Natural Products

A LABORATORY GUIDE

SECOND EDITION

RAPHAEL IKAN

NATURAL PRODUCTS
A Laboratory Guide

Second Edition

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PREFACE TO THE SECOND EDITION

In the twenty-five years since the publication of the first edition, natural product research technology has advanced incredibly through the fields of chemistry, food science, geochemistry, materials science, and life sciences. Comparisons of these compounds in microorganisms, algae, animals, higher plants, and marine invertebrates are now documented. With the advent of such techniques as Raman spectroscopy, magic-angle spinning spectroscopy, high-resolution electron microscopy, x-ray crystallography, and chromatographic methods, separation of positional and regioisomers, sterioisomers, and even isotopic isomers are now possible.

This new edition has been updated to include the following: The use of biomarkers (organic compounds in the geospherical record with carbon skeletons) reflecting the upsurge in geoporphyrin research primarily due to MS, yeast RNA nucleic acid studies; reversed-phase HPLC of amino acids; brewing industry applications (HPLC evaluation of carotenoids in orange juice and of "debittered" citrus); HPTLC of carbohydrates; synthesis of a sweetening agent from citrus peels, synthesis and degradation of alkaloids and of sterols, GC/MS uses with sterols, petroleum products, and aromatic constituents of wine and grape juice, flash chromatography of essential oils, optical purity of enantiomers affecting flavors, fragrances, and pheromones, as well as studies of lattice inclusion compounds ^{1}H- and ^{13}C-NMR, MS, IR, and UV data are presented for most natural products.

Used for twenty-five years in natural products organic chemistry courses, the successful first edition has been updated and improved to meet the needs of current research.

I wish to express my thanks to the following publishers and societies for their kind permission to reproduce tables, figures, and procedures: Academic Press, Association of Official Agricultural Chemists, Elsevier Publishing Company, John Wiley and Sons, Macmillan and Company, Methuen and Company, Naturwissenschaften Editors, Pergamon Press, Perkin Elmer Company, The American Chemical Society, The American Society of Biological Chemists, The Biochemical Society (London), The

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Sincere thanks are due to my colleagues, Drs. Bernard Crammer, Vera Weinstein and Jeana Gross (Institute of Chemistry, Hebrew University, Jerusalem), and Dr. Uzi Ravid (Department of Natural Products, Agricultural Research Organization, Newe Ya'ar).

PREFACE TO THE FIRST EDITION

The syllabus of the practical course in organic chemistry at most universities mainly covers the synthesis of aliphatic, alicyclic, aromatic, and heterocyclic compounds, as well as qualitative and quantitative analysis. For some time, the Department of Organic Chemistry at the Hebrew University, Jerusalem, has felt that it would be of value to the students, either at the undergraduate or graduate level, to take a comprehensive course in natural products, so as to become acquainted with the methods of isolating these products from plant and animal matter and of determining their structures by physical methods, degraduation and, finally, synthesis.

The study of natural products has always been the starting point of the discipline of chemistry in every country of the globe, and, in view of the importance of these organic compounds in agriculture, medicine, and industry, every student of chemistry today feels the need to acquire further knowledge in this field.

In 1961, I organized a course on the subject and prepared a manual (in Hebrew) entitled "Natural Products Laboratory Guide." In the subsequent years I have repeatedly given this course to graduate students specializing in organic and physical chemistry, biochemistry, biology, and agricultural chemistry, as well as to undergraduate students (in the last trimester of their third year of studies).

Over the seven years during which I conducted this course, I have found that it was received enthusiastically by the participants at all levels. It stimulated many of them to choose the field of natural products as the theme for their M.Sc. and Ph.D. theses.

Visiting scientists from abroad who have become acquainted with the course and its methods were favorably impressed and encouraged me to publish the manual in a form suitable for wide distribution.

The book in its present form includes the following "biogenetic" chapters:

1. Acetogenins: flavonoids, lipids, lignans, quinones.
2. Carbohydrates: mono-, oligo- and polyaccharides.
3. Isoprenoids: cartenoids, steroids, terpenoids.
4. Nitrogens compounds: alkaloids, amino acids, nucleic acids, peptides porphyrins, proteins, pteridines.

Each section of these chapters includes a general introduction and methods of isolation, degraduation, and transformation, as well as applications of chromatographic procedures. The introduction to each chapter is brief and attempts only to supply or recall knowledge in the particular field. The student, who does not always find the time to read the relevant books or reviews, will find in this introduction the required material in a "concentrated" form. Furthermore, at the end of each chapter there is a list of recommended books for additional study. Typical experiments were selected for each chapter, taking into consideration the following factors: availability of starting materials and the performance time. Most of the procedures were tailored to the modest facilities of the students' laboratory.

Each experiment is described under the following headings: Introduction, References, Recommended Reviews, Principle, Apparatus, Materials, Time, Procedure (often including spectral data). At the end of each section appears a list of questions.

A large number of experiments are described in order to give the instructor a reasonable degree of freedom.

I am deeply indebted to Professor E. D. Bergmann for his encouragement, constructive comments, and criticism. I thank also Professor J. Klein and Dr. J. Radell for their helpful guidance in the first stages of this work, and Drs. Y. Lapidot and N. de Groot for their valuable suggestions on nucleic acids.

Acknowledgments are due Mr. R. Amoils for editing the manuscript, and to Mr. Y. Lakitchewitch for drawing the formulas. Last, but certainly not least, I wish to thank my wife for her expert typing of the manuscript, and our parents for their interest and encouragement.

CONTENTS

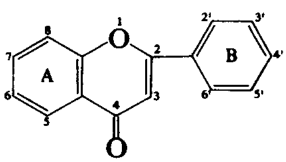
ACETOGENINS 1

The compounds grouped together in this chapter are different in most chemical properties, but similar in that many of them possess an aromatic ring bearing hydroxyl substituents. Many of these compounds possess a diversity of physiological properties. All of these natural products appear to be biosynthetically related in being derived by condensation of several molecules of acetate. Speculation regarding biosynthesis of all such polyacetate compounds may be found in several biogenetics books.

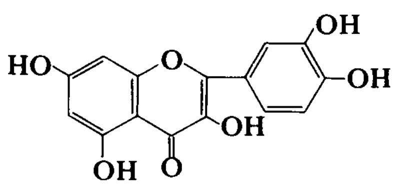
I. FLAVONOIDS

A. Introduction

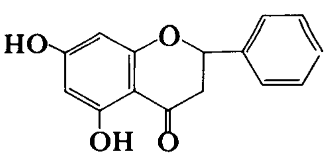
The flavonoid compounds can be regarded as C_6-C_3-C_6 compounds, in which each C_6 moiety is a benzene ring, the variation in the state of oxidation of the connecting C_3 moiety determining the properties and class of each such compound. The classes are shown below.



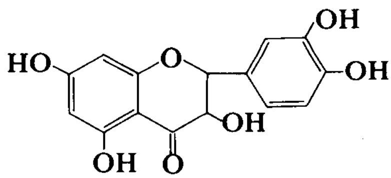
Flavone



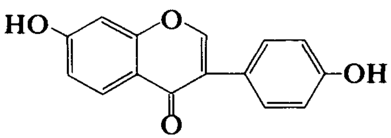
Flavonol



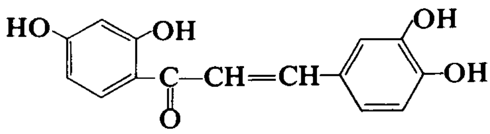
Flavanone



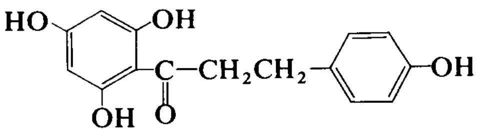
Flavanonol



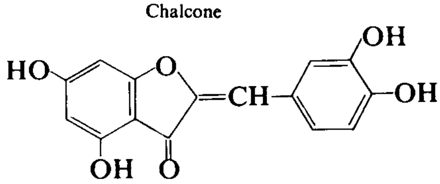
Isoflavone



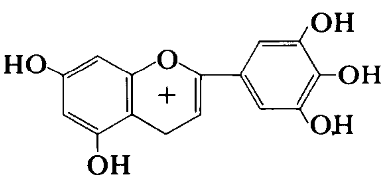
Chalcone



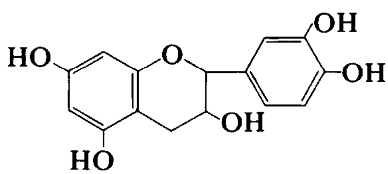
Dihydrochalcone



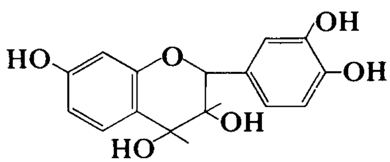
Aurone



Anthocyanidin



Catechin



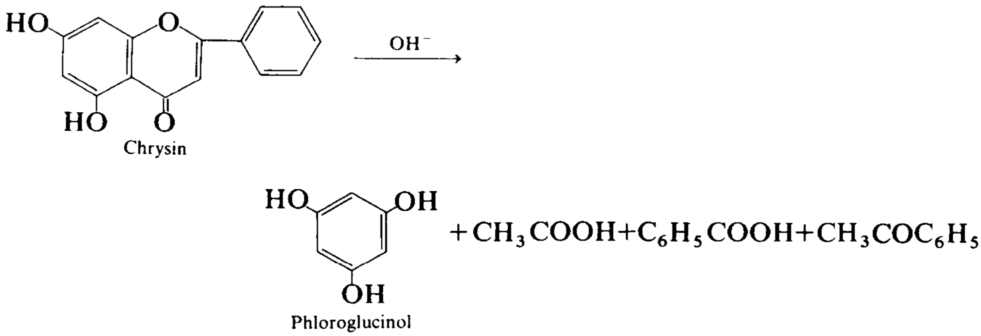
Flavan-3,4-diol

Flavonoid compounds and the related coumarins usually occur in plants as glycosides in which one or more of the phenolic hydroxyl groups are combined with sugar residues. The hydroxyl groups are nearly always found in positions 5 and 7 in ring A, while ring B commonly carries hydroxyl or alkoxyl groups at the 4′-position, or at both 3′- and 4′-positions. Glycosides of flavonoid compounds may bear the sugar on any of the available hydroxyl groups.

Flavonoids occur in all parts of plants, including the fruit, pollen, roots, and heartwood. Numerous physiological activities have been attributed to them. Thus, small quantities of flavones may act as cardiac stimulants; some flavones, e.g., hesperidin, appear to strengthen weak capillary blood vessels; highly hydroxylated flavones act as diuretics and as antioxidants for fats. It is also claimed that flavones behave like auxins in stimulating the germination of wheat seeds.

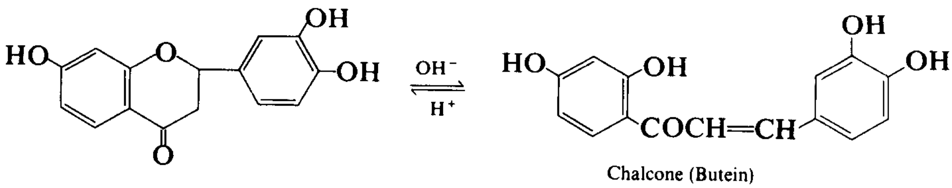
The possible function of this coloring matter in insect-pollinated flowers and edible fruits is to make these organs more conspicuous in order to aid seed dispersion by animals.

The fundamental method in structural studies is alkaline hydrolysis. Thus, alkaline degradation of chrysin yields phloroglucinol, acetic acid, benzoic acid, and a small amount of acetophenone.



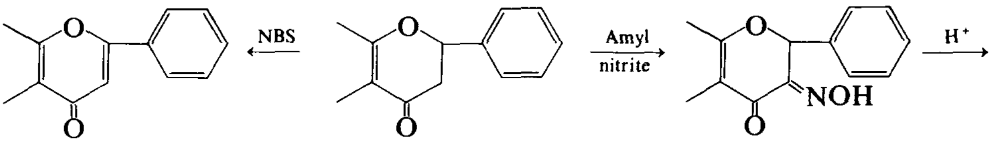
By spectroscopic measurement it is now possible to determine the structures of some flavonoid compounds on the basis of their spectra alone. Color reactions also play an important role in identification of flavonoids at the preliminary stage of analysis.

The possibility of interconversion between the various structures in this group is of considerable importance for the structural elucidation of flavonoid compounds. Thus, chalcones and flavanones are isomeric and readily undergo interconversion.

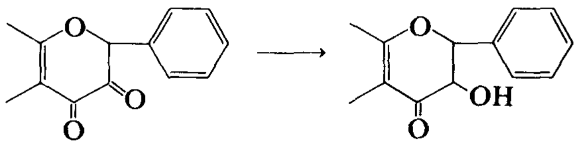


Flavanone (Butin)

Flavanones may be converted into flavonols and flavones.



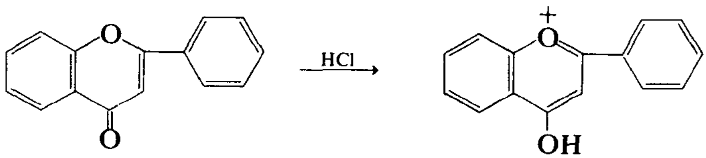
Flavone Flavanone



Flavonol

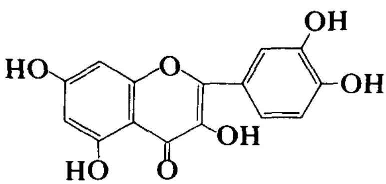
1. Flavones

In flavones, ring C is basic and forms a pyrylium salt with hydrochloric acid.

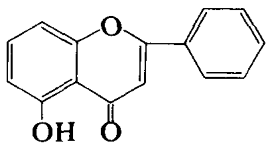


Flavone

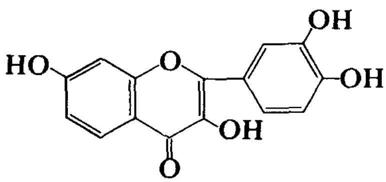
Consequently, the carbonyl group of flavone does not react normally with some carbonyl reagents such as hydroxylamine. However, it does react normally with Grignard reagents. The most widespread flavone is quercetin.



Quercetin



Primuletin



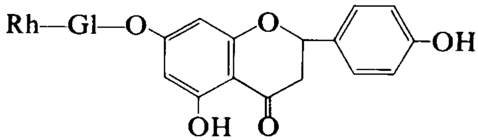
Fisetin

Some flavones, such as primuletin and fisetin, have only one hydroxyl group in ring A.

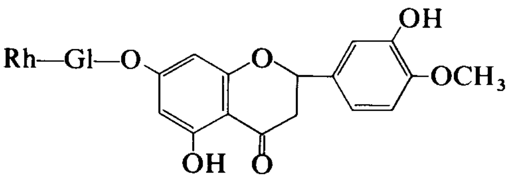
2. Flavanones

Flavanone has not yet been found in nature. Hydroxylated flavanones, however, do occur in nature, either in the free form or as glycosides. In plants they frequently coexist with the corresponding flavones, e.g., hesperidin and diosmin in the bark of *Zanthoxylum avicennae*, the rhoifolin and naringin in the peel of *Citrus aurantium*.

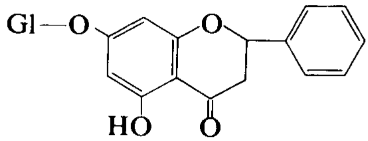
Unlike the unsaturated flavones, the saturated flavanones show reactivity of the 4-carbonyl group. The behavior of flavanones toward alkalis differs from that of flavones; the former decompose into benzaldehyde, acetic acid, and phenol under drastic conditions, whereas the latter yield phenol and cinnamic acid. Dehydrogenation of flavanones, e.g., conversion of hesperitin into diosmin, is of importance, as it makes possible the rapid identification of a new flavanone by reference to a known flavone. The following flavanones merit mentioning:



Naringin



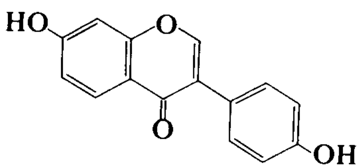
Hesperidin



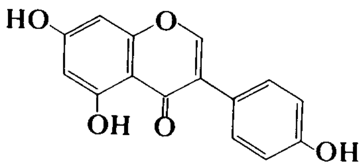
Prunin

3. Isoflavones

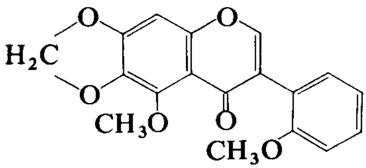
The isoflavones are 3-phenylchromones. At present about 35 isoflavones are known, of which the following are examples:



Daidzein

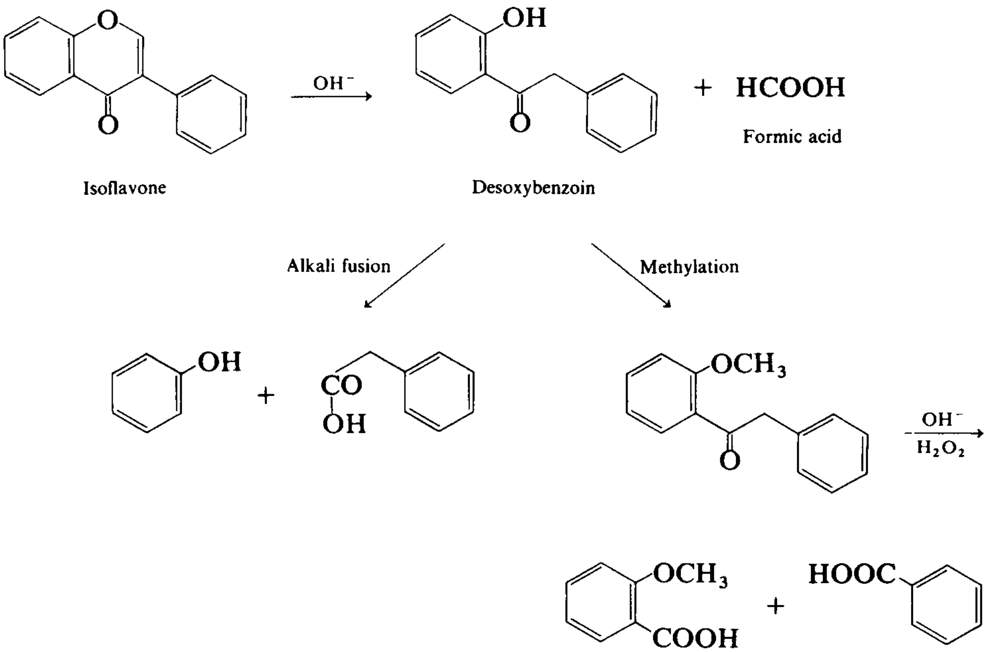


Genistein



Tlanlancuayin

Isoflavones are degraded by alkali as follows:



Isoflavones have shown estrogenic, insecticidal, and antifungal activity; some of them are potent fish poisons.

4. Anthocyanins

The innumerable shades of blue, purple, and violet, and nearly all the reds that appear in the cell sap of flowers, fruits, leaves, and stems of plants are due to anthocyanin pigments in the dissolved state. The sugar-free pigments are called anthocyanidins. The structure common to all anthocyanidins is the flavylium (2-phenylbenzopyrylium) ion.