

Plant Polyphenols 2

Chemistry, Biology,
Pharmacology, Ecology

Edited by

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PREFACE

This book has been developed from the proceedings of the 3rd Tannin Conference honoring Professor Edwin Haslam, Department of Chemistry, University of Sheffield, for his efforts and outstanding contributions to the field of plant polyphenols. The meeting was held in Bend, Oregon, July 20–25, 1998, with the objective of promoting collaboration between chemists and biologists to improve our understanding of the biological significance and to expand possibilities for use of plant polyphenols. Special efforts were made to summarize current research on the influence of these compounds on human health. As in our 2nd Tannin Conference held in Houghton, Michigan, June 1991, we strove to build the "international" character of this meeting and were rewarded by an attendance of over 150 guests from 23 countries who enjoyed more than 50 lectures and 60 presentations. Our thanks go to all those who contributed to our meeting.

A special thanks is extended to Professor G. Wayne McGraw, Department of Chemistry, Louisiana College, Pineville, Louisiana, who served as the financial manager of our meeting. He, his wife Bobbie McGraw, and Helen Hemingway worked hard to make this meeting run as smoothly as possible. We also would like to thank the Bend Chamber of Commerce, Central Oregon Community College, The Riverhouse, Interstate Tours, Wanderlust Tours, and the people of Bend for welcoming our group to Central Oregon.

In an attempt to keep this volume a manageable size, we were forced to select about 50 of the contributions for this publication under the following topics: Hydrolyzable Tannins; Condensed Tannins and Related Compounds; Biotechnology; Antioxidant Properties and Heart Disease; Conformation, Complexation, and Antimicrobial Properties; Polyphenols and Cancer; Polyphenols in Commerce; Polyphenols and Ecology. We thank all the authors who have patiently worked with us to develop this book. Janie Gurgainers worked especially hard organizing files and correspondence. Dr. Hemingway thanks Dr. Bénédicte Berké and Dr. Timothy G. Rials for their help and patience. We also would like to thank Kluwer Academic/Plenum Publishers for their willingness to help build a continuing series of books that bring together the work of chemists and biologists from a wide array of disciplines, but with a common interest in plant polyphenols.

A comparison of the contributions to the proceedings of our 1st Tannin Conference, published by Plenum Press as "Chemistry and Significance of Condensed Tannins" in 1989, the 2nd Tannin Conference published by Plenum Press as

"Plant Polyphenols; Synthesis, Properties, Significance" in 1992, and the contents of this book show recognition of the significance of these compounds on the part of biologists and biochemists and increasing relevance in medically oriented disciplines.

To be a difference, a difference must make a difference. Over the past twelve years, our science has grown to clearly demonstrate that plant polyphenols make real differences to people's lives.

Georg G. Gross
Richard W. Hemingway
Takashi Yoshida
Susan J. Branham

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Pharmacology, Ecology

INTRODUCTION

**PROFESSOR EDWIN HASLAM, RECIPIENT OF THE 3rd TANNIN
CONFERENCE AWARD**

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1. SYNOPSIS OF PROFESSOR HASLAM'S CAREER

It is with sincere pleasure that we dedicate this volume, *"Plant Polyphenols 2: Chemistry, Biology, Medicine, Ecology,"* to Professor Haslam who has been a beacon in the study of the chemistry and application of plant polyphenols. It is unimaginable that anyone who is active in this field or even has a mild interest would not be familiar with Professor Haslam's seminal overviews, *"Chemistry of Vegetable Tannins"* and *"Plant Polyphenols; Vegetable Tannins Revisited"*. To this distinguished duo, a third volume has appeared this past year titled *"Practical Polyphenolics—From Structure to Molecular Recognition, and Physiological Action"*. Those of us present at the 2nd Tannin Conference held June 17–21, 1991, in Houghton, Michigan, will recall his overview of the chemistry of gallic acid derivatives at that meeting, so we were looking forward to his 1998 update in this new book.

Professor Haslam received B.Sc. and Ph.D. degrees from the University of Sheffield and a subsequent Ph.D. degree from Cambridge University. Between the years of 1957 and 1980, he served as Assistant Lecturer, Lecturer, Senior Lecturer, and Reader in the Department of Chemistry at the University of Sheffield. In 1980, he was appointed to a Personal Chair (*ad hominem*) in Chemistry, and in 1985, he became the Head of the Chemistry Department at the University of Sheffield. He has received a number of honors and awards during his career. Among these are the following:

- 1955 Turner Research Prize in Chemistry—University of Sheffield
- 1955–1957 Sir William Ramsay Memorial Research Fellowship, Emmanuel College, Cambridge

- 1975 Hugh Kelly Senior Research Fellowship (First Election), Rhodes University, Grahamstown, South Africa
- 1977 Tate and Lyle Prize and Award, Phytochemical Society of Europe
- 1984 Visiting Professor, University of the South Pacific, Suva, Fiji
- 1987 Proctor Memorial Lecturer, Society of Leather Trades Chemists
- 1996 Wolstenholme Lecturer, Society of Leather Trades Chemists

During his career, he has published more than 150 research papers (see bibliography) principally in the *Journals of the Royal Society of Chemistry*, *Tetrahedron*, and *Phytochemistry*. He has published six books, three of which have already been mentioned, and served as Editor of Volume 5, "Biological Compounds" in the six-volume series, *Comprehensive Organic Chemistry*. Although he has been "retired" for some time, a glance at his list of publications does not show any letup in his research activities. At a time when some of us have reached retirement age (including the writer) or are facing "downsizing," the current American fad for supposedly improving the profitability of industry or academe, Professor Haslam serves as an excellent role model for not giving in to the social pressures to step aside on account of calendar age.

It is interesting to note that Haslam's first published paper (coauthored with R.D. Haworth) was on the constitution of sesamol, a bifuran lignan with methylenedioxy substituted aromatic rings. This compound is one of the constituents of sesame oil and can serve as a synergist for pyrethrum insecticides. Although not a tannin, the discipline involved in isolation and structural determination of this compound was a good prelude to Haslam's subsequent work on the constituents of the gallotannins. From there, his work broadened to include the biosynthesis and interactions of plant polyphenols with proteins, carbohydrates, etc. Overall, Professor Haslam's research has fallen into four broad categories.

- Phenolic metabolism in plants. This has involved studies of the structure, chemical properties, and biosynthesis of simple phenols such as hydroxycinnamoyl esters and glycosides, gallic acid and galloyl esters, piceatannol (a stilbene derivative), larixinol, catechins, and hydroquinone. This work included the taxonomic distribution, structure, chemical properties, conformation or shape and biosynthesis of the two principal classes of vegetable tannins, i.e., hydrolyzable tannins (mainly esters of gallic and hexahydroxydiphenic acid), and condensed tannins (polymers based on proanthocyanidins and, to a lesser extent, polyphenolic norlignans, stilbenes, etc).
- Molecular recognition and interactions: phenols and polyphenols. For the attendees of the 3rd Tannin Conference, this area of Professor Haslam's work was probably of the greatest interest and importance. The means whereby simple and complex polyphenols engage in non-covalent



Figure 1. Professor Edwin Haslam who was presented with the 3rd Tannin Conference award.

interactions (complexation) with other molecules, such as other phenols (for example, anthocyanidins), peptides and proteins, cyclodextrins, saccharides and polysaccharides, simple heterocycles (such as caffeine, theobromine, and theophylline) have been studied. This work has included the influence of environmental factors, i.e., metal ions and solvent composition, on these interactions. The importance of these interactions has been shown in various areas of applied science. Included are: (a) food and nutrition (taste, astringency, maturation, and appearance of beverages such as teas, wines, cocoa, etc.); (b) the interaction of phenols and polyphenols with proline-rich salivary proteins; (c) the development of astringency; (d) the mechanism of vegetable-tannages of leather and the deterioration of leather; (e) the role of polyphenols in health, food, and herbal medicines; (f) the mechanism of anthocyanin co-pigmentation in fruits and flowers; and (g) quinone tanning.

- Aromatic amino acid metabolism and the shikimate pathway. Biochemists are indebted to Professor Haslam's studies on the chemistry of intermediates in the shikimate pathway, i.e., shikimic acid, quinic acid, dehydro-derivatives, and chorismic acid. Research was carried out on the mechanism of action and stereochemistry of enzymes in the pathway: 3-dehydroquinase synthase, 3-dehydroquinase dehydratase, 5-enolpyruvylshikimate-3-phosphate synthase, chorismate mutase, L-phenylalanine ammonia lyase, and L-tryptophan synthase (alpha reaction).
- Secondary metabolism in plants and microorganisms. Studies have been made of the role and function of secondary metabolism and of the secondary metabolites themselves (alkaloids, terpenes, phenols, mycotoxins, etc.) in the life of the organisms that produce them.

Each reader in the field of plant polyphenols will find something in Professor Haslam's work that had or will have a bearing on their work. In the case of this writer, Haslam's first book on vegetable tannins was extremely helpful in unraveling some of the problems associated with the isolation and utilization of tannins from conifer bark. For other investigators, I could imagine that they would be especially interested in the role of polyphenol complexation in foods. Still others will be vitally interested in his studies of biosynthetic pathways and so on. In any event, it was this writer's wish that all attendees at the 3rd Tannin Conference availed themselves of the opportunity to become personally acquainted with Professor Haslam. There is a temptation for scientists to get so caught up in their own research that they fail to appreciate the personality of the other workers in their field. In the case of Eddie Haslam, this would be a great shame for he is one of the truly great scientists in our corner of the broad spectrum of chemistry and biology.

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CHE FARÒ SENZA POLIFENOLI?

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1. INTRODUCTION

Many have spoken and written concerning the motivations which inspire a scientist in his work. For some there is an inherent curiosity about the workings of nature, and for others, the inspiration comes from the desire to make significant and lasting contributions to human knowledge. It would nevertheless be idle to deny that for a scientist the recognition of his work by his peers is an event which engenders the deepest personal satisfaction. I should therefore like to express to all those who have been responsible for this award and the decision to bestow it upon me, my sincere thanks. Much, much more importantly by honoring me you also honor my many colleagues who, over the years, have participated in this endeavor. It gives me enormous pleasure to record my gratitude to them.

When anyone is called upon to ride the rough torrents of an occasion such as this, there are a number of thoughts uppermost in his mind. Not the least of these is the topic for his address. The enigmatic title which I have selected doubtless reflects the uncertainty with which I have approached this task. Should this be a valedictory oration and call from the battlements? Should it be the epic tale of a scientific Odyssey, an adventurous journey, undertaken many years ago with the best of intentions? Or should it be a "Definitive State of the Art" commentary? I hope that you will find a judicious mixture of all three.

"It is not enough to have knowledge, one must use it" Goethe

Willingly or not we are all guilty of the modern compulsion to know more and more about less and less; we ride the information superhighway, consult our web-sites and surf the internet. But information does not equate with knowledge, nor does knowledge equate with wisdom. In recent years technological revolutions have made empirical research so fast and exhilarating that scientists often have

This lecture and chapter are dedicated to the memory of the late Larry G. Butler (1934-1997), Professor of Biochemistry at Purdue University.

little or no time for reflection. However, the relentless flood of data and information rarely brings with it the answer to the important question: "What are we going to do with it all?" Waist deep in data (and often none the wiser for it) disillusionment may set in. Indeed if the data get so far ahead of their assimilation into a conceptual framework, then the data itself may prove an encumbrance. There are evermore grants for producing data, but hardly any for standing back in contemplation. In our case we do not allow ourselves, sufficiently often, the apparent luxury of taking time out to reflect and ponder on what the chemistry and biochemistry of plant polyphenols are all about and where this most arcane of topics is headed. Polyphenol chemistry has surely reached the stage at which we can begin to stand back in active contemplation of the data before us and within the framework of ideas generated look beyond the present to many of the unanswered questions associated with the presence of polyphenols in plant materials. We are like the pioneers who settled this country. We have left the Eastern seaboard heading for the mountains of Oregon and the gates of the golden West; quite what is our position in the vast desert in between is something on which we can all speculate.

In what follows, however, I do not claim objectivity, indeed, I am instantly suspicious of any academic, writer or politician who lays claim to this rare and elusive quality. I am very much inclined to the view that the perception of any situation depends to a large extent on the background—the ambitions—the limitations—and the prejudices of the beholder. I hope that you will bear this in mind as I proceed to erect a few signposts to the future in this metaphorical desert, but I also hope that it provokes you to think, to debate, and doubtless to disagree not only with the inscriptions but also with the way in which they are directed.

The first significant research into the nature of vegetable tannins (plant polyphenols) was that of a quintet of distinguished German chemists—the Nobel laureate Emil Fischer and Karl Freudenberg, in the early years of this century, and later Otto Schmidt, Walter Mayer, and Klaus Weinges (all based in Heidelberg). The vastly expanded array of physical techniques for the separation and structure determination of natural products which began to be made available in the 1950's transformed the subject. Structural problems which previously took decades to solve now often yield a solution in a matter of weeks or months. In consequence isolation and structure determination, *by themselves*, no longer remain the principal goal; they are mere prologue. In this context my own attitudes and scientific aspirations were very accurately reflected in the words of that giant Californian redwood T.A. (Ted) Geissman made over 30 years ago:

"Certainly structures are important—but the determination of structure in itself is ceasing to be of much interest or importance, and often turns out to be an exercise in the manipulative skills of the investigator. Some syntheses are in the same class . . . My own tendency (in which, of course, I am not alone) is to look at biological relationships: taxonomy, phylogeny, biosynthesis and biotransformations . . . The future of phytochemistry is to use the chemical information as the starting point for inquiry into questions that lie in the realms of biology."

Future scientists may well think it odd that, over the past 30 years, we have learned so much about the structure of polyphenol molecules but yet so little about

their intrinsic properties, about many of the important processes in which they become involved, both *in vivo* and *in vitro*, and about their function and the control of their synthesis in living systems. In this context aspects of the following areas will be considered:

- (1) —structural problems still awaiting resolution
- (2) —the control (enzymic/chemical) of biosynthesis
- (3) —the concept of molecular recognition intrinsic to polyphenol complexation and
- (4) —the vexatious question of the role of plant polyphenols in plant metabolism

2. STRUCTURE AND STRUCTURAL PROBLEMS

The word tannin has a long and well-established usage in the scientific literature. The importance of vegetable tannins to a range of scientific disciplines has been recognized for some time. However, a firm definition of what constitutes a vegetable tannin is not easy to give; probably the most acceptable, concise and simple definition is still that of Bate-Smith and Swain:¹

"Water soluble phenolic compounds having molecular weights between 500 and 3,000 and, besides giving the usual phenolic reactions, they have special properties such as the ability to precipitate alkaloids, gelatin and other protein"

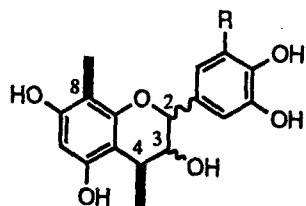
Many still prefer the term vegetable tannin, which they find valuable simply because of its lack of precision. Scientifically and terminologically, plant polyphenols is to be preferred as a descriptor for this class of higher plant secondary metabolites if serious attempts are to be made to interpret their diverse characteristics at the molecular level (however, see Postscript).

2.1 Properties and Classification

It is now possible to describe in broad terms the nature of plant polyphenols. They are secondary metabolites widely distributed in various sectors of the higher plant kingdom. They are distinguished by the following general features:

- (a) *Water solubility.* Although when pure some plant polyphenols may be difficult to dissolve in water, in the natural state polyphenol—polyphenol interactions usually ensure some minimal solubility in aqueous media.
- (b) *Molecular weights.* Natural polyphenols encompass a substantial molecular weight range from 500 to 3–4,000. Suggestions that polyphenolic metabolites occur which retain the ability to act as tannins but possess molecular weights up to 20,000 must be doubtful in view of the solubility proviso.

- (c) *Structure and polyphenolic character.* Polyphenols, per 1,000 relative molecular mass, possess some 12–16 phenolic groups and 5–7 aromatic rings.
- (d) *Intermolecular complexation.* Besides giving the usual phenolic reactions, they have the ability to precipitate some alkaloids, gelatin and other proteins from solution. These complexation reactions are not only of intrinsic scientific interest as studies in molecular recognition and possible biological function, but, as noted earlier, they have important and wide-ranging practical applications.
- (e) *Structural characteristics.* Plant polyphenols are based upon two major and one minor structural theme, namely:
- (1) *condensed proanthocyanidins.* The fundamental structural unit in this group is the phenolic flavan-3-ol (“catechin”) nucleus. Condensed proanthocyanidins exist as oligomers (soluble), containing two to five or six “catechin” units, and polymers (insoluble). The flavan-3-ol units are linked principally through the 4 and the 8 positions. In most plant tissues the polymers are of greatest quantitative significance but there is also usually found a range of soluble molecular species—monomers, dimers, trimers, etc.²⁻⁵ The “monomer” units of procyanidins (R=H) and prodelphinidins (R=OH) are phenolic flavan-3-ols which are linked primarily through their 4 and 8 positions respectively. The stereochemistry at C-2 is most commonly encountered as the 2*R* configuration.



Oligomeric condensed proanthocyanidins have been held⁶ to be most commonly responsible for the many distinctive properties of plants typically attributed to “condensed tannins”. Mole⁷ has however recently commented on some of this earlier work and has suggested that fewer plant families are characterised by the presence of “tannins” than heretofore thought. In the context of the later discussion on the polymeric proanthocyanidins it is pertinent to point out that, simply on the basis of solubility differences, Sir Robert and Lady Robinson⁸ in the 1930's originally subdivided the leucoanthocyanins (condensed proanthocyanidins) into the three classes indicated below:

- (i) *those that are insoluble in water and the usual organic solvents or give only colloidal solutions,*
- (ii) *those readily soluble in water but not readily extracted therefrom by means of ethyl acetate, and*
- (iii) *those capable of extraction from aqueous solution by ethyl acetate.*

Insofar as the total complement of condensed proanthocyanidins (procyanidins and prodelphinidins) found in plant tissues is concerned, the soluble oligomeric forms (monomers, dimers, trimers...) are in metabolic terms but the “tip of the iceberg”. According to the Robinsons' classification they represent category (iii) above.

For the generality of plants it is now quite clear that condensed proanthocyanidins which fall within the two other categories (i and ii) invariably strongly predominate over the more freely soluble forms. They are, metaphorically speaking, the base of the “metabolic iceberg”. Indeed in the tissues of some plants such as ferns and fruit such as the persimmon (*Diospyros kaki*), there is an overwhelming preponderance of these forms. They are also of frequent occurrence in plant gums and exudates.

- (2) *galloyl and hexahydroxydiphenoyl esters and their derivatives.* These metabolites are almost invariably found as multiple esters with D-glucose,^{2,3,9-12} and a great many can be envisaged as derived from the key biosynthetic intermediate β -1,2,3,4,6-penta-O-galloyl-D-glucose. Derivatives of hexahydroxydiphenic acid are assumed to be formed by oxidative coupling of vicinal galloyl ester groups in a galloyl D-glucose ester,¹² dependent upon the positions on the D-glucopyranose ring between which oxidative coupling of the galloyl ester groups takes place then a particular chirality is induced in the twisted biphenyl of the resultant hexahydroxydiphenoyl ester.^{2,3} Acid hydrolysis of hexahydroxydiphenoyl esters gives rise to the formation of the bis-lactone of hexahydroxydiphenic acid, the planar and virtually insoluble ellagic acid from which the characteristic nomenclature of ellagitannins is derived.

Gallic acid is most frequently encountered in plants in ester form. These may be classified into several broad categories:

- (i) Simple esters.
- (ii) Depside metabolites (*syn* gallotannins).
- (iii) Hexahydroxydiphenoyl and dehydrohexahydroxydiphenoyl esters (*syn* ellagitannins) based upon: