

Phytochemistry

VOLUME II

Organic Metabolites

EDITED BY

Lawrence P. Miller



PHYTOCHEMISTRY

ORGANIC METABOLITES

VOLUME **II**

LAWRENCE P. MILLER, Editor

Boyce Thompson Institute for Plant Research
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Preface

Advances in plant chemistry are progressing at such a rapid pace that it is impossible for a worker in one phase of the subject to keep up with discoveries being made in fields other than his own. It is the objective of this treatise to bring together in an authoritative way the current status in all important aspects of phytochemistry. To do this, the editor has been successful in receiving the cooperation of leaders in their respective disciplines from all over the world. It is hoped that the treatise will be of value to research workers and graduate students.

In a work of this magnitude, with many authors (38) involved, there have naturally been some unavoidable delays in completing the manuscript for submission to the publisher. The editor is grateful to the authors who have been patient in accepting these delays, especially when caused by others.

The editor is indebted to Dr. George L. McNew, Managing Director of the Boyce Thompson Institute for Plant Research, who has been most cooperative in encouraging this endeavor. He has provided desk space for Mrs. Miller and myself after our official retirement from the Institute, has supplied stenographic help, and has assumed the financial responsibility for some of the typing that could not be done at the Institute. He has also critically reviewed the sections written by the editor and had the Institute Librarian, Miss Joan DeFato, check the literature citations in the editor's chapters. The editor's sections have also been reviewed by the Publications Committee of the Boyce Thompson Institute, especially Drs. Dewayne Torgeson, Alva A. App, Herman Gershon, Richard C. Staples, Mrs. Bettie Brooks, and Miss Joan DeFato. Their suggestions have been valuable.

The cooperation of many different libraries in the literature studies is gratefully acknowledged. First and foremost is the library of the Boyce Thompson Institute. In addition to checking the literature citations, mentioned above, the Librarian has been very helpful in obtaining interlibrary loans.

Other libraries used include those of the Rockefeller University, The Chemists Club, the Library of the Pharmacy Department of Columbia University, The New York Botanical Garden, The New York Academy of Medicine, the Library of the University of Florida, the Indianapolis Public Library, and the Library of Congress.

The editor is grateful to Dr. R. C. Jack who, in addition to his contribution of the Chapter on Lipids in Volume II, furnished advice and suggestions. The editor and publisher have also benefited from the comments of a reviewer who read the entire treatise and made many suggestions. Special thanks are due to Miss E. Jeanne Ross who typed most of the manuscript of the editor's sections. Not only did she not make any mistakes in her copying, but she also spotted errors in the copy submitted to her. The editor is indebted to Mr. George Narita, Editor, and Mrs. Alberta W. Gordon, Managing Editor, Professional and Reference Book Division, Van Nostrand Reinhold Company, for the excellent cooperation and help received, and to Mrs. Kathryn W. Torgeson for preparation of the subject index.

It is impossible to acknowledge in appropriate language the help given by my wife, Florence Flemion Miller, herself also a trained scientist. She has given me constant assistance throughout, with the literature and library work, with some of the writing and indexing, and in keeping me devoted to the project. In view of my interest in many different activities and my tendency to stray from one to the other, without her this treatise would have been delayed further and would perhaps not have appeared at all.

LAWRENCE P. MILLER

Contents

PREFACE	vii
1. AMINO ACIDS	1
<i>L. Fowden</i>	
2. FLOWERING PLANT PROTEINS	30
<i>D. Boulter</i>	
3. PURINES AND PYRIMIDINES AND THEIR DERIVATIVES	61
<i>Dalton Wang</i>	
4. ALKALOIDS	118
<i>D. W. Hughes and K. Genest</i>	
5. STEROIDS	171
<i>Erich Heftmann</i>	
6. LIPIDS	227
<i>R. C. Jack</i>	
7. TERPENES	254
<i>H. J. Nicholas</i>	

x Contents

8. RUBBER, GUTTA PERCHA, AND CHICLE	310
<i>B. L. Archer and B. G. Audley</i>	
9. FLAVONOIDS	344
<i>J. B. Harborne</i>	
10. MISCELLANEOUS VOLATILE PLANT PRODUCTS	381
<i>H. J. Nicholas</i>	
AUTHOR INDEX	401
PLANT AND ANIMAL INDEX	416
SUBJECT INDEX	421

CONTENTS TO VOLUME I

THE PROCESS AND PRODUCTS OF PHOTOSYNTHESIS

PREFACE

1. PLANTS AS ORGANIC LABORATORIES

Lawrence P. Miller

2. PHOTODYNAMICS; THE CHLOROPLAST IN
PHOTOSYNTHESIS

J. J. Wolken

3. THE ROLE OF PHOTOSYNTHESIS IN GREEN
PLANTS

James A. Bassham

4. CHLOROPHYLL

O. T. G. Jones

5. CAROTENOIDS

T. W. Goodwin

CARBOHYDRATE SECTION

6. MONO- AND OLIGOSACCHARIDES

Lawrence P. Miller

7. STARCH AND INULIN

D. J. Manners

8. HEMICELLULOSES AND GUMS

Gordon A. Towle and Roy L. Whistler

9. CELLULOSE AND CHITIN

James Teng and Roy L. Whistler

10. THE PECTIC SUBSTANCES

J. J. Doesburg

11. GLYCOSIDES

Lawrence P. Miller

AUTHOR INDEX

PLANT AND ANIMAL INDEX

SUBJECT INDEX

CONTENTS TO VOLUME III

INORGANIC ELEMENTS AND SPECIAL GROUPS
OF CHEMICALS

PREFACE

1. THE ROLE OF MINERALS IN
PHYTOCHEMISTRY

Lawrence P. Miller and Florence Flemion

2. SULFUR COMPOUNDS

D. V. Richmond

3. NONVOLATILE ORGANIC ACIDS

Dalton Wang

4. NATURALLY OCCURRING ACETYLENES

F. Bohlmann

5. LIGNIN

Walter J. Schubert

6. CUTINS AND SUBERINS

J. T. Martin

7. SURFACE WAXES

A. B. Caldicott and G. Eglinton

8. VITAMINS

F. A. Robinson

9. MITOCHONDRIA AND PLANT RESPIRATION*

Walter D. Bonner, Jr.

10. ENDOGENOUS PLANT GROWTH
REGULATORS

A. J. Vlitos and B. H. Most

11. THE GIBBERELLINS

J. MacMillan and R. J. Pryce

12. MOLECULAR TAXONOMY

H. Erdtman

13. IMPORTANCE OF SECONDARY PLANT
CONSTITUENTS AS DRUGS

Norman R. Farnsworth

14. IMPORTANCE OF PLANT CHEMICALS IN
HUMAN AFFAIRS

George H. Stout and Richard Evans Schultes

15. PHYTOCHEMISTRY: RETROSPECT AND
PROSPECT

L. Fowden

AUTHOR INDEX

PLANT AND ANIMAL INDEX

SUBJECT INDEX

Amino Acids

L. FOWDEN

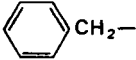
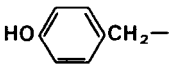
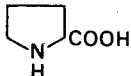
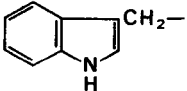
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AMINO ACIDS OCCURRING IN PROTEINS

Biochemical and genetic studies have established that twenty amino acids are incorporated into proteins following their initial activation by twenty specific aminoacyl-sRNA synthetase enzymes. These regular constituents of proteins are listed in Table 1. In addition, cystine is normally encountered in plant proteins, while *trans*-4-hydroxy-L-proline is found as a component of the structural proteins forming an integral part of the cell walls of higher plants (Lamport, 1965). The presence of these two substances in protein does not reflect the action of additional activating enzymes, but is most probably dependent upon the secondary modification of cysteine and proline residues already bound in polypeptides. Although claims that ϵ -N-methyl-lysine and bromo- and iodo-tyrosines occur in the protein of bacterial flagella and of certain red and brown algae, respectively, do not satisfy all the criteria considered necessary by Greenstein and Winitz (1961) for regarding an amino acid as being protein-bound, their presence in protein again could be reasonably explained by substitution at previously bound lysine and tyrosine residues. The presence of a small proportion of α -amino adipic acid in the protein of maize seed (Windsor, 1951), if confirmed, would necessitate a different method of incorporation, perhaps involving the nonspecific activation of this glutamic acid homologue by the plant's glutamyl-sRNA synthetase.

Chromatographic methods allow most of these regular protein constituents to be identified readily as components of the free amino acid pool of all plants, but the concentrations of the basic and sulphur-containing amino acids and of

Table 1.1 The Structures of Amino Acids Regularly Incorporated into Proteins Following their Activation by Aminoacyl-sRNA Synthetases.

Amino acid (type)	R— in structure $R \cdot CH(NH_2) \cdot COOH$	Amino acid (type)	R— in structure $R \cdot CH(NH_2) \cdot COOH$
Alkyl:		Basic:	
Glycine	H—	Lysine	$H_2N \cdot (CH_2)_4—$
Alanine	$CH_3—$		
Valine	$(CH_3)_2 \cdot CH—$		
Leucine	$(CH_3)_2 \cdot CH \cdot CH_2—$		$H_2N \cdot C \cdot NH \cdot (CH_2)_3—$ $HN=$
		Arginine	$\cdot HN—C \cdot CH_2—$ $HC \quad \quad \quad CH$ $\quad \quad \quad \backslash \quad /$ $\quad \quad \quad N$
Isoleucine	$H_3C \quad \quad \quad CH—$ $CH_3 \cdot H_2C \quad \quad \quad \backslash$	Histidine	
Acidic:		Sulphur:	
Aspartic acid	$HOOC \cdot CH_2—$	Cysteine	$HS \cdot CH_2—$
Glutamic acid	$HOOC \cdot (CH_2)_2—$	Methionine	$CH_3 \cdot S \cdot (CH_2)_2—$
Amino acid amides:		Aromatic:	
Asparagine	$H_2NOC \cdot CH_2—$	Phenylalanine	
Glutamine	$H_2NOC \cdot (CH_2)_2—$		
Imino acid:		Tyrosine	
Proline*			
Hydroxy:		Tryptophan	
Serine	$HO \cdot CH_2—$		
Threonine	$CH_3 \cdot CH(OH)—$		

* Complete structure.

tryptophan and proline are usually considerably lower than those of the remaining members of the group. However, even these acids are occasionally accumulated in remarkable amounts, as seen for histidine in ripening banana fruits (Steward et al., 1960a,b), for arginine in apple trees (Oland and Yemm, 1956) or in seed of several *Vicia* species (Bell and Tirimanna, 1965), and for proline in *Caragana* wood (unpublished observation of Brown and Fowden). A chromatogram prepared from an extract of *Caragana* revealed, after development with ninhydrin, a yellow streak running from the origin nearly to the solvent front, attributable entirely to proline! There is no general relationship between the concentration of amino acids forming the soluble nitrogen pool of a plant tissue and the amounts of each combined in the protein of the tissue. Indeed, ideas now current suggest that for most amino acids there may be several subcellular metabolic pools, including a somewhat metabolically-inert vacuolar pool that probably contains a large proportion of any amino

acid that a plant accumulates in unusual amount. Quite often such accumulations include types of amino acid not known to be incorporated into protein. These additional (or nonprotein) amino acid molecules rarely have a widespread distribution within the plant kingdom, although γ -aminobutyric acid is virtually ubiquitous, and in most cases they may be regarded as secondary products possessing no clear physiological function.

NONPROTEIN AMINO ACIDS

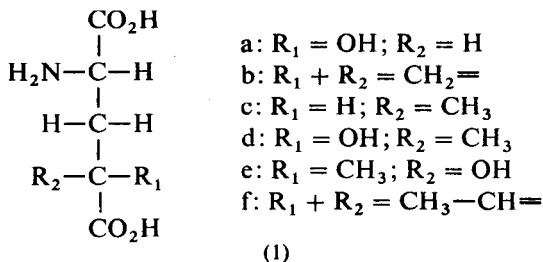
This group is now represented by about 200 plant constituents that have been strictly characterized, and by a further large number of substances identified only by chromatographic parameters during systematic surveys of plant materials (Fowden, 1970). All the types of structure seen in Table 1.1 are found also among these nonprotein amino acids, and, in addition, a number of other less usual groupings are encountered, including ethylenic linkages and cyclopropane, azetidine, pyrimidine, and pyrazole ring systems. A number of instances of homology are seen between various nonprotein amino acids and protein constituents and occasionally these, and other, nonprotein amino acids have been observed to act as specific antimetabolites (or analogues). As more compounds are described, structural relationships between groups of compounds are becoming increasingly apparent, and when such compounds originate from closely allied plant species, they naturally lead to the creation of biogenetic hypotheses and to a consideration of any taxonomic implications. These concepts will be discussed in later sections of this chapter.

Dicarboxylic Amino Acids and Amides

DERIVATIVES OF GLUTAMIC ACID Interest in naturally occurring derivatives of glutamic acid began when Dakin (1919) claimed that β -hydroxyglutamic acid was a constituent of protein. Although his preparation was resolved subsequently into a number of components, recently *threo*- β -hydroxyglutamic acid has been characterized as a constituent of the peptidoglycan present in the cell walls of *Microbacterium lacticum* (Schleifer et al., 1967). However, γ -substitution in glutamic acid is known now to be more common, and a number of compounds of this latter type occur unbound in relatively large amounts in a variety of plant species.

γ -Hydroxyglutamic acid, 1a, was isolated initially from *Phlox decussata* (Virtanen and Hietala, 1955). Later it was shown to have a sporadic distribution among a few members of the Liliaceae (Fowden and Steward, 1957), and the material isolated from one of these, *Hemerocallis* (day-lily), was assigned

the *threo*-L configuration (Fowden, 1959). *P. decussata* also contains γ -hydroxy-L-glutamine (Brandner and Virtanen, 1963).



β,γ -Dihydroxyglutamic acid has been isolated from seeds of lettuce (*Lepidium sativum*) and from leaves of rhubarb (*Rheum rhaponticum*) by Virtanen and Ettala (1957), who reported it to be widely distributed in plants. The configurations of the three asymmetric carbon atoms are not known.

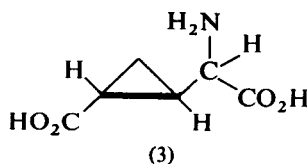
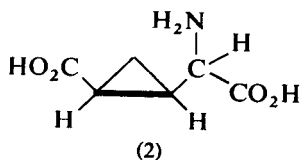
γ -Methyleneglutamic acid, 1b (and γ -methyleneglutamine) were isolated initially from peanut plants, *Arachis hypogaea* (Done and Fowden, 1952) and then from *Tulipa* (Zacharius et al., 1954) and *Amorpha fruticosa* (Tschiersch, 1962). Chromatographic evidence suggests their occurrence in a limited number of other species assigned to the Liliaceae (Fowden and Steward, 1957), and in the legumes, *Saraca indica* and *Delonix regia*. In addition, γ -methyleneglutamic acid is present in hops and in the fern, *Phyllitis scolopendrium* (Blake and Fowden, 1964). Often the unsaturated compounds are accompanied by one or both of the substances *erythro*- γ -methylglutamic acid, 1c, and 2(*S*),4(*S*)- γ -hydroxy- γ -methylglutamic acid, 1d, as seen in *Phyllitis scolopendrium* (Virtanen and Berg, 1955; Blake and Fowden, 1964) and *Tulipa gesneriana* (Blake and Fowden, 1964); in other species such as *Pandanus veitchii*, 1d occurs without the associated unsaturated compounds (Jadot et al., 1967). The diastereoisomeric 2(*S*),4(*R*)- γ -hydroxy- γ -methylglutamic acid, 1e, has been characterized in extracts of *Ledenbergia roseo-aenea* (Jadot et al., 1967).

γ -Ethylideneglutamic acid, 1f, is found together with γ -methyleneglutamic acid in the seed capsules of *Tulipa* (Fowden, 1966a) and in seed of the legume *Tetrapleura tetraptera* (Gmelin and Larsen, 1967).

OTHER ACIDIC AMINO ACIDS The occurrence of β -hydroxy- and β -methyl-aspartic acids as microbial metabolites has been reviewed (Fowden, 1964). *Erythro*- β -hydroxy-D-aspartic acid is present in the acid hydrolysate of phalloidine, a toxic peptide from *Amanita phalloides* (Wieland and Schnabel, 1962), but it has never been cited as a component of the free amino acid pool of plants.

α -Aminoadipic acid seems to have been strictly characterized only once as a plant constituent (Hatanaka and Virtanen, 1962), but chromatographic evidence suggests that it is distributed widely in plants in low concentration. Substances claimed to be the higher homologue, α -aminopimelic acid, and its γ -hydroxy derivative, were isolated from *Asplenium septentrionale* (Virtanen and Berg, 1954), but in quantities insufficient for unequivocal characterization.

Two dicarboxylic amino acids, isomeric with γ -methylene-glutamic acid, have been identified as the *cis*- and *trans*- forms of α -(2-carboxycyclopropyl)glycine (Fowden et al., 1969a). The *cis*- form, 2, was obtained from seed of *Aesculus parviflora*, while the *trans*- form, 3, occurs in *Blighia sapida* (akee seed) together with the hypoglycaemic cyclopropane derivative, β -(methylene-cyclopropyl)alanine (hypoglycin A).



Two other chemically distinct groups of acidic amino acids deserve mention. The unique feature of the first group is a *m*-carboxy substituent upon a phenyl ring. Four related substances of this type are found in members of the family Resedaceae (Kjær and Larsen, 1963): they are 3-carboxy-D-phenylglycine, 3-carboxyphenyl-L-alanine, D-(3-carboxy-4-hydroxyphenyl)glycine and 3-carboxy-L-tyrosine. 3-Carboxyphenylglycine and 3-carboxyphenylalanine also occur in *Iris tingitana* (Morris et al., 1959; Thompson et al., 1961), and the latter compound is a characteristic constituent of seeds assigned to certain subtribes of the family Cucurbitaceae (Dunnill and Fowden, 1965). The second includes a number of *S*-substituted cysteines, i.e., thioethers of structure $R \cdot S \cdot CH_2 \cdot CH(NH_2) \cdot CO_2H$, where a carboxyl group is present in group R. Examples are *S*-(2-carboxyethyl)-L-cysteine and *S*-(2-carboxyisopropyl)-L-cysteine from *Albizia* and *Acacia* seeds, dichrostachinic acid from *Dichrostachys glomerata*, and *S*-(2-carboxypropyl)-L-cysteine in a tripeptide of onion (see previous review by Fowden, 1964).

AMINO ACID AMIDES AND SUBSTITUTED AMIDES The number of simple amino acid amides isolated from plants has not increased in the striking way observed for the related dicarboxylic amino acids, and only γ -methyleneglutamine and γ -hydroxyglutamine (see above) can be added with certainty to a select list also containing asparagine and glutamine.