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# Plant Physiology

New Series Volume 9

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# Hormonal Regulation of Development I

## Molecular Aspects of Plant Hormones

Edited by
J. MacMillan

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## Molecular Aspects of Plant Hormones

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Contributors

J.R. Bearder A. Crozier D. Gross M.A. Hall
N.P. Kefford H.W. Liebisch J. MacMillan N. Murofushi
D.R. Reeve G. Schneider G. Sembdner J.L. Stoddart
N. Takahashi M.A. Venis T. Yokota M. Zeroni

With 126 Figures

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Editor:
Professor JAKE MACMILLAN
School of Chemistry
University of Bristol
Cantock's Close
Bristol BS8 1TS/United Kingdom

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Professor N.P. KEFFORD
Institute of Tropical Agriculture
and Human Resources
University of Hawaii
Honolulu, Hawaii 96822/USA

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# Foreword

This is the first of the set of three volumes in the Encyclopedia of Plant Physiology, New Series, that will cover the area of the hormonal regulation of plant growth and development. The overall plan for the set assumes that this area of plant physiology is sufficiently mature for a review of current knowledge to be organized in terms of unifying principles and processes. Reviews in the past have generally treated each class of hormone individually, but this set of volumes is subdivided according to the properties common to all classes. Such an organization permits the examination of the hypothesis that differing classes of hormones, acting according to common principles, are determinants of processes and phases in plant development. Also in keeping with this theme, a plant hormone is defined as a compound with the properties held in common by the native members of the recognized classes of hormone.

Current knowledge of the hormonal regulation of plant development is grouped so that the three volumes consider advancing levels of organizational complexity, viz: molecular and subcellular; cells, tissues, organs, and the plant as an organized whole; and the plant in relation to its environment.

The present volume treats the molecular and subcellular aspects of hormones and the processes they regulate. Although it deals with chemically distinct classes of hormone, this volume stresses properties and modes of studying them, that are common to all classes.

In a second volume of the set, the roles of hormones at levels of organization from the cell up to the whole plant are traced. The cellular processes of increase and change and the interrelations of cells in tissues, of tissues in organs, and of organs in the whole plant, are considered in turn. During this progressive treatment of levels of organization, the relevant basic properties of hormones are introduced and illustrated.

A third volume addresses the interrelationships of hormones with factors in the environments of the tissues, the organs and the whole plants, within which the hormones are functioning. When this volume touches upon wide-reaching topics such as photomorphogenesis or plant movements, only those aspects that relate to principles of hormonal regulation are treated. Separate volumes of the Encyclopedia of Plant Physiology, New Series, provide comprehensive treatments of topics such as a forthcoming volume on photomorphogenesis and one on plant movements (Vol. 7).

My role in the preparation of these volumes has been to propose a theme and prepare a plan to cover the current status of the field of hormonal regulation, then to circumscribe the portions of the plan that form logical volumes. Thereafter, the editors of the individual volumes have determined the manner in which the domain, for which they accepted responsibility, was treated. The editor

of the present volume is Professor J. MacMillan and, in an Introduction to his volume, he outlines his approach and that of his authors.

The base from which these volumes have developed is the old series of the Encyclopedia of Plant Physiology. The volumes in the New Series of the Encyclopedia may, therefore, concentrate on principles that may be derived from the mass of older information and on the findings of the past twenty years. The length of each volume has been deliberately restricted, but effective organization of topics and their succinct treatment assures the reader of a concise but comprehensive statement of current knowledge and thought in the field.

I thank Professor Kenneth V. Thimann for reviewing the theme and initial plan for these volumes with me.

October, 1980 N.P. KEFFORD

# List of Contributors


J.R. Bearder
School of Chemistry
University of Bristol
Cantock's Close
Bristol BS8 1TS/United Kingdom
Present address:
Shell Research Ltd.
Shell Biosciences Laboratory
Sittingbourne Research Centre
Sittingbourne, Kent ME9 8AG/
United Kingdom

A. Crozier
Botany Department
The University of Glasgow
Glasgow G12 8QQ/United Kingdom

D. Gross
Akademie der Wissenschaften der DDR
Institut für Biochemie der Pflanzen
Postfach 250
4010 Halle/GDR

M.A. Hall
Dept. of Botany and Microbiology
The University College of Wales
Aberystwyth, Dyfed,
Wales SY23 IRX/United Kingdom

N.P. Kefford
Institute of Tropical Agriculture
and Human Resources
University of Hawaii
Honolulu, Hawaii 96822/USA

H.W. Liebisch
Akademie der Wissenschaften der DDR
Institut für Biochemie der Pflanzen
Postfach 250
4010 Halle/GDR

J. MacMillan
School of Chemistry
University of Bristol
Cantock's Close
Bristol BS8 1TS/United Kingdom

N. Murofushi
Dept. of Agricultural Chemistry
The University of Tokyo
Bunkyo-ku
Tokyo/Japan

D.R. Reeve
Botany Department
The University of Glasgow
Glasgow G12 8QQ/United Kingdom

G. Schneider
Akademie der Wissenschaften der DDR
Institut für Biochemie der Pflanzen
Postfach 250
4010 Halle/GDR

G. Sembdner
Akademie der Wissenschaften der DDR
Institut für Biochemie der Pflanzen
Postfach 250
4010 Halle/GDR

J.L. Stoddart
Plant Biochemistry Department
University College of Wales
Welsh Plant Breeding Station
Aberystwyth, Dyfed SY23 3EB/
United Kingdom

N. TAKAHASHI
Dept. of Agricultural Chemistry
The University of Tokyo
Bunkyo-ku
Tokyo/Japan

M.A. VENIS
Shell Research Ltd.
Shell Biosciences Laboratory
Sittingbourne Research Centre
Sittingbourne, Kent ME9 8AG/
United Kingdom

T. YOKOTA
Dept. of Agricultural Chemistry
The University of Tokyo
Bunkyo-ku
Tokyo/Japan

M. ZERONI
Closed System Agriculture
The Jacob Blaustein Institute
for Desert Research
Sede Boger/Israel

# List of Abbreviations

Cytokinin abbreviations are given in Fig. 4.2 (p. 290).
Abbreviations, used in Figures and Tables only, are given therein.
Symbols used in Chapter 3 are listed at end of chapter.

| | |
|---|---|
| ABA | abscisic acid |
| ACC | 1-aminocyclopropane-1-carboxylic acid |
| AFID | alkali flame ionisation detector |
| AD-Co-Thr | N-[9-(β-D-ribofuranosyl)-purine-6-carbamoyl]threonine (Fig. 1.7, p. 39) |
| Alar | B-995, Succinic acid 2,2-dimethylhydrazide |
| AMO-1618 | 2′-isopropyl-4′-(trimethylammonium chloride)-5′-methylphenyl piperidine-1-carboxylate (Fig. 4.18, p. 315) |
| AMP | adenosine-5′-monophosphate |
| cAMP | cyclic adenosine-3′,5′-monophosphate |
| ATP | adenosine-5′-triphosphate |
| AVG | L-2-amino-4-(2′-aminoethoxy)-trans-3-butenoic acid (aminoethoxyvinylglycine) |
| B1 | buffer-insoluble (cellulase) |
| B-995 | Alar, succinic acid 2,2-dimethylhydrazide |
| BA | $N^6$-benzyladenine |
| BAR | 6-(o-hydroxybenzylamino)-9β-D-ribofuranosylpurine |
| BOA | 2-benzoxazoline |
| BS | buffer-soluble (cellulase) |
| BSA | bis-trimethylsilylacetamide |
| BSTFA | bis-trimethylsilyltrifluoroacetamide |
| c | cis |
| C-value | relative amount of DNA per nucleus; normal diploid cell after DNA synthesis and before mitosis has a 4C-value |
| $C_n$ | number of carbon atoms |
| C-n | position of carbon atom in molecule |
| CAPA | 2-chloro-4-aminophenoxyacetic acid |
| CCC | 2-chloroethyltrimethyl ammonium chloride |
| CCD | counter current distribution |
| CD | circular dichroism |
| CCCP | carbonylcyanide m-chlorophenylhydrazone |
| Chloramben | 2,5-dichloro-3-aminobenzoic acid |
| CI | chemical ionization |
| Ci | Curie |
| CoASH | coenzyme A |
| cv | cultivar |
| 2,4-D | 2,4-dichlorophenoxyacetic acid |
| DEAE | diethylaminoethyl |
| DMBOA | 6,7-dimethoxy-2-benzoxazoline |
| 2,4-DNP | 2,4-dinitrophenol |
| DP | degree of polymerization |
| 2,4-DP | 2-(dichlorophenoxy)propionic acid |
| DTE | dithioerythritol |
| EEi | elastic extensibility |
| EI | electron impact |
| ent | enantiomer |
| EtIAA | ethyl indole-3-acetate |
| ER | endoplasmic reticulum |
| FID | flame ionization detector |
| FMN | flavin mononucleotide |
| FUDR | 5-fluorodeoxyuridine |
| G1 | period of cell cycle following mitosis and prior to DNA synthesis |
| G2 | period of cell cycle following DNA synthesis and prior to mitosis |

| | |
|---|---|
| xg | X acceleration due to gravity |
| GA | gibberellin |
| GC-MS | combined gas chromatography-mass spectrometry |
| GC-CIMS | combined gas chromatography-chemical ionization mass spectrometry |
| GLC | gas liquid chromatography |
| GLP | growth-limiting protein |
| GMP | guanosine-5′-monophosphate |
| cGMP | cyclicguanosine-3′,5′-monophosphate |
| GPC | gel permeation chromatography |
| HFB | heptafluorobutyl |
| HPLC | high performance liquid chromatography |
| IAA | indole-3-acetic acid |
| IAAld | indole-3-acetaldehyde |
| IAcry | indole-3-acrylic acid |
| IAld | indole-3-carboxaldehyde |
| IAM | indole-3-acetamide |
| IAN | indole-3-acetonitrile |
| IBA | indole-3-butyric acid |
| ICA | indole-3-carboxylic acid |
| ICI | Imperial Chemical Industries Ltd. |
| IEt | indole-3-ethanol |
| ILA | indole-3-lactic acid |
| IPA | indole-3-propionic acid |
| IPyA | indole-3-pyruvic acid |
| IR | infrared |
| c-IRP | chromosomal IAA receptor protein |
| n-IRP | nuclear IAA receptor protein |
| KBM | α-keto-γ-(methylthio)butyric acid |
| KP | pellet obtained by centrifugation at $n \times 1000$ g. |
| LC | liquid chromatography |
| M | molar |
| MBOA | 6-methoxy-2-benzoxazolinone |
| MCPA | 2-methyl-4-chlorophenoxyacetic acid |
| MDMP | (D)-2-(4-methyl-2,6-dinitroanilino)-N-methylpropionamide |
| Me | methyl |
| MeneOx | methyleneoxindole |
| MeOx | methyloxindole |
| MF | mass fragmentography |
| MID | multiple ion detection |
| MTA | 5′-methylthioadenosine |
| MTR | 5′-methylthioribose |
| NAA | naphthyl-1(or 2)-acetic acid |
| NAD | nicotinamide-adenine dinucleotide |
| NADH | reduced nicotinamide-adenine dinucleotide |
| NADP | nicotinamide-adenine dinucleotide phosphate |
| NADPH | reduced nicotinamide dinucleotide phosphate |
| NMR | nuclear magnetic resonance |
| NPA | N-(naphth-1-yl)phthalamic acid |
| *o* | ortho |
| ORD | optical rotatory dispersion |
| p | para |
| PAA | phenylacetic acid |
| PAL | phenylalanine ammonia lyase |
| PC-C (PCT) | phosphorylcholine-cytidyltransferase |
| PC-G (PGT) | phosphorylcholine-glyceride transferase |
| PEi | plastic extensibility |
| PEP | phosphoenol pyruvate |
| Phosphon D | tributyl-2,4-dichlorobenzylphosphonium chloride (Fig. 4.18, p.315) |
| Phosphon S | tributyl-2,4-dichlorobenzylammonium chloride (Fig. 4.18, p. 315) |
| PM | plasma membrane |
| PMB | p-mercuribenzoate |
| PMBS | p-mercuribenzenesulphonate |
| PMSF | phenylmethylsulphonylfluoride |
| PP | pyrophosphate |
| ppb | parts per billion |

| | |
|---|---|
| PPC | paper chromatography |
| ppm | parts per million |
| PVP | polyvinylpyrrolidone |
| RER | rough endoplasmic reticulum |
| RF | radio-frequency |
| $R_F$ | ratio of travel of compound to travel of solvent front |
| RHS | right hand side |
| SAM | S-adenosylmethionine |
| SDS | sodium dodecylsulphate |
| SF | supernatant factor |
| SIM | selected ion monitoring |
| SKF-525A | 2,2-diphenylpentanoic acid 2-(dimethylamino)ethyl ester |
| SKF-7997 | tris-(N,N-diethylaminoethyl)-phosphate trichloride |
| SMM | S-methylmethionine |
| S | period of cell cycle during DNA synthesis |
| SID | single ion detection |
| SPC | silica gel chromatography |
| t | trans |
| TBA | trichlorobenzoic acid |
| TIBA | 2,3,6-triidobenzoic acid |
| TLC | thin layer chromatography |
| TMCS | trimethylchlorosilane |
| TMS | trimethylsilane |
| TMSi | trimethylsilyl |
| Trimarol | 2,4-dichlorophenylphenyl-5-pyrimidinylmethanol |
| UDP | uridinediphosphate |
| UV | ultra-violet light |
| WCOT | wall-coated open tubular (column for GLC) |
| WEC | continuous extensibility (of cell-wall) |
| Y | yield threshold |

## Nomenclature and Numbering

**Abscisic Acid and Derivatives.** The numbering of carbon atoms is shown in Table 1.16 (p. 49).

**Auxins.** Auxins are named thus, indole-3-acetic acid (IAA), naphthalene-1-acetic acid (NAA) etc. Numbering of the indole ring system is shown in Table 1.1 (p. 13).

**Gibberellins and Related Diterpenes.** The ent-gibberellane and ent-kaurane system of nomenclature is used and is explained in Chapter 1.4 (pp. 34–35). The numbering of the carbon skeleton is given in Fig. 1.4 (p. 38) and Fig. 1.5 (p. 38). Since the use of the ent-operator is confusing, in relation to drawn structures, when specifying $\alpha$- and $\beta$-stereochemistry for substituents, the authors of Chapter 4 have used the device of specifying the substituent and its stereochemistry outside the ent-operator. For example, ent-7$\alpha$-hydroxykaurenoic acid becomes 7$\beta$-hydroxy-ent-kaurenoic acid. Although strictly incorrect, this device has the advantage that the specified stereochemistry corresponds to the drawn structures.

**Cytokinins.** The nomenclature, numbering, and abbreviations are given in Fig. 4.2 (pp. 290–291). The abbreviations are based upon those established for nucleic acid derivatives (IUPAC-IUB Commission on Biochemical Nomenclature, 1970, J. Biol. Chem., 245, 5171–6).

# Contents

**Introduction**
J. MacMillan . . . . . 1

**1 Plant Hormones and Other Growth Substances – Their Background, Structures and Occurrence**
J.R. Bearder (With 10 Figures)

1.1 Introduction . . . . . 9
1.2 Ethylene . . . . . 9
1.3 Auxins . . . . . 12
1.4 Gibberellins . . . . . 22
1.5 Cytokinins . . . . . 38
1.6 Abscisic Acid and Related Compounds . . . . . 48
1.7 Other Plant Constituents Which Affect Plant Growth . . . . . 56
a) Aromatic Compounds . . . . . 58
b) Nitrogen-Containing Compounds . . . . . 74
c) Terpenoid Compounds . . . . . 76
d) Aliphatic Compounds . . . . . 79
e) Other Compounds . . . . . 80
References . . . . . 80

**2 Extraction, Purification, and Identification**
T. Yokota, N. Murofushi, and N. Takahashi (With 38 Figures)

2.1 Methods of Extraction, Purification, and Isolation . . . . . 113
2.1.1 Extraction of Active Principles from Plant Materials . . . . . 113
a) General Remarks . . . . . 113
b) Auxins . . . . . 114
c) Gibberellins . . . . . 116
d) Cytokinins . . . . . 117
e) Abscisic Acid and Related Compounds . . . . . 117
2.1.2 Fractionation Based on Solvent Partitioning and Ion Exchange Resin . . 118
a) General Remarks . . . . . 118
b) Auxins . . . . . 120
c) Gibberellins . . . . . 124
d) Cytokinins . . . . . 125
e) Abscisic Acid and Related Compounds . . . . . 128
2.1.3 Column Chromatography and Other Purification Techniques . . . . . 129
a) General Remarks . . . . . 129
b) Adsorption Column Chromatography . . . . . 130
c) Partition Column Chromatography . . . . . 132
d) Sephadex Column and Gel Permeation Column Chromatography . . 138
e) Insoluble Polyvinylpyrrolidone Column Chromatography . . . . . 139
f) Countercurrent Distribution . . . . . 142
g) Other Techniques . . . . . 143

2.1.4 Examples of Purification of Plant Hormones . . . . . 144
a) Isolation of Auxins from Young Citrus Fruits . . . . . 144
b) Isolation of Indole-3-Ethanol from Cucumber Seedlings . . . . . 145
c) Isolation of Gibberellins $A_1$, $A_5$, $A_6$, and $A_8$ from Immature *Phaseolus* Seeds . . . . . 146
d) Isolation of Gibberellins and Gibberellin Conjugates from Mature *Phaseolus* Seeds . . . . . 147
e) Isolation of Cytokinins from Immature Sweet Corn . . . . . 150
f) Isolation of Glucosylzeatin and Glucosyl Ribosylzeatin from *Vinca rosea* Crown Gall . . . . . 154
g) Isolation of Abscisic Acid from Young Cotton Fruits . . . . . 154
2.2 Identification Without Isolation . . . . . 154
2.2.1 Criteria of Identification and Reliability . . . . . 154
2.2.2 Paper and Thin-Layer Chromatography . . . . . 156
a) General Remarks . . . . . 156
b) Identification of Plant Hormones by Paper and Thin-Layer Chromatography . . . . . 157
2.2.3 Gas-Liquid Chromatography . . . . . 168
a) General Remarks . . . . . 168
b) Identification of Plant Hormones by Gas-Liquid Chromatography . . 169
2.2.4 High Performance (Pressure) Liquid Chromatography . . . . . 175
a) General Remarks . . . . . 175
b) Identification of Plant Hormones by High Performance Liquid Chromatography . . . . . 176
2.2.5 Combined Gas-Liquid Chromatography-Mass Spectrometry . . . . . 181
a) General Remarks . . . . . 181
b) Identification of Plant Hormones by GC-MS . . . . . 182
c) Selected Ion Monitoring . . . . . 184
2.2.6 Optical Rotatory Dispersion and Circular Dichroism . . . . . 190
References . . . . . 190

## 3 Quantitative Analysis of Plant Hormones

D.R. Reeve and A. Crozier (With 13 Figures)

3.1 Introduction . . . . . 203
3.2 Theoretical Considerations . . . . . 203
3.2.1 Basic Analytical Errors – Accuracy and Precision . . . . . 203
3.2.2 Analysis of Samples from Open-Ended Systems . . . . . 209
3.2.2.1 Open Versus Closed Systems . . . . . 209
3.2.2.2 Accuracy as a Probability Term . . . . . 209
3.2.2.3 Verification of Accuracy . . . . . 214
a) Successive Approximation . . . . . 215
b) Information Content . . . . . 226
c) Some General Considerations . . . . . 236
3.3 Assays . . . . . 240
3.3.1 Bioassays . . . . . 240
3.3.2 Immunological Assays . . . . . 245
3.3.3 Physicochemical Detectors . . . . . 247
3.4 Chromatographic Procedures . . . . . 254
3.5 Internal Standards . . . . . 262
3.6 Analytical Procedures . . . . . 264
3.6.1 Indole-3-Acetic Acid . . . . . 264
3.6.2 Gibberellins . . . . . 267
3.6.3 Cytokinins . . . . . 269
3.6.4 Abscisic Acid . . . . . 270
3.7 Summary . . . . . 272
3.8 List of Symbols . . . . . 274
References . . . . . 276

## 4 Biosynthesis and Metabolism of Plant Hormones

G. Sembdner, D. Gross, H.-W. Liebisch and G. Schneider (With 34 Figures)


4.1 Biosynthetic Pathways . . . . . 281
4.1.1 Auxins . . . . . 281
a) Tryptophan as Primary Precursor . . . . . 281
b) Pathways of IAA Formation from Tryptophan . . . . . 283
c) Indole-3-Pyruvic Acid Pathway . . . . . 283
d) Tryptamine Pathway . . . . . 287
e) Other Possible Pathways . . . . . 288
f) Chlorinated Auxins . . . . . 289
4.1.2 Cytokinins . . . . . 289
a) Biosynthesis of tRNA-Cytokinins . . . . . 289
b) Biosynthesis of Free Cytokinins . . . . . 292
4.1.3 Plant Hormones of Terpenoid Origin: General Terpenoid Pathway . . . 293
4.1.4 Abscisic Acid . . . . . 295
a) Mevalonic Acid as Precursor of ABA . . . . . 295
b) Compounds with a Pre-Formed Carbon Skeleton of ABA . . . . . 298
c) Formation of ABA by Degradation of Carotenoids . . . . . 300
4.1.5 Gibberellins . . . . . 301
a) General View . . . . . 301
b) Enzymic Cyclization of Geranylgeranyl Pyrophosphate to ent-Kaurene 304
c) Oxidation of ent-Kaurene . . . . . 307
d) Contraction of Ring B in ent-Kaurene . . . . . 309
e) Conversion of $C_{20}$-Gibberellins into $C_{19}$-Gibberellins . . . . . 311
f) Predominant Steps in Regulation of Biosynthesis . . . . . 314
4.1.6 Ethylene . . . . . 317
a) Ethylene Formation in Model Systems . . . . . 318
b) Synthesis in Vivo – Physiological Pathways . . . . . 319
c) Enzymes – Reaction Mechanisms . . . . . 324
d) Regulation of Ethylene Biosynthesis . . . . . 326
4.2 Metabolism (Interconversion and Catabolism) . . . . . 336
4.2.1 Auxins . . . . . 336
a) Routes and Products of IAA Catabolism . . . . . 336
b) Enzymes of IAA Catabolism . . . . . 340
c) Physiological Significance of IAA Catabolism . . . . . 345
d) Control of IAA Catabolism in Vivo . . . . . 346
4.2.2 Cytokinins . . . . . 347
4.2.3 Abscisic Acid . . . . . 350
a) 6′-Hydroxymethyl ABA . . . . . 350
b) Phaseic Acid . . . . . 352
c) 4′-Dihydrophaseic Acid and Its 4′-Epimer . . . . . 353
4.2.4 Gibberellins . . . . . 354
a) Interconversion of $C_{19}$-Gibberellins in the Fungus . . . . . 354
b) Metabolism of Gibberellins in Higher Plants . . . . . 355
c) Catabolism of Gibberellins . . . . . 359
d) Control of Gibberellin Metabolism by Other Plant Hormones and by Environmental Factors . . . . . 360
4.2.5 Ethylene . . . . . 362
4.3 Conjugation . . . . . 363
4.3.1 Auxins . . . . . 363
a) Peptidyl IAA Conjugates . . . . . 364
b) Glycosyl IAA Conjugates . . . . . 366
c) myo-Inositol IAA Conjugates . . . . . 367
4.3.2 Cytokinins . . . . . 368
a) Metabolic Conjugation Pathways . . . . . 368
b) Enzymic Investigations . . . . . 371
4.3.3 Abscisic Acid . . . . . 373

4.3.4 Gibberellins . . . 376
a) Endogenous Gibberellin Conjugates . . . 376
b) Chemical Synthesis of Gibberellin Conjugates . . . 376
c) Metabolism of Gibberellin Conjugates . . . 377
d) Enzymatic Hydrolysis and Biological Activity of Gibberellin Conjugates 381
e) Biological Function of Gibberellin Conjugates . . . 382
4.4 Localization of Biosynthesis and Metabolism . . . 382
4.4.1 Auxins . . . 383
4.4.2 Cytokinins . . . 384
4.4.3 Abscisic Acid . . . 387
4.4.4 Gibberellins . . . 387
4.4.5 Ethylene . . . 389
References . . . 390

**5 Molecular and Subcellular Aspects of Hormone Action**
J.L. STODDART and M.A. VENIS (With 21 Figures)

5.1 Introduction . . . 445
5.1.1 Receptor Characteristics . . . 445
5.1.2 General Methodology of Receptor Studies . . . 446
a) Analysis of Binding Data . . . 446
b) Acquisition of Binding Data . . . 446
5.2 Auxins . . . 448
5.2.1 Structure-Activity Relationships . . . 448
5.2.2 Potential Location of Auxin Receptors . . . 450
5.2.3 Nuclear and Cytoplasmic Receptors . . . 453
a) Auxin Mediator Proteins . . . 453
b) Auxin-Binding Proteins . . . 455
5.2.4 Membrane-Bound Receptors . . . 457
a) The Corn Coleoptile System . . . 457
b) Membrane-Bound Sites in Other Tissues . . . 469
5.3 Cytokinins . . . 470
5.3.1 Structure-Activity Relationships . . . 470
5.3.2 Cytokinins in Transfer RNA (tRNA) . . . 472
5.3.3 Cytokinin Binding to Sub-Cellular Components . . . 474
5.4 Gibberellins . . . 478
5.4.1 Structure-Activity Relationships . . . 478
5.4.2 Transcriptional Control: Analogies with Animal Steroid Hormones . . . 483
a) Steroid Hormones . . . 484
b) Gibberellins . . . 486
5.4.3 Effects on Membrane Organization . . . 490
5.4.4 Action in Artificial Membrane Systems . . . 492
5.4.5 Changes in Cell Extensibility . . . 497
5.5 Abscisic Acid . . . 498
5.6 Ethylene . . . 498
5.6.1 Structure-Activity Relationships . . . 499
5.6.2 Aspects of Molecular Action . . . 499
5.7 Concluding Remarks . . . 501
References . . . 501

**6 Molecular Effects of Hormone Treatment on Tissue**
M. ZERONI and M.A. HALL (With 10 Figures)

6.1 Introduction . . . 511
6.2 Effects of Hormones on Processes Involved in Growth . . . 513

6.2.1 Effects of Auxins on Cell Extensibility . . . . . 514
a) Kinetic Studies on Cell Extension . . . . . 516
b) Effects on Nucleic Acids and Proteins . . . . . 519
c) The Acid Growth Effect . . . . . 521
d) Effect on Metabolism of the Cell Wall . . . . . 524
6.2.2 Other Growth Regulators . . . . . 530
a) Gibberellins . . . . . 530
b) Cytokinins . . . . . 530
c) Ethylene . . . . . 532
d) Abscisic Acid . . . . . 532
e) 3-Methyleneoxindole . . . . . 532
f) Fusicoccin . . . . . 533
6.2.3 Conclusions . . . . . 534
6.3 Effects of Growth Regulators on Ion Transport and Regulation of Membrane Properties . . . . . 537
6.3.1 Hormones and Stomata . . . . . 541
6.3.2 Hormone-Directed Transport . . . . . 544
6.4 Effects of Hormones on Non-Growing Systems . . . . . 544
6.4.1 The Aleurone Layer System . . . . . 544
6.5 Effects of Hormones on Differentiating Systems . . . . . 553
6.6 Conclusions . . . . . 567
References . . . . . 568

**Author Index** . . . . . 587

**Subject Index** . . . . . 651

# Introduction

J. MacMillan

The scope of this volume has been deliberately limited and selective for the following reasons. Firstly, in accord with the underlying philosophy of the New Series of the Encyclopedia of Plant Physiology, a prime consideration has been to provide a volume of reasonable size enabling it to be found on the bookshelves of individuals as well as on library shelves. Secondly, since the publication in 1961 of the original Encyclopedia Volume XIV – Growth and Growth Substances – almost 10000 research papers on plant hormones and plant growth regulation have been published and literature citations alone could have filled a reasonably sized volume. Length has been kept within reasonable proportions in two other ways. In the early stages of planning it was intended to include chapters on bio-assay methods and on structure-activity relationships. However it became evident after discussing these plans with colleagues and prospective authors that there was little that could be added on these topics, per se, that had not been adequately covered in existing reviews. Thus the planned chapter on bio-assays has been replaced by one on quantitative analysis, including bio-assay methods, and the discussion on structure-activity relationships has been restricted to those aspects which are of direct relevance to the discussion of hormone receptors.

In the original Encyclopedia, Volume XIV on Growth and Growth Substances (1961) contained 1357 pages, over 500 of which were devoted to auxins and only 35 pages to the gibberellins; ethylene received passing mention and the cytokinins and abscisic acid were, of course, not included. In contrast, the present volume is concerned, in roughly equal measure, with the five presently recognized groups of plant hormones and Chapter 1 includes a brief review of other plant constituents which affect plant development. Since the five main groups of hormones may be presumed to act on basic processes of plant growth and development, either concertedly, consequentially, or separately, it seems logical to discuss the main groups of hormones together in relation to the properties common to each group. The six chapters in this volume have been organized in this way, rather than in the traditional method of discussing each group of hormones in separate chapters.

In Chapter 1 the five groups of hormones are introduced with a brief history. The individual members of each group, and their occurrence, are then listed. Only those hormones which have been unequivocally identified, either by isolation or by mass spectrometry, are given. As stated earlier the chapter also includes plant constituents which have been found to affect plant development.

As pointed out by the authors of Chapter 2, the recognition of the existence of a particular hormone ultimately depends upon its isolation and the determination of the biological and chemical properties of the pure hormones. The methods

by which plant extracts are fractionated and by which the pure hormones can be obtained are therefore the subject of Chapter 2. Examples of the isolation of each group are included. Once the hormones have been isolated and their properties have been determined, they may then be identified without isolation by methods which are discussed in this chapter. These methods, particularly combined gas chromatography-mass spectrometry, are being increasingly used to quantify the hormones in plant extracts and many examples of the use of deuterium-labelled hormones, as internal standards, in quantitative analysis by mass spectrometry have been described in the last two years.

The topic of quantitative analysis is critically examined in Chapter 3, in which the criteria required for accurate analysis are assessed. This subject is cogent, not only in the context of the present volume, but is equally important for the general problem of quantitative analyses of single organic compounds in bulk samples. Two approaches to the problem are discussed. One is that of successive approximation towards an accurate value. The other is the analysis of an open-ended system in terms of information theory to determine the number of bits of information, required for a given accuracy, and to assess the number of bits of information provided by the various analytical procedures. The chapter is deliberately provocative but it is intended to encourage critical appraisal of the accuracy of the methods used. Conversely it may stimulate workers to examine critically the accuracy which is required to answer the questions which they are asking.

Biosynthesis of plant hormones and their further metabolism have been areas of very rapid progress in the last two decades. These topics are reviewed in depth in Chapter 4. In Chapter 5, the important topic of receptor sites of the hormones is discussed and, in the final Chapter 6, the molecular effects of the hormones on plant tissue are reviewed.

There are many inherent short-comings in a multi-author volume. Some overlap of material between chapters is unavoidable. It is hoped that unnecessary overlap has been eliminated and that any overlap which remains is helpful to the reader. Also, the greater the number of authors the longer is the gestation period. Some papers came to our attention too late to be included in the main body of the text; others were published too late to be included; and some are known to be in press and not yet published. To mitigate these omissions, the opportunity has been taken to include stop-press items in this introduction.

## General

The Symposia papers, delivered at the Tenth International Conference on Plant Growth Substances, Madison, July 1979, will appear in Proceedings in Life Sciences (ed. by SKOOG, Springer 1980) and summaries of the other papers and poster demonstrations are contained in the Abstracts of that meeting. Papers presented at a symposium on the gibberellins, organized jointly by the British Plant Growth Regulator Group and the Society of Experimental Biology, are to be published (LENTON, 1980). The chemistry (HEDDEN, 1979) and the metabo-