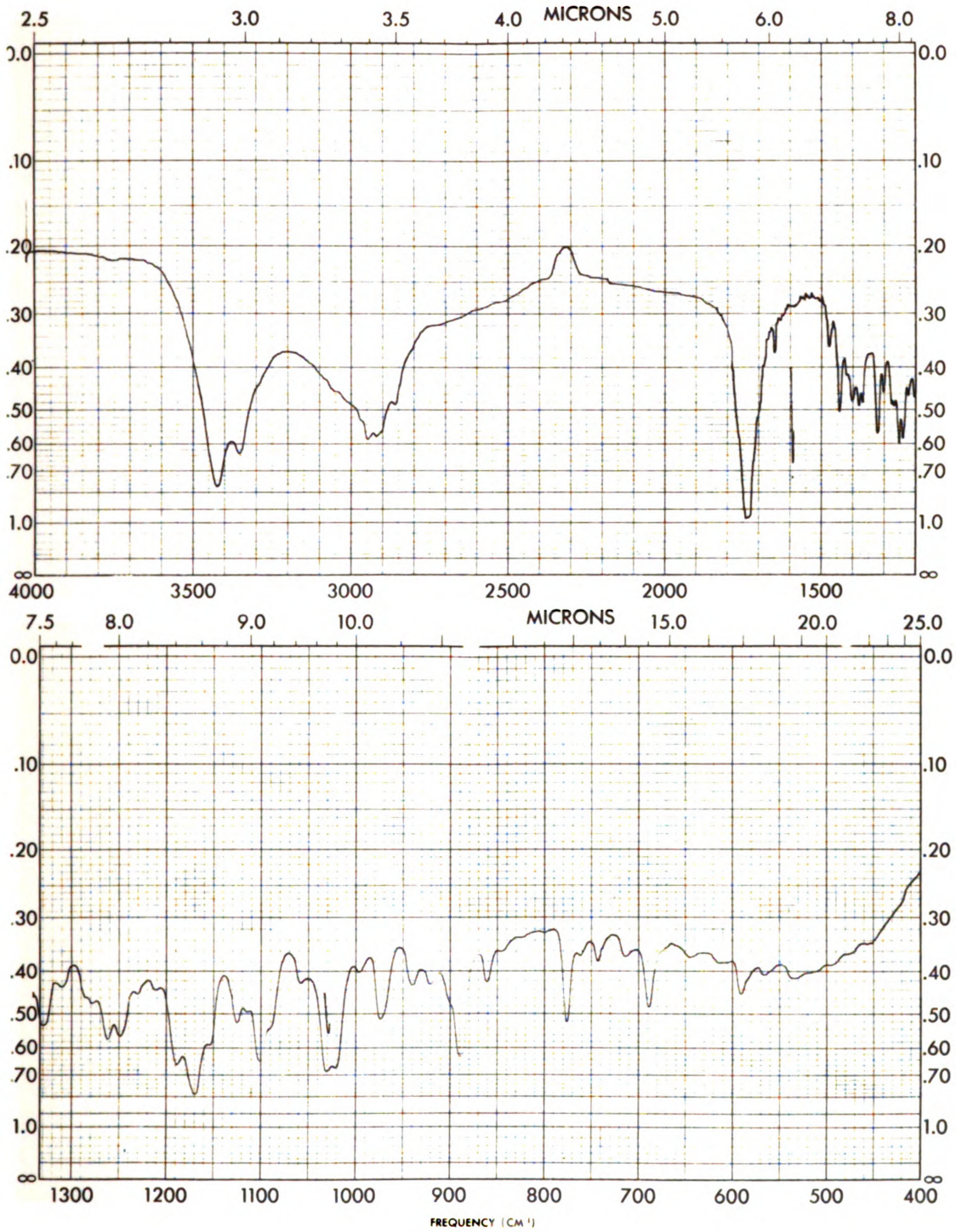

2.5
3.0
3.5
4.0
MICRONS
5.0
6.0
8.0
0.0
.10
.20
.30
.40
.50
.60
.70
1.0
∞
4000
3500
3000
2500
2000
1500
7.5
8.0
9.0
10.0
MICRONS
15.0
20.0
25.0
1300
1200
1100
1000
900
800
700
600
500
400
FREQUENCY (CM⁻¹)

Figure 18

Figure 19

Mass spectra of M_1 (upper) and M_2 (lower), gibberellin-like compounds isolated from gynoecious C. melo seed.

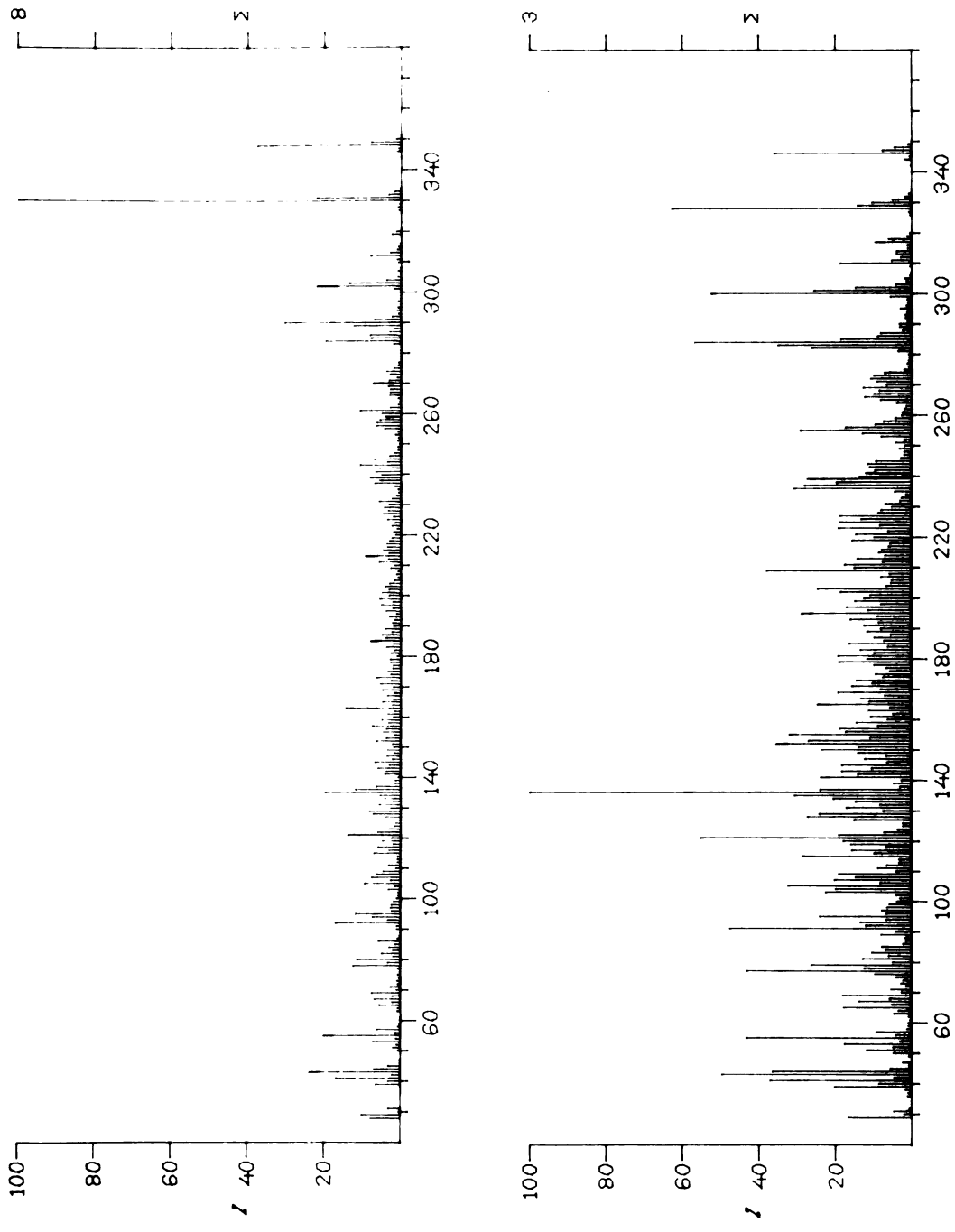


Figure 19

and GA_3. The slight differences in certain peak heights are probably due to small differences in either ion source temperature or amounts of impurities.

Table 13 contains an analysis of the spectrum of M_3 (Figure 20). The molecular ion (m/e 330) is identical in molecular weight to both GA_5 and GA_7; there are many peaks in common with the spectra of both methyl A_5 and methyl A_7. However, the peak at m/e 267 (M^+-63) which is characteristic of methyl A_7 is weak in Figure 20, and the peak at m/e 286 (M^+-44) is very strong, a characteristic of the spectra of methyl esters of the gibberellins which lack a C-2 hydroxyl. Thus, M_3 is GA_5.

Table 13

Analysis of mass spectrum in Figure 20

m/e	M^+-m/e	assignment
330	0	molecular ion
312	18	loss of H_2O
286	44	" CO_2
284	46	" CO_2, H_2
268	62	" CO_2, H_2O
136	194	" rings A and B

VIII. Identification of Neutral Fraction Gibberellins in C. sativus

Methanol extraction of five kg monoecious C. sativus seed (Figure 3) yielded ca. 20 mg of biologically active material in the neutral fraction. Repeated thin-layer chromatography and bioassay of the neutral fraction material revealed one band at R_f 0.7 (benzene: n-butanol: acetic acid, 70:25:5, v/v) which had gibberellin-like activity.

Figure 20

Mass spectrum of M_3, a gibberellin-like compound isolated from gynoecious C. melo seed.

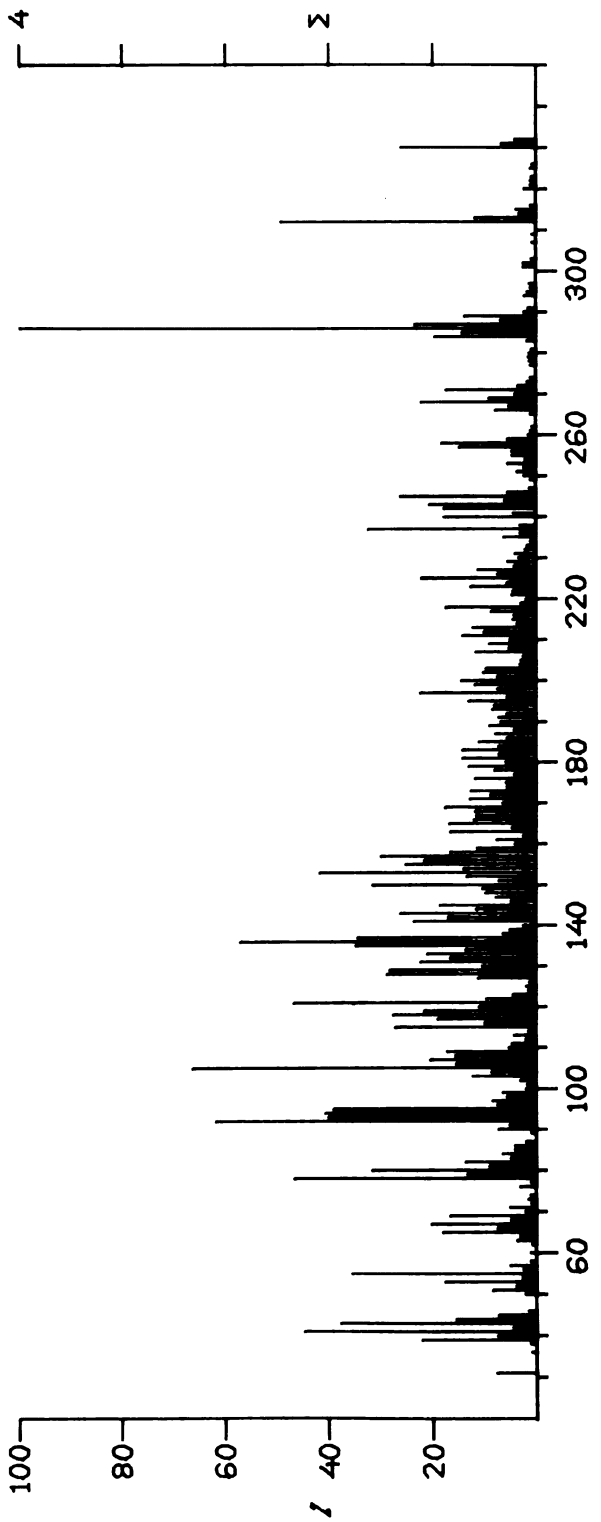


Figure 20

Eluates of this band were further purified on TLC and two compounds were found to be present. An apparently pure compound (m.p. 140-141°), designated N_1, was resolved from the mixture by crystallization and the remainder was designated N_2 (m.p. 140-148°). N_1 produced a pale brown spot on TLC plates when developed with alcoholic H_2SO_4; N_2 produced overlapping pale brown and pale green spots. N_1 and N_2 (Figure 21) are slightly less polar than most of the free acidic gibberellin standards.

A. Identification of N_1.

After recrystallization from ethyl acetate-petroleum ether, the apparently pure N_1 was subjected to direct probe mass spectrometry. The spectrum is seen in Figure 22; Table 14 contains an analysis of the spectrum. The fragmentation pattern and molecular ion (m/e 388) indicated that N_1 might be a propyl ester of gibberellin A_3, but spectra of gibberellin propyl esters have not been reported. Therefore, the n-propyl ester of A_3 was synthesized according to the method of Sell et al. (1959). The product had a melting point of 140° and its mass spectrum (Figure 23) is essentially identical to that of N_1.

Table 15 contains a comparison of the authentic GA_3 n-propyl ester, N_1, and N_2 in three TLC systems. The R_f values of N_1 and authentic n-propyl A_3 correspond closely in each system.

The infrared spectra of N_1 and authentic GA_3 n-propyl ester (Figures 24 and 25, respectively), are also essentially identical.

Table 14

Analysis of mass spectrum in Figure 21 (N_1)

m/e	M^+-m/e	assignment
388	0	molecular ion
370	18	loss of H_2O
342	46	" CO_2, H_2
329	59	" $C_3H_7O\cdot$
328	60	" C_3H_7OH
325	63	" H_2O, CO_2, H
301	87	" $C_3H_7OC{=}O\cdot$
300	88	" $C_3H_7OC{=}OH$
238	150	" $C_3H_7OC{=}OH$, H_2O, CO_2
237	151	" $C_3H_7OC{=}OH$, H_2O, CO_2H
136	252	" rings A and B
43 (base peak)	345	$CH_3CH_2CH_2\cdot$

Table 15

TLC R_f values of authentic GA_3 n-propyl ester, N_1, and N_2 in three solvent systems.

Solvent system	PrA_3	N_1	N_2[1]
benzene:n-butanol:CH_3COOH (70:25:5)	.68	.68	.72
chloroform:ethyl acetate:CH_3COOH (60:40:5)	.41	.41	.41
di-isopropyl ether:CH_3COOH (95:5)	.48	.49	.50

TLC plates were Silica Gel F_{254} with fluorescent indicator. Spots developed with 5% alcoholic H_2SO_4.

[1] Faster of two overlapping spots

Figure 21

Thin-layer chromatogram of N_1 and N_2, neutral gibberellin-like compounds isolated from C. sativus seed. [authentic samples of gibberellins A_1, A_2, A_3, A_4, A_5, A_7, A_9 spotted for comparison; plate: Brinkman Pre-coated Silica Gel F_{254}, 0.25 mm thickness, sprayed with alcoholic H_2SO_4; solvent system: benzene: n-butanol:acetic acid (70:25:5, v/v)]

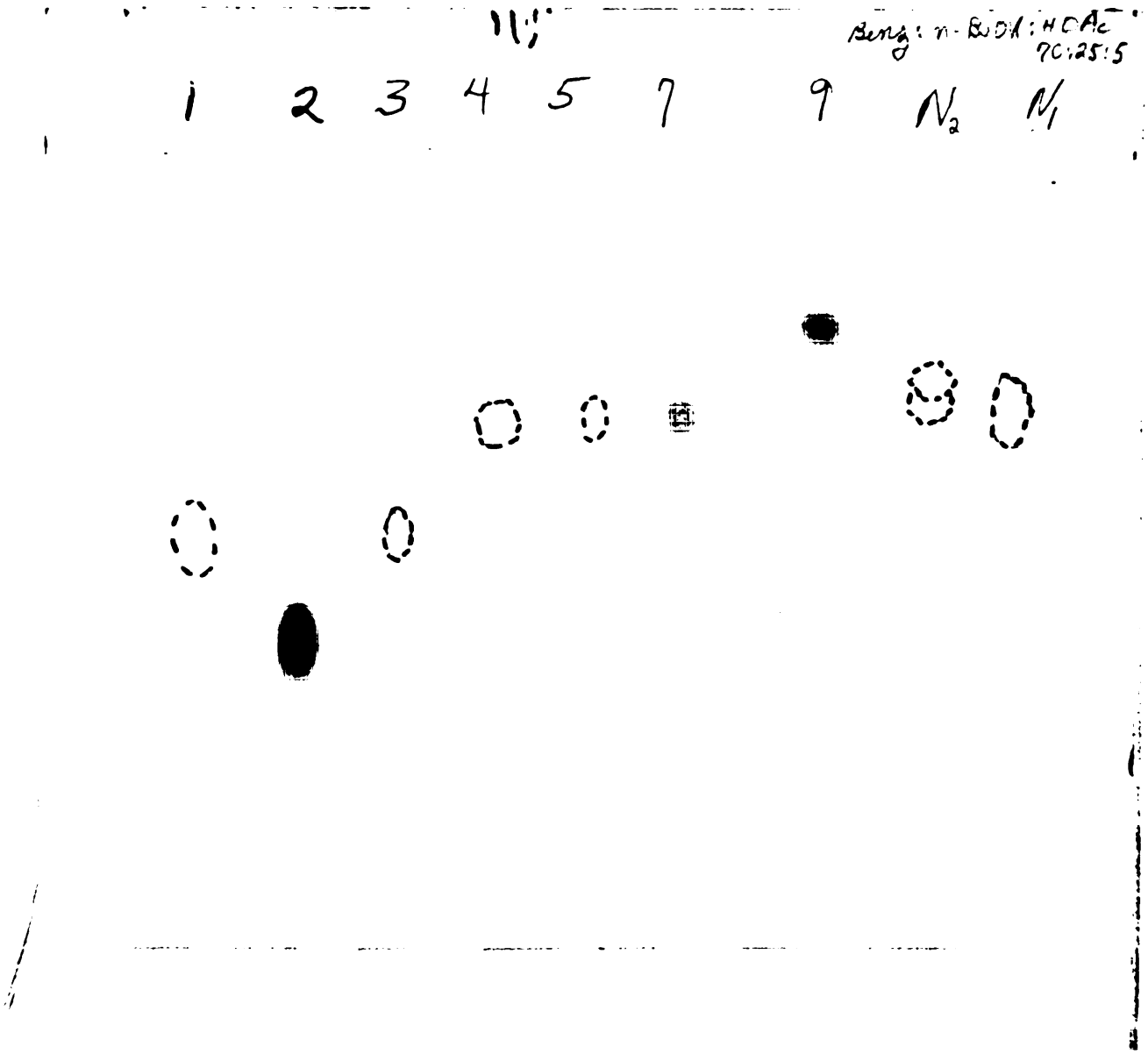


Figure 21

Figure 22

Mass spectrum of N_1, a neutral gibberellin-like compound isolated from C. sativus seed.

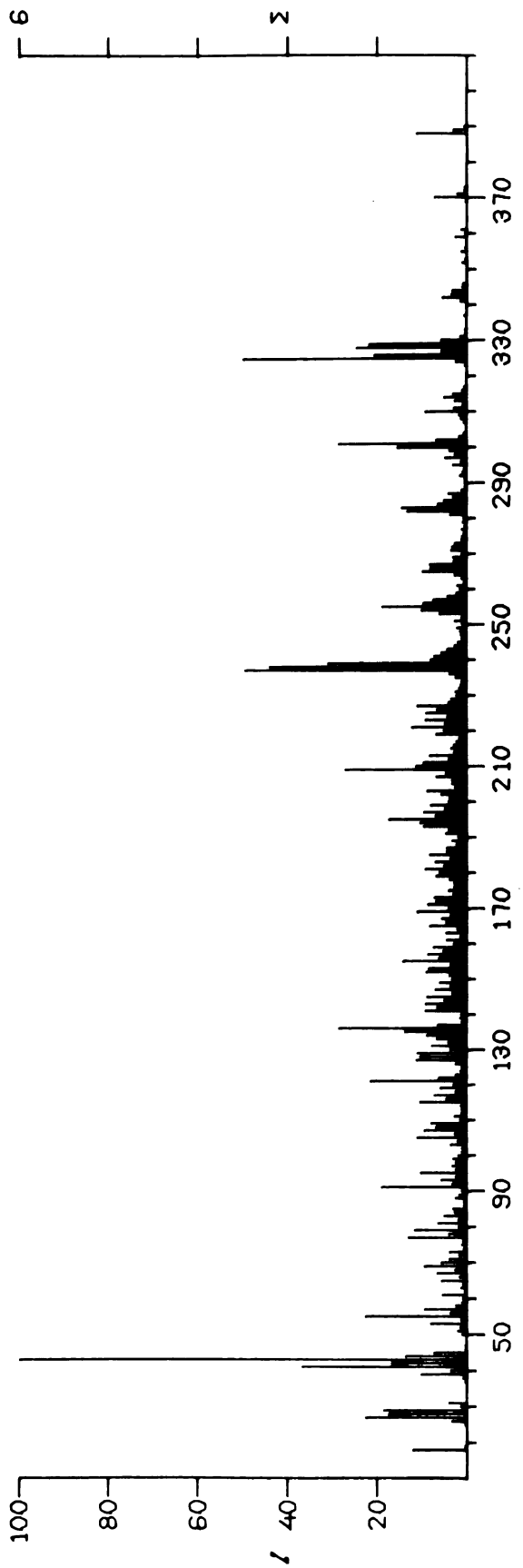


Figure 22

Figure 23

Mass spectrum of authentic gibberellin A_3 n-propyl ester.

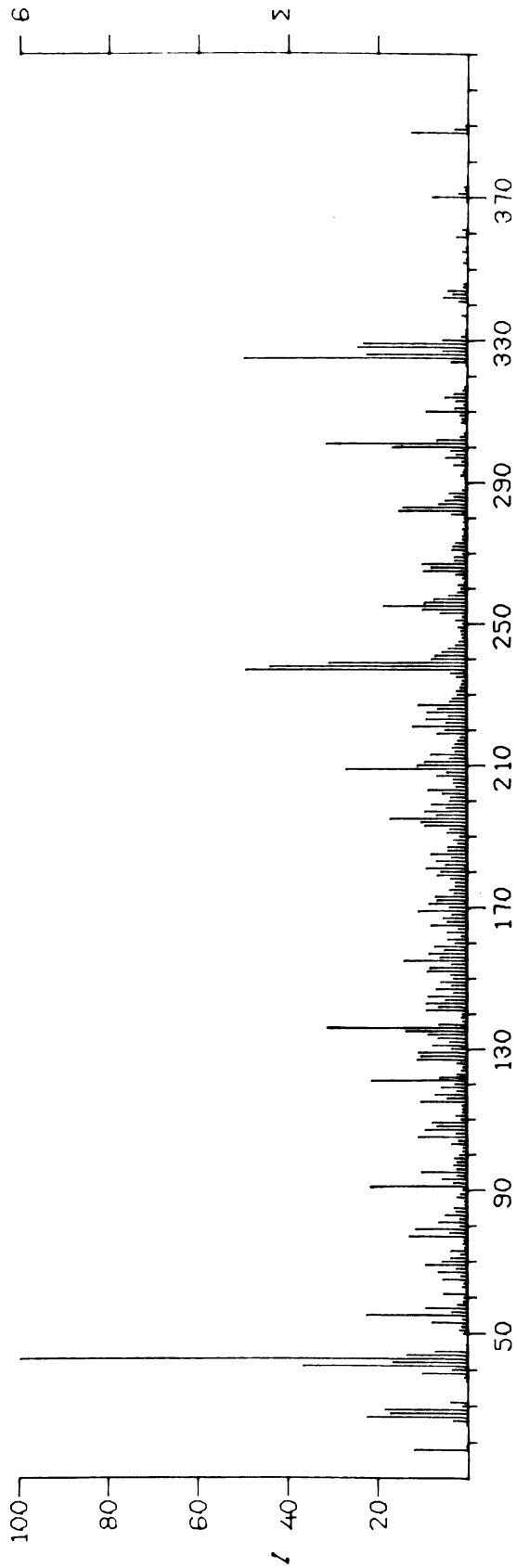


Figure 23

Figure 24

Infrared spectrum of N_1, a neutral gibberellin-like compound isolated from C. sativus seed.

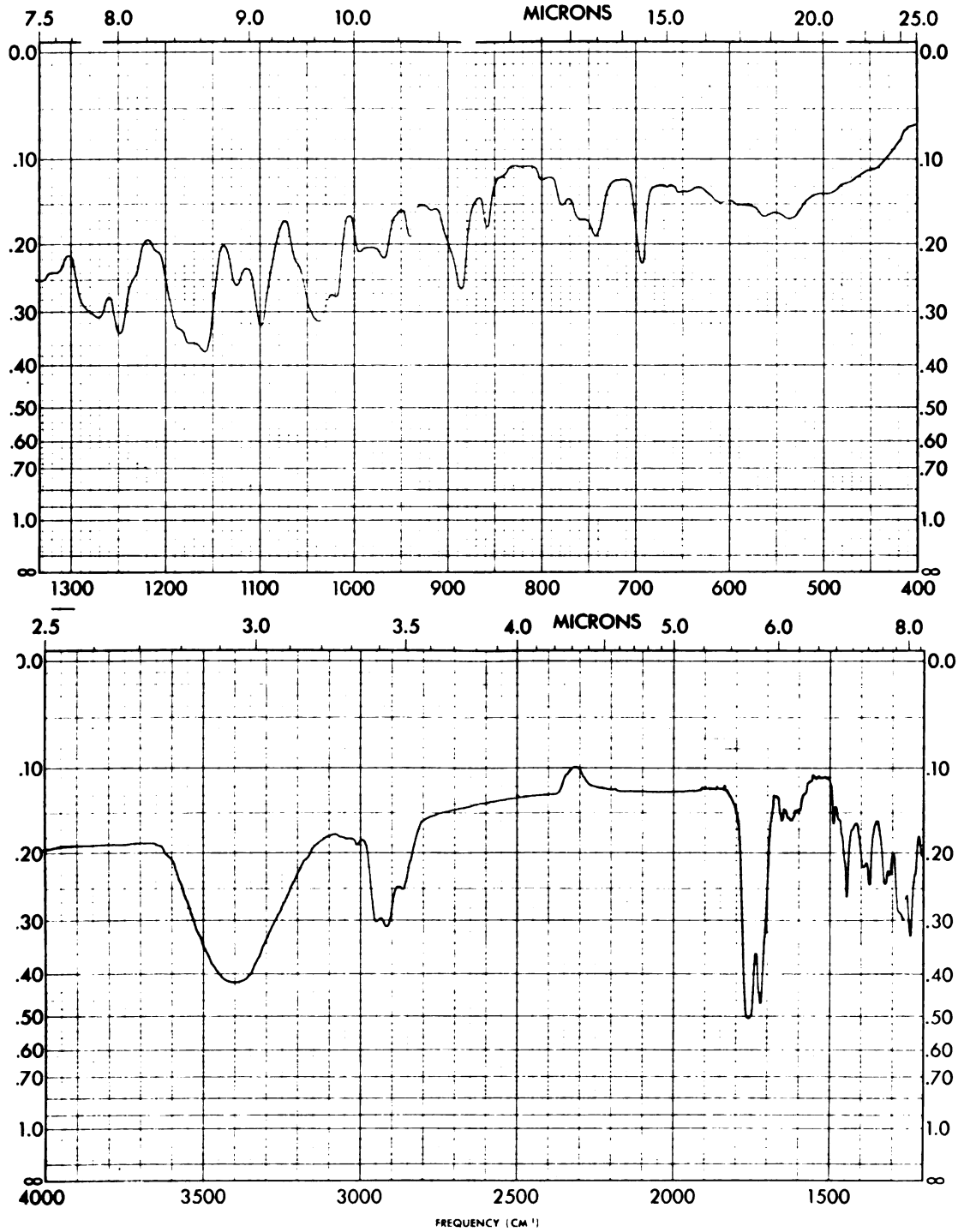

7.5
8.0
9.0
10.0
MICRONS
15.0
20.0
25.0
0.0
.10
.20
.30
.40
.50
.60
.70
1.0
∞
1300
1200
1100
1000
900
800
700
600
500
400
2.5
3.0
3.5
4.0
MICRONS
5.0
6.0
8.0
4000
3500
3000
2500
2000
1500
FREQUENCY (CM⁻¹)

Figure 24

Figure 25

Infrared spectrum of authentic gibberellin A_3 n-propyl ester.

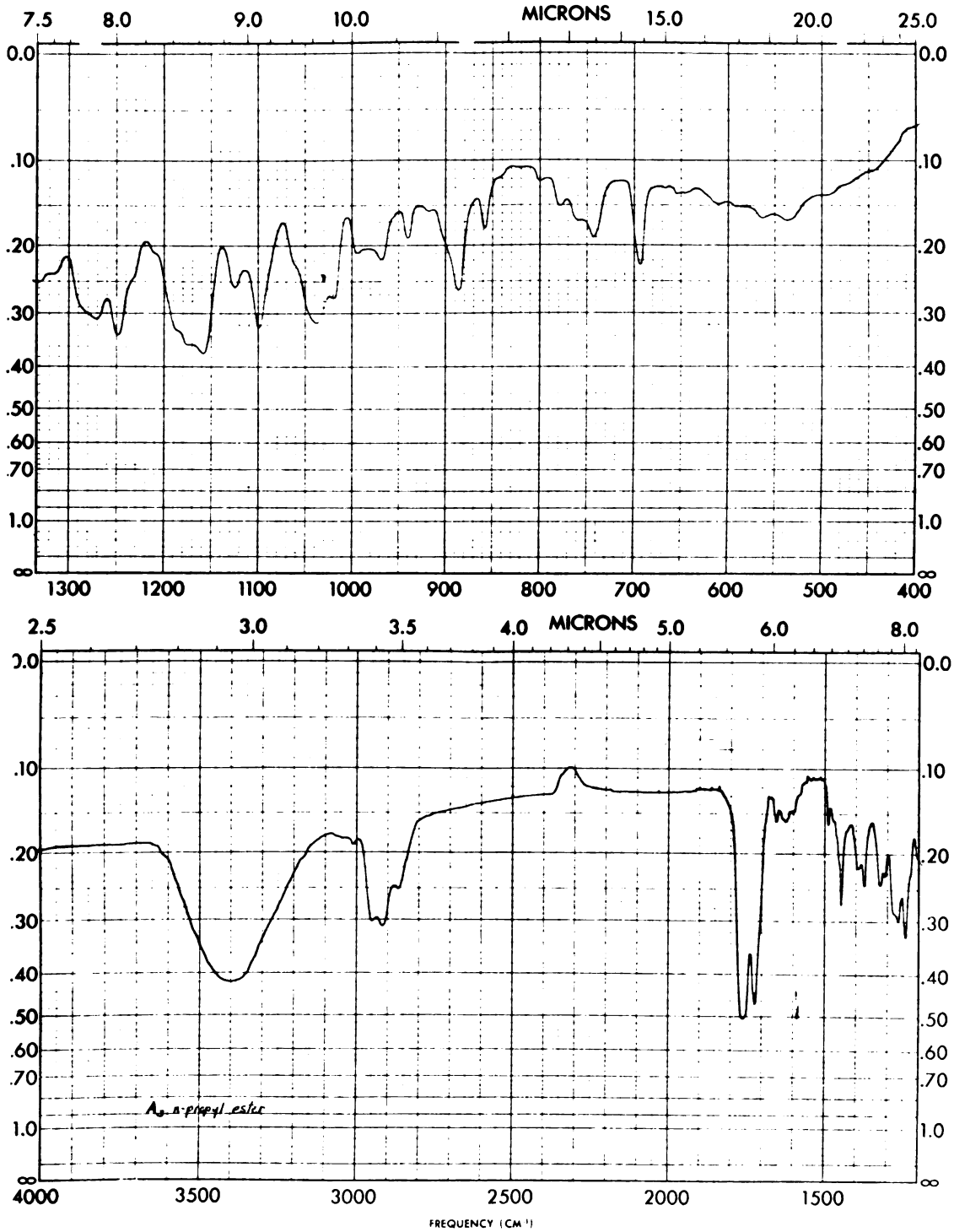

7.5
8.0
9.0
10.0
MICRONS
15.0
20.0
25.0
0.0
.10
.20
.30
.40
.50
.60
.70
1.0
∞
1300
1200
1100
1000
900
800
700
600
500
400
2.5
3.0
3.5
4.0
MICRONS
5.0
6.0
8.0
4000
3500
3000
2500
2000
1500
FREQUENCY (CM⁻¹)

Figure 25

B.

pr
cr
je
co
tr
of
wi
pr
ty
Th
ha
ma
p

I

i
l
w
c
a
s
p

B. Identification of N_2.

N_2, which appeared to contain N_1 and another compound, proved difficult to resolve completely by either TLC or crystallization. After trimethylsilylation, N_2 was subjected to combined GLC-mass spectrometry. Figures 26 and 27 contain mass spectra of the two compounds resolved from trimethylsilyl (TMS) -N_2; Tables 16 and 17 contain analyses of these spectra. The fragmentation pattern in Figure 26, with molecular ion of 532 and fragments due to loss of propyl and propylcarboxyl groups as well as several peaks typical of TMS derivatives, indicates the presence of the TMS diether of GA_3 propyl ester. The spectrum in Figure 27 has a molecular ion of 534 and other major peaks are two mass units greater than those of Figure 26, indicating the presence of the TMS diether of GA_1 propyl ester.

IX. Biological Activity of a Neutral Gibberellin (N_1) from *C. sativus*

The n-propyl ester of GA_3 (N_1) was tested for activity in four bioassays (Table 18). Activity in each assay was 10% or less of the activity of GA_3. These figures correspond well with those for synthetic propyl A_3 in the dwarf pea, cucumber hypocotyl, and d_1 dwarf corn bioassays (Brian *et al.*, 1967). In addition, Sell *et al.* (1959) reported that synthetic n-propyl A_3 was considerably less effective in promoting lettuce seed germination than GA_3.

Figure 26

Mass spectrum of TMS-N_2 (first component), a neutral gibberellin-like fraction isolated from C. sativus seed. [N_2 was trimethylsilylated and subjected to GLC-mass spectrometry]

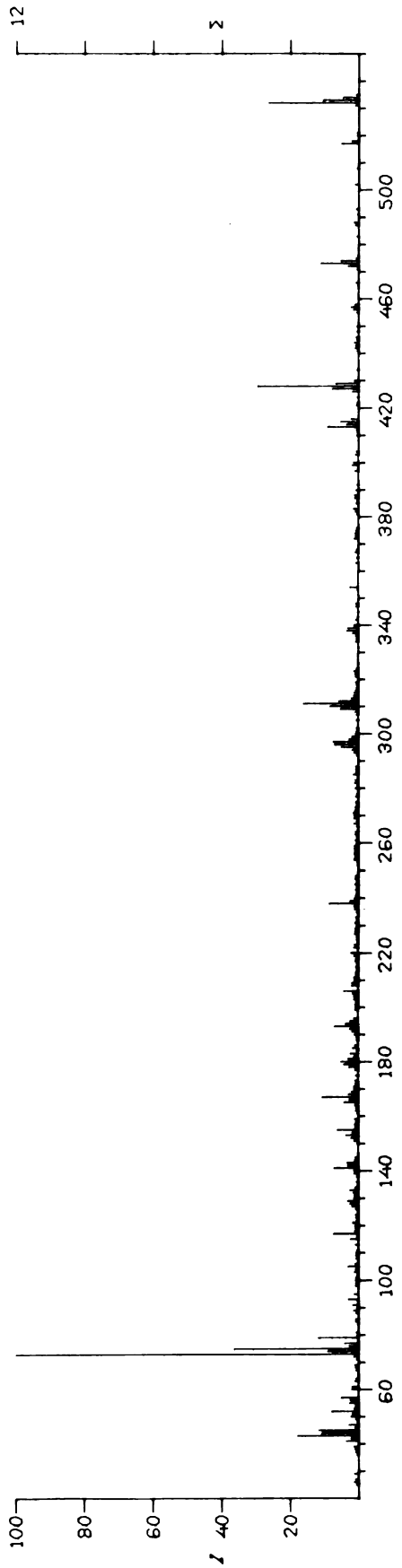

12
2
500
460
420
380
340
300
260
220
180
140
100
60
100
80
60
40
20
I

Figure 26

Figure 27

Mass spectrum of TMS-N_2 (second component), a neutral gibberellin-like fraction isolated from C. sativus seed.

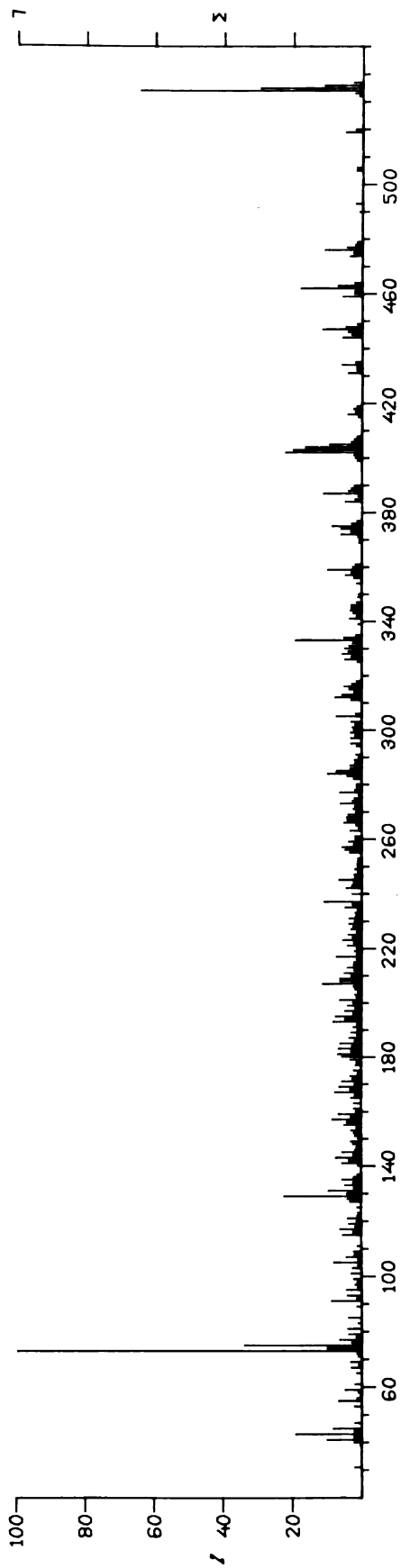


Figure 27

Table 16

Analysis of mass spectrum in Figure 26

m/e	M^+-m/e	assignment
532	0	molecular ion
517	15	loss of $CH_3\cdot$
502	30	" 2 x $CH_3\cdot$
488 (weak)	44	" CO_2
473	59	" $C_3H_7O\cdot$
472	60	" C_3H_7OH
445	87	" $C_3H_7OC{=}O\cdot$
444	88	" $C_3H_7OC{=}OH$
442	90	" $(CH_3)_3SiOH$
428	104	" CO_2, C_3H_7OH
129	403	$C_6H_{13}OSi$
75	457	$(CH_3)_2SiOH$
73	459	$(CH_3)_3Si\cdot$

Table 17

Analysis of mass spectrum in Figure 27

m/e	M^+-m/e	assignment
534	0	molecular ion
519	15	loss of $CH_3\cdot$
504	30	" 2 x $CH_3\cdot$
490 (weak)	44	" CO_2
474	60	" C_3H_7OH
447	87	" $C_3H_7OC{=}O\cdot$
446	88	" $C_3H_7OC{=}OH$
444	90	" $(CH_3)_3SiOH$
405	129	" $C_6H_{13}OSi$
402	132	" $C_3H_7OC{=}OH$, CO_2
129	405	$C_6H_{13}OSi$
75	459	$(CH_3)_2SiOH$
73	461	$(CH_3)_3Si\cdot$

Table 18

Biological activity of the GA_3 n-propyl ester (N_1) from C. sativus. [relative to an equal concentration of GA_3 (0.1 mg/ml) in four bioassays]

Bioassay	N_1 activity/GA_3 activity
dwarf pea	<.005
cucumber hypocotyl	.1
dwarf corn (d_1)	.05
barley amylase	.01

X. Characterization of a Highly Polar Gibberellin from C. sativus

Methanol extraction of five kg of monoecious C. sativus seed (Figure 3) and subsequent purification yielded several mg of active material in the n-butanol fraction which was not due to traces of free acidic gibberellins. Following repeated TLC purification and crystallization, this material was found to be more polar than several gibberellin standards on TLC (Figure 28). The crystalline material (m.p. 170-180°) was suspected to be a gibberellin-polyol conjugate since it gave a positive Molisch test and strong bands at ca. 3400 and 1000 to 1100 cm^{-1} in the infrared. Titration of the material as a monobasic acid yielded a molecular weight of 500.

The remaining material was hydrolyzed with emulsin, purified by TLC, and compared with several D-sugar standards in two solvent systems (Figure 29). The hydrolyzed material was also compared with GA_1 and β-D-glucose standards in two solvent systems (Figure 30). The R_f values of the two spots

Figure 28

Thin-layer chromatogram of a highly polar acidic gibberellin (B_1) isolated from C. sativus seed. [authentic gibberellins A_1, A_2, A_3, A_4, A_5, A_7, A_9 spotted for comparison; plate: Brinkmann Pre-coated Silica Gel F_{254}, 0.25 mm thickness, sprayed with alcoholic H_2SO_4; solvent system: benzene:n-butanol:acetic acid (70:25:5, v/v)]

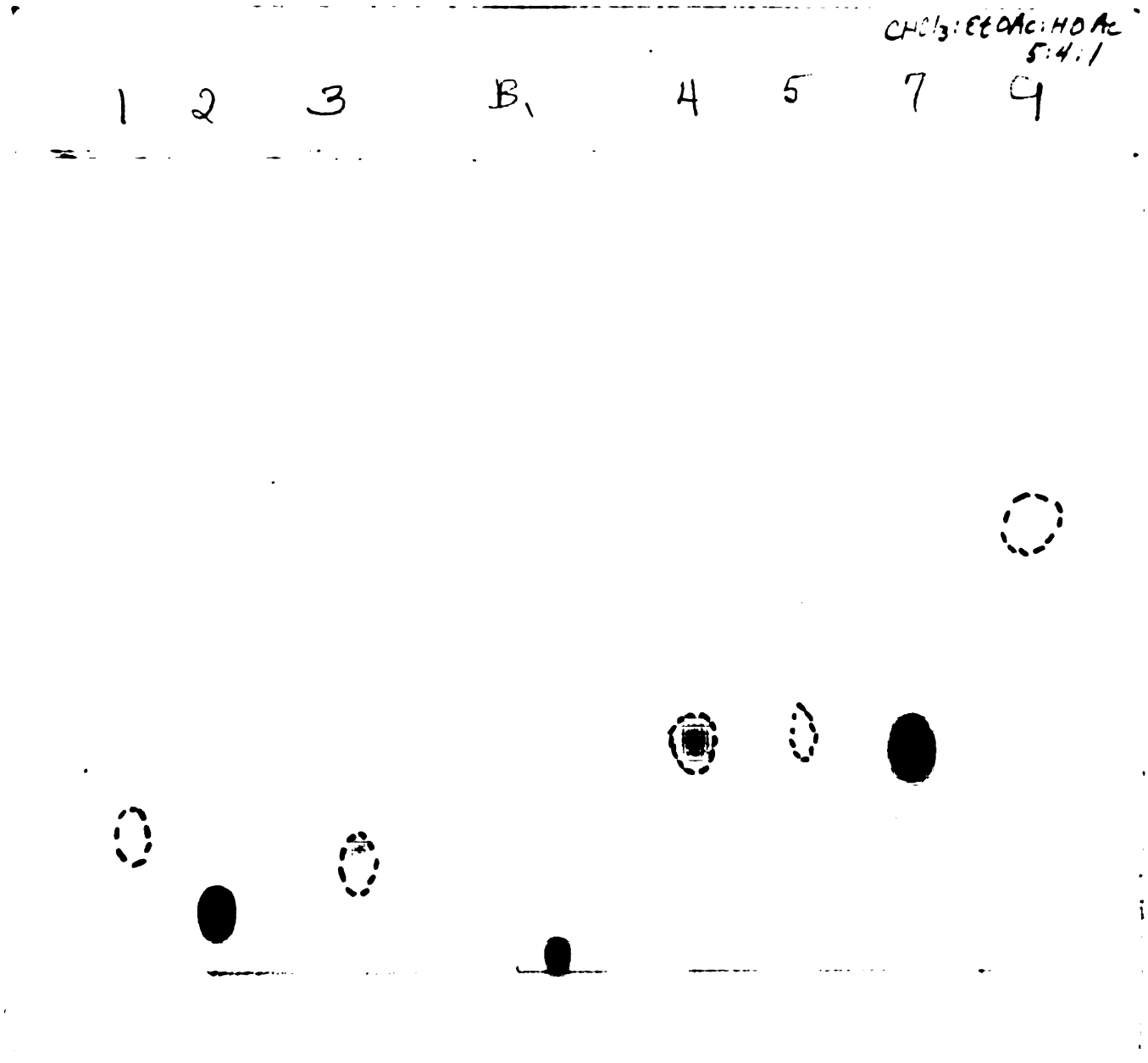


Figure 28

Figure 29

Thin-layer chromatograms of the hydrolysis products of a highly polar acidic gibberellin isolated from C. sativus seed, and several sugar standards. [D-sugar standards: glucose, ribose, fructose, mannose, galactose, glucosamine; plates: Brinkmann Pre-coated Silica Gel F_{254}, 0.25 mm thickness, sprayed with periodate-benzidine; solvent systems: A, iso-propanol: acetic acid:water (3:1:1, v/v), B, n-butanol: acetic acid:water (4:1:2, v/v)]

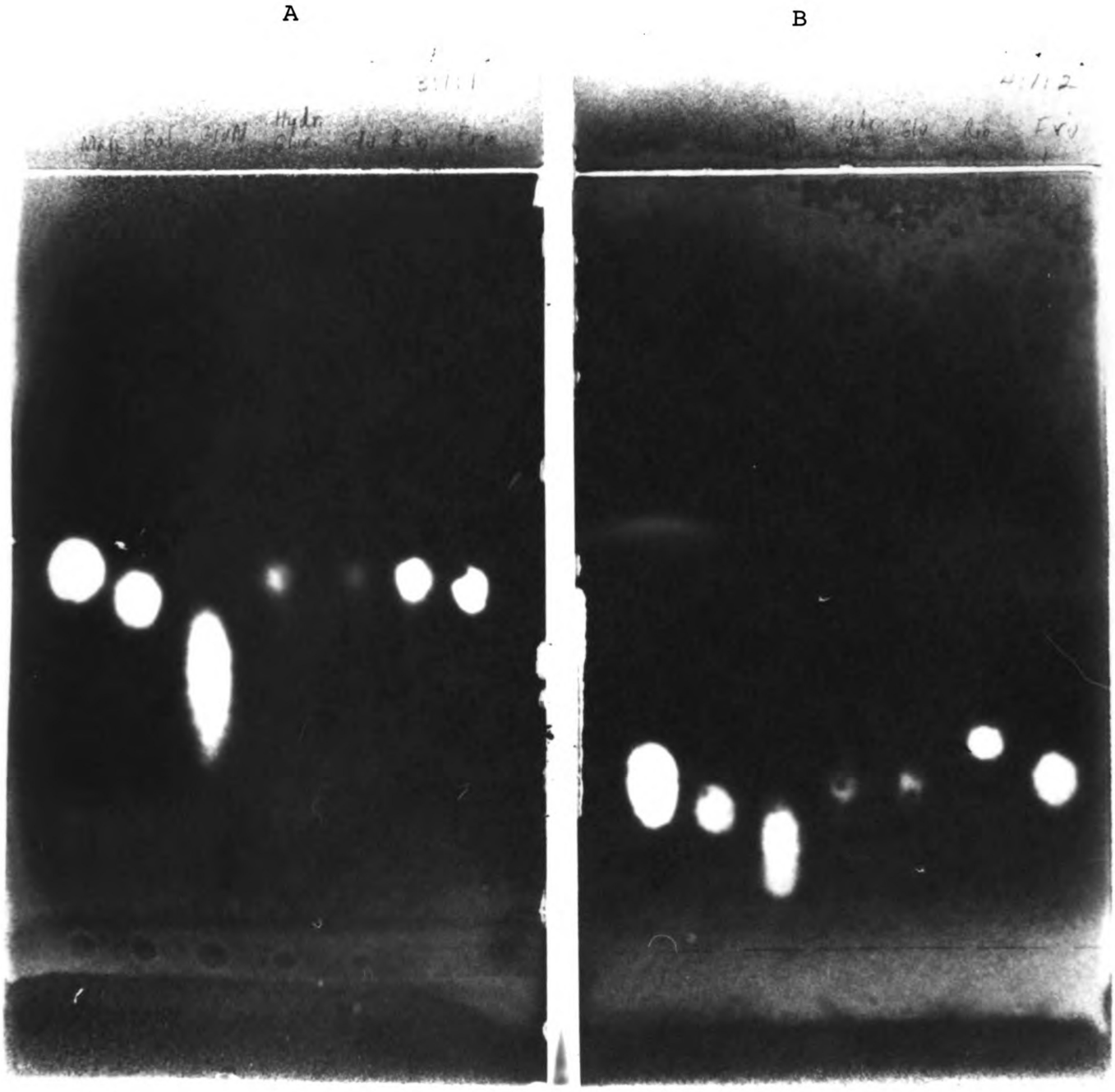


Figure 29

Figure 30

Thin-layer chromatograms of the hydrolysis products of a highly polar acidic gibberellin isolated from C. sativus seed, D-glucose, and GA_1. [plates: Brinkmann Pre-coated Silica Gel G, 0.25 mm thickness, sprayed with Folin-Ciocalteau reagent; solvent systems: A, iso-propanol:ammonium hydroxide:water (10:1:1, v/v), B, iso-propanol:acetic acid:water (3:1:1, v/v)]

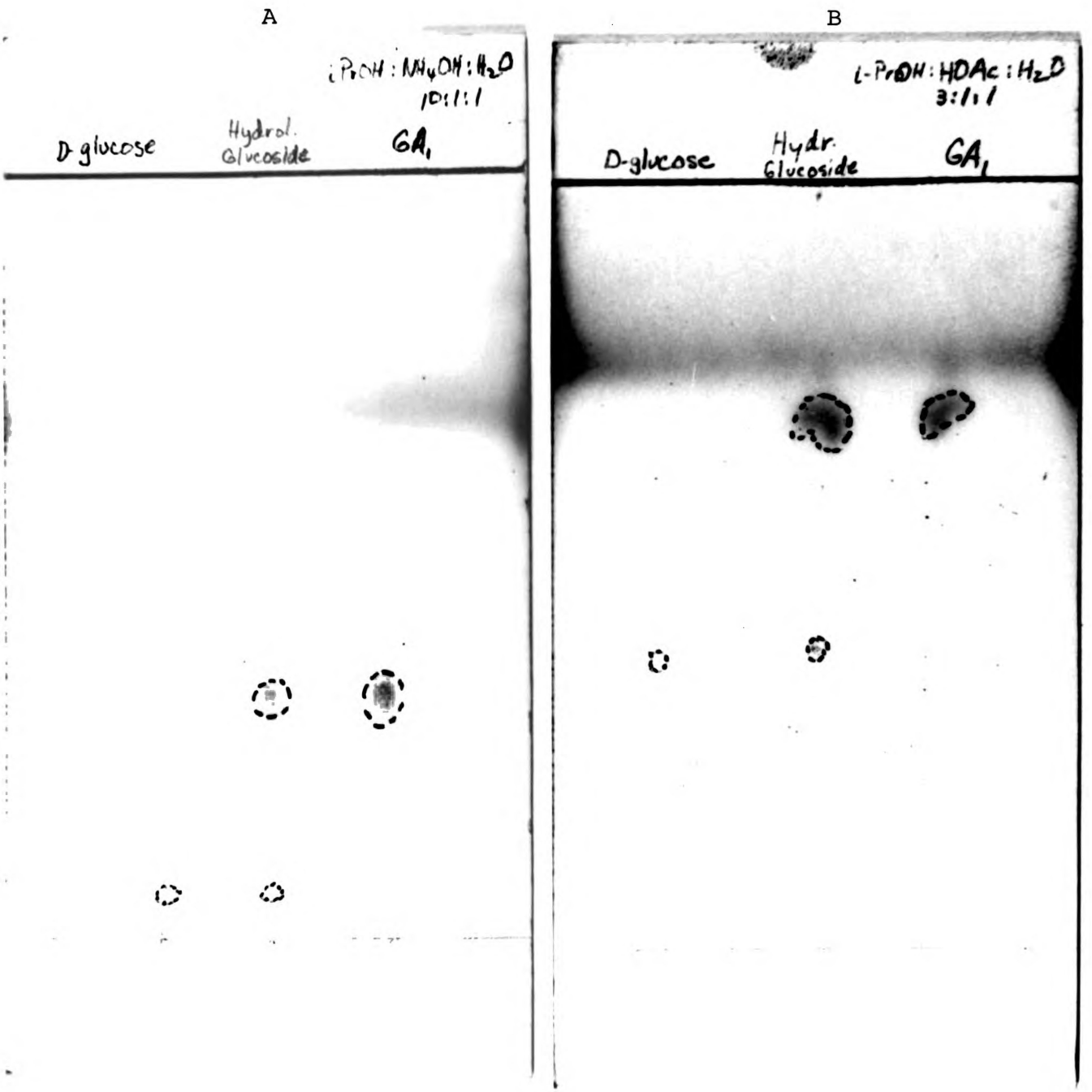

A
iPrOH : NH4OH : H2O
10:1:1
D-glucose
Hydrol. Glucoside
GA1
B
t-PrOH : HOAc : H2O
3:1:1
D-glucose
Hydr. Glucoside
GA1

Figure 30

produced by the hydrolyzed material agree closely with those for β-D-glucose and GA_1. The gibberellin spot did not correspond to other gibberellin standards. These results indicate that the highly polar gibberellin-like material consists of a GA_1-β-D-glucose conjugate. The molecular weight obtained by titration indicates the conjugate contains equimolar amounts of GA_1 and glucose since the molecular weight of GA_1-β-D-glucose would be 510.

DISCUSSION

Extraction of gibberellins from seeds and etiolated seedlings of three C. sativus sex types has demonstrated that the gynoecious type is gibberellin deficient relative to the more male types. This difference in both free acidic and total (free acidic plus enzyme-released) gibberellin activity was observed at all growth stages. Extracts of monoecious and andromonoecious types contained 50 to 800 percent more free acidic gibberellin activity, depending on growth stage, than did the gynoecious (Figure 9).

The more difficult task of measuring gibberellin activity in green plants was undertaken to avoid extrapolating results with etiolated seedlings to green plants. Long days increase maleness in C. sativus (Ito and Saito, 1957a) and gibberellin production is often associated with young leaves as well as root tips. Thus, green plants may contain higher levels of gibberellin than etiolated seedlings at the same growth stage.

Since C. sativus floral parts are initiated bisexually and then develop into either pistillate, staminate, or perfect flowers (Atsmon and Galun, 1960), hormone levels at the time of flower differentiation may be particularly important in determining sex type. Flower buds appear in

the axil of the first true leaf and often even in the cotyledonary axil. In addition, formation of staminate flowers on MSU 713-5 in response to gibberellin application is greatest if the treatment occurs at the cotyledon or first leaf stage (Kubicki, 1969h; Pike and Peterson, 1969). Sex type can generally be determined this early since the ovary is usually inferior. Axils of the first leaves of monoecious plants usually bear recognizable staminate flowers which abort while quite small; normal staminate flowers appear at the second to fourth nodes. Although environmental factors cause some variation, the first true leaf (fully expanded) usually occurs at fourteen to twenty days, the second at twenty-one to twenty-six days. Thus, fully developed flowers often occur by the fourth week. The possible relationship of time of flower differentiation and gibberellin activity at that growth stage could not be satisfactorily investigated with etiolated seedlings which remain at the hypocotyl stage until three weeks old.

Relative gibberellin levels in green plants were found to parallel those in etiolated seedlings (Figure 10). The high levels of gibberellin activity in the more male sex types at the one-week stage are particularly significant since this is the stage where flower initiation at the cotyledonary and first leaf axils is likely to occur.

Gynoecious C. sativus (MSU 713-5) seedlings planted in early summer and subjected to a severe cold spell of a few days duration were observed to produce unexpected

staminate flowers (C. E. Peterson, unpublished results). This is in contrast to normal field conditions where lower temperatures favor female sex expression in monoecious C. sativus. It was postulated that the effect of this environmental shock might be due to a change in either synthesis or metabolism of gibberellin or some other endogenous growth regulator. In addition, vernalization (chilling) of hazel seeds followed by incubation at 20°C increased the quantity of organic solvent-extractable gibberellin (Ross and Bradbeer, 1968). In view of these observations it appeared that chilling of C. sativus seed might affect gibberellin levels as well as sex expression.

Greenhouse-grown monoecious and gynoecious plants were produced from chilled, germinated seeds. The staminate to pistillate ratio in monoecious plants was unaffected relative to non-chilled plants, but there was a definite induction of staminate flowers in gynoecious plants produced from chilled seed (Table 5). This induction corresponded closely to increased gibberellin activity (relative to control) in one-week old gynoecious seedlings (Table 6). Thus, staminate flower induction in chilled gynoecious C. sativus plants may be due to increased gibberellin activity. The mechanism by which chilling increases the synthesis or release of gibberellin remains unknown.

Several lines of evidence, summarized below, now exist which support the theory that endogenous gibberellins are an integral part of C. sativus sex expression regulation and

deserve the title "male hormone" of this species. Exogenously applied gibberellins have successfully induced staminate flowers in many gynoecious lines. Longer hypocotyl and internode lengths, indicators of endogenous gibberellin levels, have been correlated with more male sex types. Known inhibitors of gibberellin biosynthesis, such as CCC, increase femaleness. Shoot diffusates of four day old monoecious seedlings and root exudates of six week old monoecious plants contained higher levels of gibberellin than did those of gynoecious. Greater amounts of applied gibberellin have been recovered from monoecious than from gynoecious plants. Organic and buffer extraction of seeds, etiolated seedlings, and green plants in a wide range of growth stages has demonstrated that a monoecious and an andromonoecious line consistently contained higher levels of both free and "bound" gibberellin activity than a gynoecious line. Vernalization of gynoecious C. sativus seed increased both male tendency and gibberellin activity.

Kubicki (1969a) hypothesized that C. sativus sex type is determined by the balance of hormone substances "M" and "F" in the region of the incipient flower bud. High levels of the ovary-forming substance "F" lead to pistillate or perfect flower formation while staminate flowers are formed in the presence of low levels of "F". In view of the antagonistic effects of auxin and ethylene versus gibberellin on C. sativus sex expression, this hypothesis seems reasonable.

Table 19 contains a listing of the genes which are known to affect C. sativus and C. melo sex expression. It represents a compilation of data from several investigators (Kubicki, 1969a-h; Galun, 1961; Rowe, 1969). Kubicki (1969a) associates "F" with locus acr: allele acr^+ conditions reduced accumulation of "F", acr^F conditions a high rate of accumulation of "F", and acr^1 is intermediate. According to Kubicki, the activity of this gene is increased by auxin and reduced by gibberellins, auxins being inductors, and gibberellins co-repressors in the operon system governing synthesis of "F". Exogenous gibberellin would then reduce the activity of "F" in gynoecious plants to a level at which staminate flowers can be temporarily formed. Presumably, high levels of endogenous gibberellin would function similarly.

On the other hand, it appears equally probable that endogenous gibberellin activity is directly associated with locus acr. Of the five loci known to affect sex expression, only the occurrence of acr can be correlated with gibberellin activity; acr^+acr^+ occurs in homozygous monoecious and andromonoecious lines while acr^Facr^F occurs in homozygous gynoecious lines. Homozygous gynoecious and monoecious lines contain the same alleles of loci a, m, and tr. However, locus acr may also be associated with the level of a gibberellin inhibitor, auxin, an auxin inhibitor, or some other sex determinant.

Table 19

Genetics of *Cucumis* Sex Expression

A. *C. sativus*

Phenotype	Homozygous Genotype			
	potential for female flowers	perfect or female flowers	female intensity[1]	superior ovary
androecious	aa	--	acr^+acr^+--	--
andromonoecious	AA	mm	acr^+acr^+--	trtr
monoecious	AA	MM	acr^+acr^+--	trtr
trimonoecious	AA	MM	acr^+acr^+--	TrTr
hermaphroditic	AA	mm	acr^Facr^FFF	trtr
gynoecious	AA	MM	acr^Facr^Fff	trtr

[1] *st*$^+$ according to Galun (1961)

B. *C. melo*

Phenotype	Homozygous Genotype		
	potential for male flowers	perfect or female flowers[2]	superior ovary
andromonoecious	GG	mm	$Tr_1Tr_1tr_2tr_2$
monoecious	GG	MM	$Tr_1Tr_1tr_2tr_2$
trimonoecious	--	--	$tr_1tr_1Tr_2Tr_2$
hermaphroditic	gg	mm	$Tr_1Tr_1tr_2tr_2$
gynoecious	gg	MM	$Tr_1Tr_1tr_2tr_2$

[2] *a* according to Poole and Grimball (1939)

Assuming that locus acr is associated with either gibberellin, auxin, or "F" levels, some predictions may be made. If gibberellin is associated with acr, homozygous androecious and trimonoecious lines, which contain allele acr^+, should contain gibberellin levels similar to those in monoecious and andromonoecious lines. Homozygous hermaphroditic lines ($acr^F acr^F$) should then be relatively gibberellin deficient.

Auxin can induce female flowers in androecious C. sativus plants (acr^+) (Kubicki, 1969c). A hermaphroditic line has been found to contain more auxin than a closely related andromonoecious line (Galun et al., 1965); only alleles of locus acr should be different in these lines. These findings indicate an association of auxin with the acr locus. Androecious, trimonoecious, and monoecious lines should then be auxin deficient relative to gynoecious lines. However, these predictions of relative auxin and gibberellin levels based on which hormone is associated with acr are not mutually exclusive since the same sex types predicted to contain relatively high gibberellin levels are also predicted to be auxin deficient.

In contrast to C. sativus, seeds and green plants of monoecious and andromonoecious C. melo lines were found to be gibberellin deficient relative to gynoecious and hermaphroditic lines (Tables 7 and 8). This finding is consistent with the failure of exogenous gibberellin to induce staminate flowers on gynoecious C. melo plants. Thus, gibberellin is not the "male hormone" of C. melo.

Staminate flowers have been induced on gynoecious C. melo (MSU 1G) plants by grafting melon scions onto either pumpkin rootstocks or interstocks. Thus, the active factor in staminate flower production appeared to be synthesized in, or associated with, the pumpkin foliage. Since gynoecious C. melo is not gibberellin deficient relative to more male types, and exogenous application of gibberellins has not successfully induced staminate flowers in gynoecious C. melo, a hormone or regulator other than gibberellin was suspected to be responsible for the grafting effect.

Methanol extraction of pumpkin seeds and leaves of two varieties which successfully induce staminate flowers yielded levels of gibberellin activity far lower than that in any C. melo sex type (Table 9). Thus, the relatively small amount of gibberellin produced by pumpkin stocks would not be expected to affect C. melo sex expression. This is consistent with the hypothesis that gibberellin is not the "male hormone" of C. melo.

Gene g (Table 19) plays a role in C. melo female intensity analagous to that of gene acr in cucumber, but the dominant allele G occurs in the more male sex types. Kubicki associates allele G with high levels of "M", but it appears that gibberellin levels are not associated with g since gibberellin does not increase maleness in C. melo. Since gibberellin levels are relatively high in hermaphroditic and gynoecious lines (recessive alleles gg) and low in lines containing GG, gibberellin might be

considered a female hormone in C. melo. However, this is unlikely in view of the absence of any report that exogenous gibberellin increases femaleness in C. melo. Apparently, gibberellin has little influence on sex expression in this species.

Hayashi et al. (1971) demonstrated that the primary free acidic gibberellin in six day old etiolated monoecious seedlings of C. sativus is A_1. Gas-liquid chromatography also indicated that A_3, A_4, and A_7 might be present. The free acidic gibberellins of C. sativus seed have been shown here to be A_1, A_3, A_4, and probably A_7; A_1 accounts for most of the total gibberellin activity. Since all three sex types contain the same relative proportions of these gibberellins, the quantity rather than type of gibberellin appears to be important in sex expression. Gibberellins A_4 and A_7, more active than A_1 or A_3 in promoting staminate flowers in C. sativus, contribute only a small fraction of the total extractable activity. This situation may be due to easier absorption of the less-polar A_4 and A_7 by the plant, or A_1 may represent a slightly deactivated storage or transport form while A_4 and A_7 are the actual active species.

C. melo free acidic gibberellins include A_1, A_3, and probably A_5. A_1 and A_3 are present in far greater amounts than A_5. As in C. sativus, the relative proportions of the gibberellins are the same for the four sex types investigated.

Three "bound" or conjugated gibberellins, a glucoside and two esters, were isolated from mature seeds of monoecious C. sativus (MSU 736); evidence for the presence of "bound" gibberellin in andromonoecious and gynoecious seed was also obtained. Monoecious seed was used for isolation and identification of these compounds due to overall higher levels of gibberellins in these seeds and the availability of large seed quantities necessary to isolate several milligrams of the compounds.

The original evidence for the presence of "bound" gibberellins in C. sativus seed was extraction of increased amounts of free acidic gibberellins following incubation of tissue homogenates with ficin or β-glucosidase (Table 1). An initial decrease in this "bound" gibberellin upon germination corresponded with increased amounts of free gibberellin. Following germination, developing seedlings synthesized increasing amounts of both free and "bound" gibberellins.

The release of gibberellin by enzymes was at first attributed to hydrolysis of protein-gibberellin or carbohydrate-gibberellin bonds. Such protein- or carbohydrate-gibberellin conjugates would not be extracted with organic solvents. However, precipitation of low-molecular weight compounds with protein is a non-specific phenomenon which can be observed with gelatine solutions. Thus, much of the ficin release of gibberellin may be due to hydrolysis of protein and solubilization of gibberellin non-specifically complexed with protein by other than covalent bonds.

β-Glucosidase release of gibberellin may be attributed, in part, to hydrolysis of specific carbohydrate-gibberellin covalent bonds such as occur in gibberellin-β-glucosides; GA_1-β-D-glucoside may contribute to the total enzyme-releasable activity. The two isolated gibberellin esters are probably not part of the enzyme-releasable activity. They were isolated from the neutral fraction and make up only a small part of the extractable activity.

GA_1-β-D-glucose is similar to glucosides of gibberellins A_3, A_8, A_{26}, and A_{27} isolated from several sources. Its occurrence in dry seeds and decline during germination suggest a storage function; however, the high water solubility of the glucosides suggests a transport function. The occurrence of glucosides in bleeding sap of several tree species (Sembdner et al., 1968) also suggests a transport function. These two functions may not be antagonistic; glucosides found in bleeding sap may be stored gibberellins in transport from storage areas to other tissues.

The occurrence of neutral gibberellin propyl esters as natural constituents of plants is a unique finding. The occurrence of such compounds is surprising in view of the relative absence of esters of other plant acids. The possibility that these compounds represent an artifact of the isolation procedure is small since no C_3 alcohols, acids, ethers or other C_3 compounds were utilized in the extraction process. Elimination of isopropanol in TLC and paper chromatography did not result in elimination of spots due

to the esters. The identification of the A_3 n-propyl ester is beyond doubt since the synthetic compound had the same IR and mass spectra, R_f in three solvent systems, and melting point.

The function of gibberellin esters in plant metabolism is unknown but a storage function seems likely since esterification represents both a deactivation and depolarization. A decrease in polarity makes transport in sap less likely as a function, but transport across a hydrophobic membrane may be facilitated.

Possibilities for Future Research:

The results presented in this thesis point to several promising avenues for further investigations of the relationship of plant hormones to sex expression. As sufficient quantities of seed become available, gibberellin activity should be assayed in other C. melo and C. sativus sex types. Assays of mature seed and one growth stage of green plants, as reported above for C. melo, do not require enormous amounts of material. Assay of gibberellin activity might also be extended to other Cucurbita species such as pumpkin, squash, watermelon, etc. as different sex types of these species become available.

Assay of the activity of other hormones, e.g., auxin, ethylene, and cytokinin, in a wide variety of sex types of these species would provide additional information on the role of phytohormones in sex expression. Comparison of these results with the genetic background of the plants

would be of theoretical interest in regard to the biochemical genetics of sex expression. Of more immediate importance to human welfare is the probability that a complete picture of the relationship of hormones to sex expression would allow control of sex expression through hormone application. Such control might greatly facilitate development of new hybrids as well as increase productivity.

From the biochemist's point of view the presence of gibberellin glucosides and esters in C. sativus raises interesting questions about the biosynthesis of these compounds. Labelling studies might determine the precursor of the n-propanol fragment of the GA_1 and GA_3 esters.

Perhaps most interesting is the question of the mechanism of gibberellin action in C. sativus. How does gibberellin induce male floral parts, or is this a secondary effect? Are there specific receptors for gibberellin on cell or nuclear membranes? Does gibberellin induce DNA synthesis, RNA synthesis, or bind to an enzyme to increase its activity? Each of these questions poses an interesting problem for future research.

BIBLIOGRAPHY

BIBLIOGRAPHY

Abdel-Gawad, H. A. and H. J. Ketellapper. 1968. The effect of 2-chloroethyltrimethylammonium chloride (CCC) and N^6-benzyladenine on growth and flowering of squash plants. Abstract No. 98, 65th Ann. Meeting Am. Soc. Hort. Sci., Davis, Calif.

Abeles, F. B. 1967. Mechanism of action of abscission accelerators. Physiol. Plantarum 20, 442-54.

Abeles, F. B., R. E. Holm, H. E. Gahagan. 1967. Abscission: the role of aging. Plant Physiol. 42, 1351-56.

Abeles, F. B. and B. Rubenstein. 1964. Regulation of ethylene evolution and leaf abscission by auxin. Plant Physiol. 39, 963-69.

Alvim, P. De T. and I. Quinones. 1967. Control of sex in cucumber blossoms for increased productivity. Ciencia Cult. (Sao Paulo) 19, 562-6.

Andreae, W. A. and N. E. Good. 1955. The formation of indoleacetylaspartic acid in pea seedlings. Plant Physiol. 30, 381-2.

Atal, C. K. 1959. Sex reversal in hemp by application of gibberellin. Current Sci. (India) 28, 408-9.

Atsmon, D. and E. Galun. 1960. A morphogenic study of staminate, pistillate and hermaphroditic flowers in Cucumis sativus L. Phytomorphology 10, 110-15.

Atsmon, D. and E. Galun. 1962. Physiology of sex in Cucumis sativus L. Leaf age patterns and sex differentiation of floral buds. Ann. Botany (London) 26, 137-46.

Atsmon, D., A. Lang, E. N. Light. 1968. Contents and recovery of gibberellins in monoecious and gynoecious cucumber plants. Plant Physiol. 43, 806-10.

Atsmon, D., E. N. Light, A. Lang. 1967. Relationships between gibberellins and sex expression in cucumber. (i) Effects of environmental conditions and gibberellin application on floral bud development. Plant Research '67, MSU/AEC Plant Research Laboratory. p. 57-8.

Aung, L. H., A. A. De Hertogh, G. Staby. 1969. Temperature regulation of endogenous gibberellin activity and development of Tulipa gesneriana L. Plant Physiol. 44, 403-6.

Bandurski, R. S., M. Ueda, P. B. Nicholls. 1969. Esters of indole-3-acetic acid and myo-inositol. Ann. N. Y. Acad. Sci. 165, 655-67.

Barendse, G. W. M., H. Kende, A. Lang. 1968. Fate of radioactive gibberellin A_1 in maturing and germinating seeds of peas and Japanese morning glory. Plant Physiol. 43, 815-22.

Binks, R., J. MacMillan, R. J. Pryce. 1969. Plant Hormones-VIII. Combined gas chromatography-mass spectrometry of the methyl esters of gibberellins A_1 to A_{24} and their trimethylsilyl ethers. Phytochemistry 8, 271-84.

Bose, T. K. and J. P. Nitsch. 1970. Chemical alteration of sex-expression in Luffa acutangula. Physiol. Plantarum 23, 1206-11.

Brantley, B. B. and G. F. Warren. 1960. Sex expression and growth in muskmelon. Plant Physiol. 35, 741-5.

Brian, P. W., J. Grove, T. Mulholland. 1967. Relations between structure and growth promoting activity of the gibberellins and some allied compounds in four test systems. Phytochemistry 6, 1475-99.

Bukovac, M. J. and S. H. Wittwer. 1961. Gibberellin modification of flower sex expression in Cucumis sativus L. Adv. Chem. Ser. 28, 80-88.

Burg, S. P. and E. A. Burg. 1966. The interaction between auxin and ethylene and its role in plant growth. Proc. Nat. Acad. Sci. 55, 262-69.

Catarino, F. M. 1964. Effects of kinetin on sex expression in Kalanchoe crenata. Port. Acta Biol., Ser. A. 8, 267-84.

Champault, A. 1969. Masculinization of female Mercurialis annua flowers by in vitro cultivation of nodes isolated in the presence of auxins. C. R. Acad. Sci., Ser. D. 269, 1948-50.

Choudhury, B. and Y. S. Babel. 1969. Sex modification by chemicals in bottle gourd Lagenaria siceraria. Sci. Cult. (Calcutta) 35, 320-22.

Choudhury, B. and S. C. Phatak. 1959. Sex expression and sex ratio in cucumber (Cucumis sativus L.). Indian J. Hort. 16, 164-69.

Choudhury, B. and S. C. Phatak. 1960. Further studies on sex expression and sex ratio in cucumber as affected by plant regulator sprays. Indian J. Hort. 17, 210-16.

Clark, R. K. and D. S. Kenney. 1969. Comparison of staminate flower production on gynoecious strains of cucumbers, Cucumis sativus L., by pure gibberellins (A_3, A_4, A_7, A_{13}) and mixtures. J. Am. Soc. Hort. Sci. 94, 131-32.

Conrad, K. and K. Mothes. 1961. Sex-linked differences in auxin content in dioecious hemp plants. Naturwissenschaften 48, 26-27.

Cooke, A. R. and D. J. Randall. 1968. Induction of flowering by 2-haloethanephosphonic acid. Nature 218, 974.

Cooper, W. C., G. Rasmussen, B. Rogers, P. Reece, W. Henry. 1968. Control of abscission in agricultural crops and its physiological basis. Plant Physiol. 43, 1560-76.

Coyne, D. P. 1970. Effect of 2-chloroethylphosphonic acid on sex expression and yield in butternut squash and its usefulness in producing hybrid squash. Hort. Sci. 5, 227-8.

Czao, C. S. 1957. The effect of external factors on the sex ratio of cucumber flowers. Acta Sci. Nat. Univ. Pekin. 3, 233-45.

Davidyan, G. 1967. The effect of gibberellin on growth, development, and sex of hemp. Sel'skokhoz. Biol. 2, 90-4.

Durand, B. 1966. Effect of a kinetin on the sexual characteristics of Mercurialis annua. C. R. Acad. Sci., Ser. D. 263, 1309-11.

Fuchs, Y. and M. Lieberman. 1968. Effects of kinetin, IAA, and gibberellin on ethylene production, and their interactions in growth of seedlings. Plant Physiol. 43, 2029-36.

Galun, E. 1956. Effect of seed treatments on sex expression in the cucumber. Experientia 12, 218-19.

Galun, E. 1959a. Effects of gibberellic acid and naphthaleneacetic acid on sex expression and some morphological characters in the cucumber plant. Phyton (Buenos Aires) 13, 1-8.

Galun, E. 1959b. The role of auxins in the sex expression of the cucumber. Physiol. Plantarum. 12, 48-61.

Galun, E. 1961. Study of the inheritance of sex expression in the cucumber. The interaction of major genes with modifying genetic and non-genetic factors. Genetica 32, 134-63.

Galun, E., Y. Jung, A. Lang. 1962. Culture and sex modification of male cucumber buds in vitro. Nature 194, 596-98.

Galun, E., Y. Jung, A. Lang. 1963. Morphogenesis of floral buds of cucumber in vitro. Develop. Biol. 6, 370-87.

Galun, E., S. Izhar, D. Atsmon. 1965. Determination of relative auxin content in hermaphrodite and andromonoecious Cucumis sativus. Plant Physiol. 40, 321-6.

Gargiulo, A. 1968. Change of sex in grapes. Transformation of masculine flowers into hermaphrodites by application of a synthetic kinin. Vitis 7, 294-8.

Glushchenko, G. 1970. Effect of growth substances on the male reproductive system of plants. Sel'skokhoz. Biol. 5, 37-43.

Gupta, G. R. P. and S. C. Maheshwari. 1970. Cytokinins in seeds of pumpkin. Plant Physiol. 45, 14-18.

Halevy, A. H. and Y. Rudich. 1967. Modification of sex expression in muskmelon by treatment with the growth retardant B-995. Physiol. Plantarum 20, 1052-58.

Hall, W. C. 1949. Effects of photoperiod and nitrogen supply in growth and reproduction in the gherkin. Plant Physiol. 24, 753-69.

Harada, H. and T. Yokota. 1970. Isolation of gibberellin A_8-glucoside from shoot apices of Althaea rosea. Planta 92, 100-04.

Hashimoto, T. and L. Rappaport. 1966a. Variations in endogenous gibberellins in developing bean seeds. I. Occurrence of neutral and acidic substances. Plant Physiol. 41, 623-28.

Hashimoto, T. and L. Rappaport. 1966b. Variations in endogenous gibberellins in developing bean seeds. II. Changes induced in acidic and neutral fractions by GA_1. Plant Physiol. 41, 629-32.

Hashizume, H. 1960. Changes in endogenous growth substances during flower initiation and sex differentiation in Cryptomeris japonica. Tottori Nagakkaiho 12, 150-4.

Hayase, H. and M. Tanaka. 1966. Experimental modification of sex expression in the cucumber plant. II. The node range of staminate flowers in the gynoecious cucumber line MSU 713-5 induced by gibberellin treatment. Hokkaido Nogyo Shikensho Iho. No. 90, 24-30.

Hayase, H. and M. Tanaka. 1967. Experimental modification of sex expression in the cucumber plant. IV. Induction of staminate flowers in the gynoecious MSU 713-5 by application of gibberellin and (2-chloroethyl)-trimethylammonium chloride (CCC) simultaneously or alternately. Hokkaido Nogyo Shikensho Iho No. 91, 26-30.

Hayashi, F., S. Blumenthal-Goldschmidt, L. Rappaport. 1962a. Acid and neutral gibberellin-like substances in potato tubers. Plant Physiol. 37, 774-80.

Hayashi, F. and L. Rappaport. 1962b. Gibberellin-like activity of neutral and acidic substances in the potato tuber. Nature 195, 617-18.

Hayashi, F., H. M. Sell, C. E. Peterson. 1967. Gibberellin content of monoecious and gynoecious cucumber. Seventh International Congress of Biochemistry, Abstract J-180, Tokyo.

Hayashi, F., D. Boerner, C. E. Peterson, H. M. Sell. 1971. The relative activity of gibberellin in seedlings of gynoecious and monoecious cucumber (Cucumis sativus). Phytochemistry 10, 57-62.

Herich, R. 1960. Gibberellin and sex differentiation of flowering plants. Nature 188, 599-600.

Heslop-Harrison, J. 1956. Auxin and sexuality in Cannabis sativa. Physiol. Plantarum 9, 588-97.

Heslop-Harrison, J. 1957. The experimental modification of sex expression in flowering plants. Biol. Revs. Cambridge Phil. Soc. 32, 38-90.

Heslop-Harrison, J. and Y. Heslop-Harrison. 1957. Flowering plant growth and organogenesis. I. Morphogenetic effects of 2,3,5-triiodobenzoic acid on Cannabis sativa. Proc. Roy. Soc. Edinburgh 66B, 409-23.

Hillyer, I. G. and S. H. Wittwer. 1959. Chemical and environmental relationships in flowering of acorn squash. Proc. Am. Soc. Hort. Sci. 74, 558-68.

Ikekawa, N., T. Kagawa, Y. Sumiki. 1963. Biochemistry of Bakanae fungus. LXVII. Determination of nine gibberellins by gas and thin layer chromatographies. Proc. Japan Acad. 39, 507-12.

Ito, H. and T. Saito. 1956a. Factors responsible for the sex expression of Japanese cucumber. III. The role of auxin in the plant growth and sex expression (1). J. Hort. Assoc. Japan 25, 101-10.

Ito, H. and T. Saito. 1956b. Factors responsible for the sex expression of Japanese cucumber. IV. The role of auxin in the plant growth and sex expression (2). J. Hort. Assoc. Japan 25, 141-51.

Ito, H. and T. Saito. 1957a. Factors responsible for the sex expression of Japanese cucumber. VII. Effects of long day and high night temperature treatment applied for short duration at various stages of seedling development on the sex expression of flowers. J. Hort. Assoc. Japan 26, 149-53.

Ito, H. and T. Saito. 1957b. Factors responsible for the sex expression of Japanese cucumber. VIII. Effects of long day and high night temperature treatment of short duration, accompanied by growth substance spray application, on the sex expression of cucumber. J. Hort. Assoc. Japan 26, 209-14.

Iwahori, S., J. Lyons, W. Sims. 1969. Induced femaleness in cucumber by 2-chloroethanephosphonic acid. Nature 222, 271-72.

Iwahori, S., J. Lyons, O. E. Smith. 1970. Sex expression in cucumber plants as affected by 2-chloroethylphosphonic acid, ethylene, and growth regulators. Plant Physiol. 46, 412-15.

Janick, J. and E. Stevenson. 1955. Environmental influences on sex expression in monoecious lines of spinach. Proc. Am. Soc. Hort. Sci. 65, 416-22.

Jones, D. F. 1964. Examination of the gibberellins of Zea mays and Phaseolus multiflorus using thin-layer chromatography. Nature 202, 1309-10.

Jones, R. L. 1968. Aqueous extraction of gibberellins from pea. Planta 81, 97-105.

Jones, R. L. and J. E. Varner. 1967. The bioassay of gibberellins. Planta 72, 155-61.

Kamienska, A. 1966. The dynamics of gibberellin-like substances and growth inhibitors during the development of leaves of black poplar (Populus nigra L). Roczniki Nauk Rolniczych 91, 673-80.

Karchi, Z. 1970. Effects of 2-chloroethanephosphonic acid on flower types and flowering sequences in muskmelon. Jour. Am. Soc. Hort. Sci. 95, 515-18.

Kende, H. 1967. Preparation of radioactive gibberellin A_1 and its metabolism in dwarf peas. Plant Physiol. 42, 1612-18.

Khryanin, V. N. 1969. Gibberellin as a factor in the sex shift of hemp. Sel'skokhoz. Biol. 4, 753-8.

Klämbt, H. D. 1960. Indol-3-acetylasparaginsäure, ein natürlich vorkommendes Indolderivat. Naturwissenschaften 47, 398.

Klämbt, H. D. 1961. Wachstumsinduktion und Wuchsstoffmetabolismus im Weizenkoleoptilzylinder. II. Mitteilung. Stoffwechselproducte der Indol-3-essigsäure und der Benzosäure. Planta 56, 618-31.

Kooistra, E. 1967. Femaleness in breeding glasshouse cucumbers. Euphytica 16, 1-17.

Koshimizu, K., M. Inui, H. Fukui, T. Mitsui. 1968. Isolation of (+)-abscisyl-β-D-glucopyranoside from immature fruit of Lupinus luteus. Agr. Biol. Chem. (Tokyo) 32, 789-91.

Kubicki, B. 1966a. Genetic basis for obtaining gynoecious muskmelon lines and the possibility of their use for hybrid seed production. Genet. Pol. 7, 27-29.

Kubicki, B. 1966b. Sex determination in cucumbers (Cucumis sativus). I. The influence of 1-naphthaleneacetic acid and gibberellin on sex differentiation of flowers in monoecious cucumbers. Genet. Pol. 6, 153-76.

Kubicki, B. 1969a. Investigations on sex determination in cucumber (Cucumis sativus L.). IV. Multiple alleles of locus acr. Genet. Pol. 10, 23-68.

Kubicki, B. 1969b. Investigations on sex determination in cucumber (Cucumis sativus L.). V. Genes controlling intensity of femaleness. Genet. Pol. 10, 69-86.

Kubicki, B. 1969c. Investigations on sex determination in cucumber (Cucumis sativus L.). VI. Androecism. Genet. Pol. 10, 87-100.

Kubicki, B. 1969d. Investigations on sex determination in cucumber (Cucumis sativus L.). VII. Andromonoecism and hermaphroditism. Genet. Pol. 10, 101-122.

Kubicki, B. 1969e. Investigations on sex determination in cucumber (Cucumis sativus L.). VIII. Trimonoecism. Genet. Pol. 10, 123-44.

Kubicki, B. 1969f. Sex determination in muskmelon (Cucumis melo L). Genet. Pol. 10, 145-66.

Kubicki, B. 1969g. Comparative studies on sex determination in cucumber (Cucumis sativus L.) and muskmelon (Cucumis melo L.). Genet. Pol. 10, 167-83.

Kubicki, B. 1969h. Investigations on sex determination in cucumber (Cucumis sativus L.). III. Variability of sex expression in monoecious and gynoecious lines. Genet. Pol. 10, 3-22.

Kutuzova, S. N. 1968. Change in the sex of dioecious hemp under the influence of gibberellin. Sk. Tr. Aspir. Molodykh Nauch. Sotrudnikov, Vses. Narch.-Issled. Inst. Rastenievod. No. 9, 437-41.

Labarca, C., P. B. Nicholls, R. S. Bandurski. 1965. A partial characterization of indoleacetylinositols from Zea mays. Biochem. Biophys. Res. Comm. 20, 641-46.

Laibach, F. and F. Kribben. 1950a. Importance of β-indoleacetic acid in flower formation. Ber. Deut. Botan. Ges. 63, 119-20.

Laibach, F. and F. Kribben. 1950b. The effect of growth substance on the sex of flowers in a monoecious plant. Beitr. Biol. Pflanz. 28, 64-7.

Lang, A. 1970. Gibberellins: Structure and metabolism. Ann. Rev. Plant Physiol. 21, 537-70.

Lange, A. H. 1961. The effect of 2,3-dichloroisobutyrate and 2,2-dichloropropionate on the sex expression of Carica papaya. Proc. Am. Soc. Hort. Sci. 78, 218-24.

Limberk, J. 1954. The change of sex in hops (Humulus lupulus). Cesk. Biol. 3, 243-46.

Lower, R. L. and C. H. Miller. 1969. Ethrel (2-chloroethanephosphonic acid), a tool for plant hybridizers. Nature 222, 1072-73.

Mallik, P. C., R. K. Sahay, D. L. Singh. 1959. Effect of hormones on the sex ratio in mango. Current Sci. (India) 28, 410.

McComb, A. J. 1961. 'Bound' gibberellin in mature runner bean seeds. Nature 192, 575-76.

McGlasson, W. B. and H. K. Pratt. 1963. Fruit-set patterns and fruit growth in cantaloupe (Cucumis melo L., var. reticulatus Naud.). Proc. Am. Hort. Sci. 83, 495-505.

McMurray, A. L. and C. H. Miller. 1968. Cucumber sex expression modified by 2-chloroethanephosphonic acid. Science 162, 1397-8.

McMurray, A. L. and C. H. Miller. 1969. Effect of 2-chloroethanephosphonic acid (Ethrel) on the sex expression and yields of Cucumis sativus. J. Am. Soc. Hort. Sci. 94, 400-02.

Mehanik, F. 1958a. Acetylene treatment as a method of increasing the formation of fruitful female flowers in cucumber. Dok. Vses. Akad. Selskokhoz. Nauk 21, 20-23.

Mehanik, F. 1958b. The effect of acetylene on the development of female flowers in cucumber. Sad i Ogorod 10, 13-15.

Mishra, R. and B. Pradhan. 1970. Effect of (2-chloroethyl)-trimethylammonium chloride on sex expression in cucumber. J. Hort. Sci. 45, 29-31.

Mitchell, W. D. and S. H. Wittwer. 1962. Chemical regulation of flower sex expression and vegetative growth in Cucumis sativus. Science 136, 880-81.

Mockaitis, J. and A. Kivilaan. 1964. Graft induced sex changes in Cucumis melo L. Nature 202, 216.

Murakami, Y. 1961. Formation of gibberellin A_3 glucoside in plant tissues. Bot. Mag. (Tokyo) 74, 424-25.

Murakami, Y. 1962. Occurrence of 'water-soluble' gibberellin in higher plants. Bot. Mag. (Tokyo) 75, 451-52.

Murofushi, N., T. Yokota, N. Takahashi. 1970. Isolation and structures of gibberellins from immature seeds of Calonyction aculeatum. Agr. Biol. Chem. (Tokyo) 34, 1436-38.

Naugoljnyh, V. N. 1955. Chemical regulation of plant flowering. Botan. Zh. 40, 715-19.

Negi, S. and H. Olmo. 1966. Sex conversion in a male Vitis vinifera L. by a kinin. Science 152, 1624-25.

Nicholls, P. B. 1967. The isolation of indole-3-acetyl-2-O-myo-inositol from Zea mays. Planta 72, 258-64.

Nitsch, J. P., E. Kurtz, J. Liverman, F. W. Went. 1952. The development of sex expression in cucurbit flowers. Am. J. Bot. 39, 32-43.

Ogawa, Y. 1966a. Acid, neutral and "water-soluble" gibberellin-like substances occurring in developing seed of Sechium edule. Bot. Mag. (Tokyo) 79, 1-6.

Ogawa, Y. 1966b. Ethyl acetate-soluble and aqueous-soluble gibberellin-like substances in seeds of Pharbitis nil, Lupinus luteus, and Prunus persica. Bot. Mag. (Tokyo) 79, 67-76.

Ota, T. 1962. Studies on BCB (Bromocholine bromide). V. Effects of BCB on the growth and flowering of cucumber plant. J. Japan Soc. Hort. Sci. 31, 329-36.

Pegg, G. C. 1966. Changes in levels of naturally occurring gibberellin-like substances during germination of seed of Lycopersicon esculentum Mill. J. Exp. Botany 12, 214-30.

Peterson, C. E. 1960. A gynoecious inbred line of cucumber. Mich. State Univ. Agric. Exp. Sta. Quar. Bull. 43, 40-2.

Peterson, C. E. 1963. Gynoecious muskmelons for hybrid seed production. Abstract No. 332, 60th Ann. Meeting Amer. Soc. Hort. Sci., Amherst, Mass.

Peterson, C. E. and L. Anhder. 1960. Induction of staminate flowers on gynoecious cucumbers with gibberellin A_3. Science 131, 1673-74.

Phatak, S., S. H. Wittwer, S. Honma, M. J. Bukovac. 1966. Gibberellin-induced anther and pollen development in a stamenless tomato mutant. Nature 209, 635-36.

Phinney, B. O. 1956. Growth response of single-gene dwarf mutants in maize to gibberellic acid. Proc. Nat. Acad. Sci. 42, 185-89.

Pike, L. M. and C. E. Peterson. 1969. Gibberellin A_4/A_7 for induction of staminate flowers on the gynoecious cucumber (Cucumis sativus). Euphytica 18, 106-09.

Poole, C. and P. Grimball. 1939. Inheritance of new sex forms in Cucumis melo L. J. Heredity 30, 21-25.

Prakash, R. and S. Maheshwari. 1970. Studies on cytokinins in watermelon seeds. Physiol. Plantarum 23, 792-99.

Prasad, A., A. Dikshit, I. Tyagi. 1966. Effect of maleic hydrazide on sex expression and sex ratio in cucumber (Cucumis sativus). Sci. Cult. (Calcutta) 32, 596-97.

Prasad, A. and I. Tyagi. 1963. Effect of maleic hydrazide on the sex expression and sex ratio in bitter gourd (Momordica charantia L.). Sci. Cult. (Calcutta) 29, 605-06.

Pykhtina, T. K. 1968. Effect of gibberellin on the formation of staminate flowers in partially diclinous cucumber (Cucumis sativus). Vest. Mosk. Univ., Biol., Pochvoved. 23, 112-14.

Radley, M. 1958. The distribution of substances similar to gibberellic acid in higher plants. Ann. Bot. (London) 22, 297-307.

Ram, H. Y. and V. S. Jaiswal. 1970. Induction of female flowers on male plants of Cannabis sativa by 2-chloroethanephosphonic acid. Experientia 26, 214-16.

Reinhard, E. and R. Sacher. 1967a. Über die Gibberelline in Samen und Kapseln von Pharbitis purpurea. 1. Analyse der Gibberelline. Z. Pflanzenphysiol. 58, 138-50.

Reinhard, E. and R. Sacher. 1967b. Über die Gibberelline in Samen und Kapseln von Pharbitis purpurea. 2. Die Gibberelline in verschiedenen Entwicklungsstadien. Z. Pflanzenphysiol. 58, 151-64.

Reinhard, E. and R. Sacher. 1967c. Versuche zum enzymatischen Abbau det "gebundenen" Gibberelline von Pharbitis purpurea. Experientia 23, 415-16.

Robinson, R. W., S. Shannon, M. De la Guardia. 1969. Regulation of sex expression in the cucumber. Bioscience 19, 141-42.

Robinson, R. W., T. Whitaker, G. Bohn. 1970. Promotion of pistillate flowering in Cucurbita by 2-chloroethylphosphonic acid. Euphytica 19, 180-83.

Rodriguez, A. 1932. Chemical induction of pineapple flowering by ethylene. Jour. Dept. Agric., Puerto Rico 16, 5.

Rosa, J. T. 1927. Results of inbreeding melons. Proc. Am. Soc. Hort. Sci. 24, 79-84.

Rosa, J. T. 1928. The inheritance of flower types in Cucumis and Citrullus. Hilgardia 3, 233-50.

Ross, J. and J. Bradbeer. 1968. Concentrations of gibberellin in chilled hazel seeds. Nature 220, 85-86.

Rowe, P. R. 1969. The genetics of sex expression and fruit shape, staminate flower induction, and F_1 hybrid feasibility of a gynoecious muskmelon. Ph.D. Thesis, Mich. State Univ., Dept. of Horticulture p. 64-5.

Rudich, J., A. Halevy, N. Kedar. 1969. Increase in femaleness of three cucurbits by treatment with Ethrel, an ethylene releasing compound. Planta 86, 69-76.

Rudich, J., N. Kedar, A. Halevy. 1970. Changed sex expression and possibilities for F_1-hybrid seed production in some cucurbits by application of Ethrel and Alar (B-995). Euphytica 19, 47-53.

Saeed, S. and M. Akbar. 1970. Sex control in Citrullus by hormone application. Sci. Ind. (Karachi) 7, 91-2.

Saito, Y. 1957. Artificial control of sex differentiation in Japanese red pine and black pine strobiles. J. Fac. of Agric., Tottori Univ., III, 1-31.

Satyanarayana, G. and G. Rangaswami. 1959. The effect of plant regulators on sex expression in ribbed gourd (Luffa acutangula). Current Sci. (India) 28, 376-77.

Schaffner, J. H. 1921. Influence of environment on sexual expression in hemp. Botan. Gaz. 71, 197-219.

Schaffner, J. H. 1923. Sex reversal in the Japanese hop. Bull. Torrey Bot. 50, 73-79.

Schaffner, J. H. 1925. Experiments with various plants to produce change of sex in the individual. Bull. Torrey Bot. 52, 35-47.

Schreiber, K., G. Schneider, G. Sembdner, I. Focke. 1966. Gibberellins. X. Isolation of 2-O-acetylgibberellic acid as a metabolic product from Fusarium moniliforme. Phytochemistry 5, 1221-26.

Schreiber, K., J. Weiland, G. Sembdner. 1967. Gibberellins. XIII. Isolation and structure of a gibberellin glucoside. Tetrahedron Lett. 43, 425-28.

Sell, H. M., S. Rafos, M. J. Bukovac, S. H. Wittwer. 1959. Characterization of several n-alkyl esters of gibberellin A_3 and their comparative biological activity. J. Org. Chem. 24, 1822-23.

Sembdner, G. 1970. Gibberellins. XIV. Isolation of gibberellin A8-O(3)-β-D-glucopyranoside from Phaseolus coccineus fruit. Phytochemistry 9, 189-98.

Sembdner, G., G. Schneider, J. Weiland, K. Schreiber. 1964. Über ein gebundenes Gibberellin aus Phaseolus coccineus L. Experientia 20, 89-90.

Sembdner, G. and K. Schreiber. 1965. Gibberelline. IV. Über die Gibberelline von Nicotiana tabacum L. Phytochemistry 4, 49-56.

Sembdner, G., J. Weiland, O. Aurich, K. Schreiber. 1968. Isolation, structure, and metabolism of a gibberellin glucoside. S.C.I. Monograph No. 31, 70-86.

Shannon, S. and M. De la Guardia. 1969. Sex expression and the production of ethylene induced by auxin in the cucumber (Cucumis sativus). Nature 223, 186.

Shifriss, O. 1961a. Sex control in cucumbers. J. Heredity 52, 5-12.

Shifriss, O. 1961b. Gibberellin as sex regulator in Ricinus communis. Science 133, 2061-62.

Splittstoesser, W. E. 1970. Effects of 2-chloroethylphosphonic acid and gibberellic acid on sex expression and growth of pumpkins. Physiol. Plantarum 23, 762-68.

Stambera, J. and J. Zeman. 1969. Effect of gibberellin on the growth and development of the cucumber (Cucumis sativus). Rostl. Vyroba 42, 479-88.

Tamura, S., N. Takahashi, T. Yokota, N. Murofushi, Y. Ogawa. 1968. Isolation of water-soluble gibberellins from immature seeds of Pharbitis nil. Planta 78, 208-12.

Tanaka, M., R. Yosuke, H. Hayase. 1970. Effects of two growth retardants (CCC and B9) on vegetative growth, sex expression, and fruit yield in cucumber. Hokkaido Nogyo Shikenjo Iho No. 96, 47-53.

Tcakzenko, N. N. 1935. Trudy po Prikl. Bot. Gen. i Sel., Ser. 2, 9, 311-56.

Thompson, A. E. 1955. Methods of producing first generation hybrid seed in spinach. Cornell Univ. Agr. Exp. Sta. Mem. 336, 1-48.

Tibeau, M. E. 1936. Time factor in utilization of minerals by hemp. Plant Physiol. 11, 731-47.

Tiedjens, V. A. 1928. Sex ratios in cucumber flowers as affected by different conditions of soil and light. J. Agr. Res. 36, 721-46.

Varga, M. and M. Bito. 1968. On the mechanism of gibberellin-auxin interaction. I. Effect of gibberellin on the quantity of free IAA and IAA conjugates in bean hypocotyl tissues. Acta Biol. Acad. Sci. Hung. 19, 445-53.

Vergeley, E., I. Barthelmess, W. Hoffmann. 1967. The influence of short and long days and of chemicals on sex expression and growth type in hemp (Cannabis sativa). Z. Pflanzenzeucht. 57, 26-57.

Warner, H. L. and A. C. Leopold. 1969. Ethylene evolution from 2-chloroethylphosphonic acid. Plant Physiol. 44, 156-58.

Weston, E. W. 1960. Changes in sex in hop caused by plant growth substances. Nature 188, 81-82.

Wichert-Kolus, I. 1969. Effect of gibberellin on growth, development, and sex determination of Cannabis sativa plants treated with colchicine. Hodowla Rosl. aklim. Nasiennictwo 12, 711-22.

Winter, A. and K. Thimann. 1966. Bound indoleacetic acid in Avena coleoptiles. Plant Physiol. 41, 335-42.

Wittwer, S. H. and M. J. Bukovac. 1962. Staminate flower formation on gynoecious cucumbers as influenced by the various gibberellins. Naturwissenschaften 49, 305-06.

Wittwer, S. H. and I. G. Hillyer. 1954. Chemical induction of male sterility in cucurbits. Science 120, 893-94.

Wulfson, N., V. Zaretskii, I. Papernaja, E. Serebryakov, V. Kucherov. 1965. Mass spectrometry of gibberellins. Tetrahedron Lett. 47, 4209-16.

Yamaguchi, I., T. Yokota, N. Murofushi, Y. Ogawa, N. Takahashi. 1970. Isolation and structure of a new gibberellin from immature seeds of Prunus persica. Agr. Biol. Chem. (Tokyo) 34, 1439-41.

Yang, S. F. 1967. Biosynthesis of ethylene. Ethylene formation from methional by horseradish peroxidase. Arch. Biochem. Biophys. 122, 481-87.

Yang, S. F. 1969. Ethylene evolution from 2-chloroethylphosphonic acid. Plant Physiol. 44, 1203-04.

Yokota, T., N. Takahashi, N. Murofushi, S. Tamura. 1969a. Isolation of gibberellins A_{26} and A_{27} and their glucosides from immature seeds of Pharbitis nil. Planta 87, 180-84.

Yokota, T., N. Takahashi, N. Murofushi, S. Tamura. 1969b. Structures of new gibberellin glucosides in immature seeds of Pharbitis nil. Tetrahedron Lett. 1969, 2081-84.

Zenk, M. H. 1962. 1-(indole-3-acetyl)-β-D-glucose, a new compound in the metabolism of indole-3-acetic acid in plants. Nature 191, 493-94.

Zimmerman, P. W. and F. Wilcoxon. 1935. Several chemical growth substances which cause initiation of roots and other responses in plants. Contrib. Boyce Thompson Inst. 7, 209-29.