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FLAVONOID METABOLISM

Helen A. Stafford

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Author

Helen A. Stafford, Ph.D.

Professor

Biology Department

Reed College

Portland, Oregon



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PREFACE

I think that one can fairly say that the study of secondary compounds in plants in the 20th century was dominated by flavonoids. The study of their metabolism, the sum total of all the chemical reactions involved, could be interpreted narrowly; I have attempted, however, to place these metabolic reactions in the living cell in the functioning plant. This requires an interdisciplinary approach, involving localization, physiology, and genetics as well as the basic chemistry involved. I hope that my approach will interest young investigators with all types of scientific backgrounds; there is still much to be discovered about flavonoids and their regulation. My aim was to present both an overview of flavonoid metabolism, as well as an introduction into both the historical and modern literature.

My emphasis in this volume and in my own research has been on the enzymology involved in plant metabolism. This interest in plant enzymology started with my Ph.D. thesis, completed in 1951, on cytochrome oxidase in pea mitochondria with David R. Goddard at the University of Pennsylvania, and was continued with a study of NADH-NADPH-dependent dehydrogenases as a postdoctoral fellow with Birgit Vennesland at the University of Chicago. My own research interests in flavonoids started just prior to 1963 when I shifted from a study of lignin biosynthesis in *Phleum pratense* to that of the phenolics in seedlings of *Sorghum milo*. This was in part a return to my earlier interests on the effect of light on the growth of seedlings that resulted in a Master's thesis with Richard H. Goodwin at Connecticut College and a publication in 1948. The publications of Furuya, Galston, and Stowe on flavonoids as cofactors of indole acetic acid oxidase, 1962¹ and of Downs and Siegelman, 1963, on the photocontrol of anthocyanin synthesis in sorghum² were of considerable importance in shifting my interests to flavonoids. I spent the academic year of 1963—1964 as a National Science Foundation Senior Postdoctoral Fellow in the laboratory of Ted A. Geissman, Chemistry Department at the University of California at Los Angeles. Prior to this, I had joined the Plant Phenolics Group of North America (now the Phytochemical Society of North America). At the yearly symposia, I heard talks by E. Sondheimer, T. A. Geissman, Margaret K. Seikel, and J. E. Watkin, and met a variety of chemists and biochemists that I did not meet at the annual meetings of the American Society of Plant Physiologists. The volume edited by T. A. Geissman in 1962, *The Chemistry of Flavonoid Compounds*, became my bible for the status of the chemical knowledge of flavonoids. My publications on anthocyanins were the result of this visit. A second major shift in my research interests occurred in 1977 when I spent a sabbatical at the Oregon Graduate Center in Dr. T. Cheng's laboratory; I was introduced to cell suspension cultures of Douglas fir that produced large amounts of proanthocyanidins. Research on these cultures kept me busy for the rest of my laboratory research career.

I greatly appreciate the comments and criticisms of readers of versions of one or more of these chapters: Loverine Taylor, Ralf Oelmüller, Lawrence Porter, Geza Hrazdina, and Ronda Bard. My thanks also go to Stephen Sillett and Michael Robbins, Reed students, who prepared my figures on a Macintosh.

I greatly appreciate also the financial aid from the National Science Foundation that supported my research from 1955 through 1988, and I thank Reed College for permitting some released time from a heavy teaching load for research.

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THE AUTHOR

Helen A. Stafford, Ph.D., is Professor of Biology Emeritus at Reed College, Portland, Oregon. She received a B.A. degree from Wellesley in 1944, an M.A. degree from Connecticut College in 1948, and a Ph.D. in Botany from the University of Pennsylvania in 1951. After postdoctoral research with Dr. B. Vennesland in the Biochemistry Department at the University of Chicago, she was appointed Assistant Professor of Biology at Reed College in 1954 and Professor in 1965. She was a Guggenheim Fellow in 1958—1959 in Dr. K. V. Thimann's laboratory at Harvard University, and a National Science Foundation Senior Postdoctoral Fellow in 1963—64 in Dr. T. A. Geissman's laboratory at the University of California at Los Angeles. She has served on/as: a CUEBS Commission in 1968—1971; a National Science Foundation panel in Metabolic Biology, 1973—1975; the Editorial Board of *Plant Physiology*, 1964—present; the Editorial Board of *Plant Science*, 1982 to the present; President of the Phytochemical Society of North America, 1977—1978; and Editor of *Recent Advances in Phytochemistry*, 1989.

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Chapter 1

GENERAL ASPECTS OF CHEMISTRY AND METABOLISM
OF FLAVONOIDS

I. CHEMISTRY

A. Basic Chemistry

1. General Characteristics of the C_{15} Unit

The basic C_{15} unit consists of two benzene rings A and B connected by a three carbon chain. This chain is closed in most flavonoids, forming the heterocyclic ring C, but remains open in the chalcones and dihydrochalcones (Figure 1). Natural flavonoids are divided into subclasses based generally on the oxidation state of the C-ring (Table 1, Figure 2). See the 1988 *The Flavonoids* volume for recent details of the chemistry.¹

2. Hydroxylation Patterns of A-, B-, and C-Rings

The 5,7-hydroxylation pattern of the A-ring is the most common one, but a 7-hydroxy ring (generally called a 5-deoxy ring) is common in the isoflavonoid subgroups and in some proanthocyanidins. Occasionally, a 5,7,8- or 5,6,7-hydroxylation pattern is found. The B-ring generally has a 4'-, a 3',4'-, or a 3',4',5'-hydroxylation pattern. Rare flavonoids lack B-ring oxygenation. In the isoflavonoids, a 2'-hydroxylation pattern is found. The C-ring is frequently hydroxylated at the carbon 3 position, and sometimes at the 6a position in the isoflavonoids (Chapter 8). This usually six-membered ring can contain a carbonyl group, a hydroxyl group, a double bond between positions 2 and 3, or can be completely unsubstituted as in unsubstituted flavans.² The isoflavonoid pterocarpanes contain additional rings resulting from the 2'-hydroxylation of the original B-ring or from the cyclization of the added prenyl groups.

B. Basic Substitutions

1. Hydroxylation

The 5,7 (or 4',6' of the chalcone) hydroxyl groups of the A-ring are derived from the acetate moiety of the malonyl-CoA precursor. The 4'-hydroxyl of the B-ring is formed at the C_6-C_3 level in the phenylpropanoid pathway. The enzymology of hydroxylation at position 8 is unknown. Two types of hydroxylases that add the hydroxyl groups to the B- and C-rings have been demonstrated: microsomal monooxygenases that are NADPH-dependent cytochrome P-450 enzymes and a soluble dioxygenase requiring 2-oxoglutarate, Fe^{2+} , and ascorbate as cofactors. The 4'-hydroxylation producing 4-coumarate at the phenylpropanoid level is catalyzed by the action of a microsomal cytochrome P-450 cinnamic-4-hydroxylase. The 3'- and