



Natural Products

A LABORATORY GUIDE

**SECOND
EDITION**

**RAPHAEL
IKAN**

NATURAL PRODUCTS

A Laboratory Guide

Second Edition

Raphael Ikan

Department of Organic Chemistry
The Hebrew University of Jerusalem
Jerusalem, Israel



ACADEMIC PRESS, INC.

Harcourt Brace Jovanovich, Publishers

San Diego New York Boston

London Sydney Tokyo Toronto

This book is printed on acid-free paper. (∞)

Copyright © 1991, 1969 by ACADEMIC PRESS, INC.

All Rights Reserved.

No part of this publication may be reproduced or transmitted in any form or by any means, electronic or mechanical, including photocopy, recording, or any information storage and retrieval system, without permission in writing from the publisher.

Academic Press, Inc.

San Diego, California 92101

United Kingdom Edition published by

Academic Press Limited

24-28 Oval Road, London NW1 7DX

Library of Congress Cataloging-in-Publication Data

Ikan, Raphael, date

Natural products : a laboratory guide / Raphael Ikan. -- 2nd ed.

p. cm.

Includes index.

ISBN 0-12-370551-7

1. Biochemistry--Laboratory manuals. I. Title.

QD415.5.I4 1991

547.0028--dc20

PRINTED IN THE UNITED STATES OF AMERICA

91 92 93 94 9 8 7 6 5 4 3 2 1

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, stereoisomers, 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 "decaffeinated" 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 ^1H - 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

Royal Chemical Society (London), Varian Associates, Verlag Chemie, and Springer Verlag.

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, Neve 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, degradation 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: carotenoids, steroids, terpenoids.
4. Nitrogen compounds: alkaloids, amino acids, nucleic acids, peptides porphyrins, proteins, pteridines.

Each section of these chapters includes a general introduction and methods of isolation, degradation, 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

PREFACE TO THE SECOND EDITION xi

PREFACE TO THE FIRST EDITION xiii

1 ACETOGENINS

I. Flavonoids 1

- A. Introduction 1
- B. Isolation of Hesperidin from Orange Peel 9
- C. Acidic Degradation of Hesperidin 12
- D. Isolation of Naringin from Grapefruit Peel 14
- E. Synthesis of Naringin Dihydrochalcone—A Sweetening Agent 17
- F. Color Changes of Anthocyanins at Various pH Values 19
- G. Questions 22
- H. Recommended Books 22

II. Lipids 23

- A. Introduction 23
- B. Isolation of Trimyristin and Myristicin from Nutmeg 26
- C. Preparation of Azelaic Acid from Castor Oil 30
- D. Formation of *n*-Heptaldehyde and Undecenoic Acid from Castor Oil 32
- E. Ozonization of Methyl Oleate 34
- F. Preparation of Urea Inclusion Compounds 36
- G. Gas-Liquid Chromatography of Methyl Esters of Fatty Acids 41
- H. Thin-Layer Chromatography of Fatty Acids 44
 - I. Questions 46
- J. Recommended Books 47

III. Lignans 47

- A. Introduction 47
- B. Isolation of Sesamin and Sesamolin from Sesame Oil 50
- C. Questions 53
- D. Recommended Book 54

IV. Quinones 54

- A. Introduction 54
- B. Isolation of Rhein from Rhubarb Root 59
- C. Isolation and Identification of Phenols and Quinones from Defensive Secretions of Beetles 61
- D. Questions 66
- E. Recommended Books 66

- V. Phloroglucinols 66
 - A. High-Performance Liquid Chromatography of Hop Bitter Substances 67

2 CARBOHYDRATES

- I. Mono- and Oligosaccharides 70
 - A. Introduction 70
 - B. Isolation of D-Mannoheptulose from Avocado 79
 - C. α -D-Glucosamine from Crustacean Shells 82
 - D. Chromatographic Separation of Sugars on Charcoal 84
 - E. Gas-Liquid Chromatography of Carbohydrates 86
 - F. Thin-Layer Chromatography of Carbohydrates 89
 - G. Separation of Carbohydrates by High-Performance Thin-Layer Chromatography 91
 - H. Questions 92
 - I. Recommended Books 93
- II. Polysaccharides 93
 - A. Introduction 93
 - B. Isolation of Amylopectin and Amylose from Potato Starch 97
 - C. Isolation of Wood Cellulose 101
 - D. Questions 104
 - E. Recommended Books 104

3 ISOPRENOIDS

- I. Carotenoids 105
 - A. Introduction 105
 - B. Isolation of Capsanthin from Paprika 110
 - C. Isolation of Lycopene from Tomatoes 113
 - D. Thin-Layer Chromatography of Carotenoid Pigments in Oranges 117
 - E. High-Performance Liquid Chromatography of Carotenoids in Orange Juice 120
 - F. Determination of β -Carotene and Chlorophylls in Plants 123
 - G. Questions 126
 - H. Recommended Books 126
- II. Steroids 127
 - A. Introduction 127
 - B. Isolation of Stigmasterol from Soybean Oil 136
 - C. Degradation of Stigmasterol 139
 - D. Isolation and Identification of Algal Sterols 142
 - E. Isolation of Hecogenin from Agaves and Its Transformation to Tigogenin and Rockogenin 146
 - F. Preparation of Δ^5 -Cholesten-3-one and Δ^4 -Cholesten-3-one from Cholesterol 149
 - G. Preparation of Vitamin D₂ and Its Separation by Thin-Layer Chromatography 154
 - H. Determination of Sterols by Digitonin 159
 - I. Gas-Liquid Chromatography of Steroids 161

- J. Thin-Layer Chromatography of Sterols and Stanols on Silica Impregnated with Silver Nitrate 165
 - K. Questions 167
 - L. Recommended Books 168
- III. Terpenoids 168
- A. Introduction 168
 - B. Conversion of D-Limonene to L-Carvone and Carvacrol 182
 - C. Isolation and Determination of Optically Pure Carvone Enantiomers from Caraway (*Carum carvi* L.) and Spearmint (*Mentha spicata* L.) 187
 - D. Transformation of α -Pinene to Camphor 191
 - E. Photopronation of Limonene to *p*-Menth-8-en-1-yl Methyl Ether 195
 - F. Flash Chromatography of Essential-Oil Constituents 198
 - G. Isolation and Determination of Grape and Wine Aroma Constituents 201
 - H. Qualitative and Sensory Evaluation of Aromatic Herb Constituents by Direct Headspace-Gas Chromatography Analysis 205
 - I. Preparation of Abietic Acid from Wood Rosin and Its Dehydrogenation to Retene 209
 - J. Conversion of Betulin to Allo- and Oxyallobetulin 212
 - K. Isolation of Cerin and Friedelin from Cork 217
 - L. Removal of Bitter Components from Citrus Juices with β -Cyclodextrin Polymer 220
 - M. Questions 224
 - N. Recommended Books 225

4 NITROGENOUS COMPOUNDS

- I. Alkaloids 226
 - A. Introduction 226
 - B. Method of Degradation 227
 - C. Isolation of Caffeine from Tea 230
 - D. Isolation of Piperine from Black Pepper 233
 - E. Degradation of Piperine 236
 - F. Isolation of Strychnine and Brucine from the Seeds of *Strychnos nux vomica* 238
 - G. Synthesis of Mescaline 242
 - H. Gas Chromatography of Alkaloids 247
 - I. Thin-Layer Chromatography of Opium Alkaloids 250
 - J. Questions 251
 - K. Recommended Books 253
- II. Amino Acids 253
 - A. Introduction 253
 - B. Isolation of Glutamine from Red Beet 259
 - C. Preparation of D-Tyrosine from L-Tyrosine 262
 - D. Synthesis of DL- β -Phenylalanine 265
 - E. Preparation of Glycylglycine by Mixed Carboxylic-Carbonic Acid Anhydride Method 269
 - F. Thin-Layer Chromatographic Enantiomeric Resolution of Racemic Amino Acids 273
 - G. Reverse-Phase High-Performance Liquid Chromatography of Amino Acids 275

H.	Gas-Liquid Chromatography of Amino Acids	277
I.	Test Tube and Glass Rod Thin-Layer Chromatography of Amino Acids	280
J.	Thin-Layer Chromatography of Carbobenzoxy-Peptides	282
K.	Questions	284
L.	Recommended Books	285
III.	Nucleic Acids	285
A.	Introduction	285
B.	Isolation of Soluble Ribonucleic Acid from Baker's Yeast	290
C.	Preparation of Thymidine 3'-Phosphate	293
D.	Chemical and Enzymatic Preparation of Cyclic Nucleotides	296
E.	Ion-Exchange Thin-Layer Chromatography of Nucleotides	300
F.	Questions	303
G.	Recommended Books	303
IV.	Porphyrins	304
A.	Introduction	304
B.	Isolation of Hemin from Blood	309
C.	Degradation of Hemin	311
D.	Thin-Layer Chromatography of Plant Pigments	315
E.	Isolation and Identification of Petroleum Metalloporphyrins	317
F.	Questions	320
G.	Recommended Books	321
V.	Proteins	321
A.	Introduction	321
B.	Isolation of Lysozyme from Albumen	324
C.	Isolation and Separation of Proteins from Groundnuts	326
D.	Thin-Layer Chromatography of Proteins	328
E.	Questions	330
F.	Recommended Books	331
VI.	Pteridines	331
A.	Introduction	331
B.	Isolation of Pteridines from the Fruit Fly, <i>Drosophila melanogaster</i>	334
C.	Synthesis of Pteridines	338
D.	Thin-Layer Chromatography of Pteridines	341
E.	Questions	343
F.	Recommended Books	343
VII.	Pyrazines	343
A.	Introduction	343
B.	Isolation and Characterization of Pyrazines, the Volatile Constituents of Bell Peppers	343
C.	Questions	346
D.	Recommended Books	346
	BIBLIOGRAPHY	347
	SUBJECT INDEX	351
	INDEX OF CHEMICAL COMPOUNDS	357

ACETOGENINS

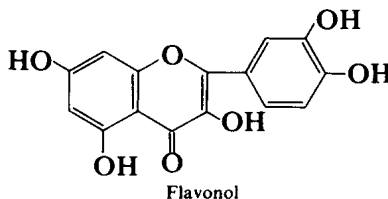
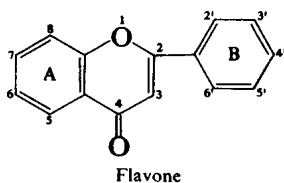


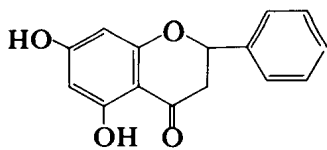
The compounds grouped together in this chapter are different in most chemical properties, but similar in that many of them possess an aromatic ring bearing hydroxyl substituents. Many of these compounds possess a diversity of physiological properties. All of these natural products appear to be biosynthetically 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

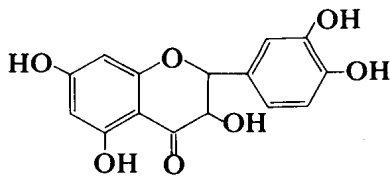
A. Introduction

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.

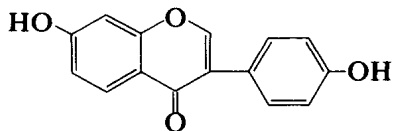




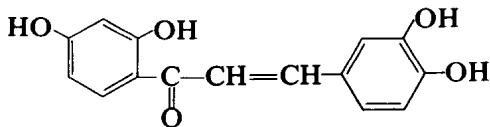
Flavanone



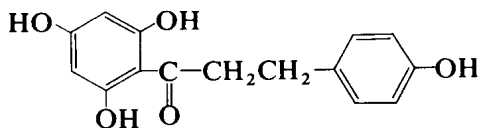
Flavanonol



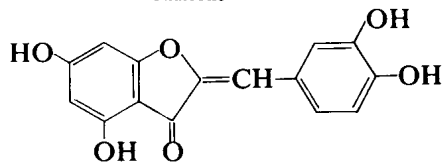
Isoflavone



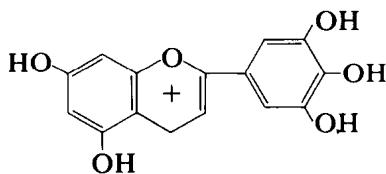
Chalcone



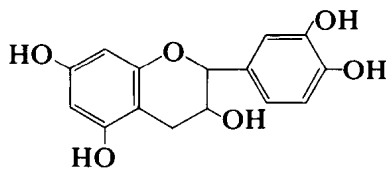
Dihydrochalcone



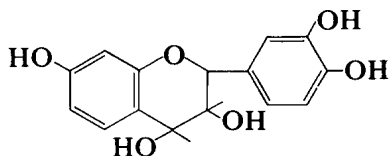
Aurone



Anthocyanidin



Catechin



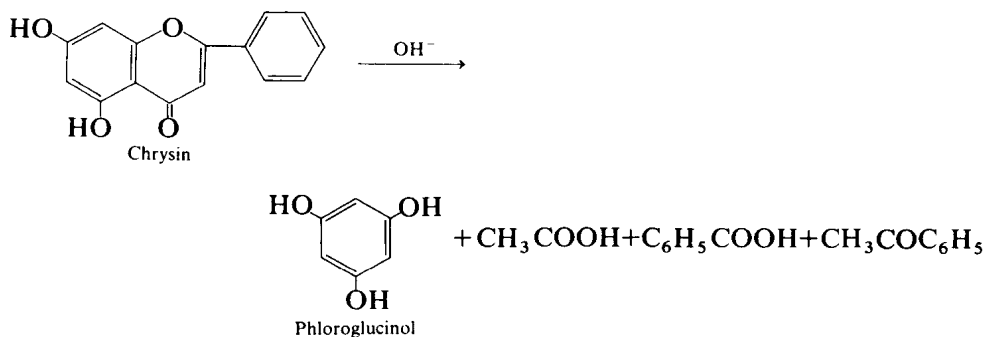
Flavan-3,4-diol

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 alkoxy 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.

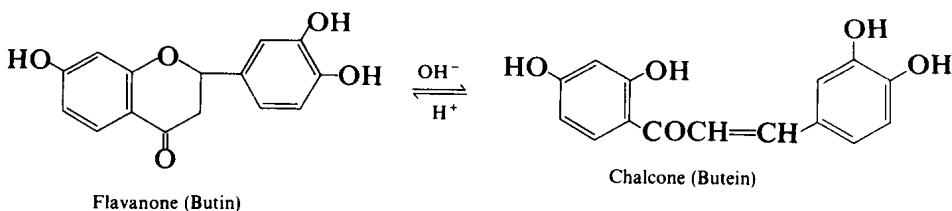
The possible function of this coloring matter in insect-pollinated flowers and edible fruits is to make these organs more conspicuous in order to aid seed dispersion by animals.

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.

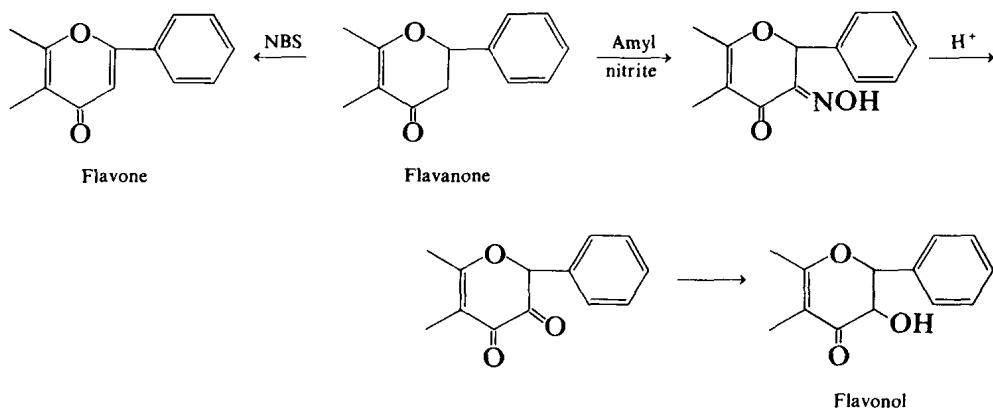


By spectroscopic measurement it is now possible to determine the structures of some flavonoid compounds on the basis of their spectra alone. Color reactions also play an important role in identification of flavonoids at the preliminary stage of analysis.

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.

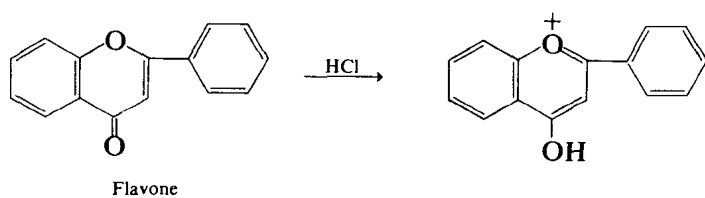


Flavanones may be converted into flavonols and flavones.

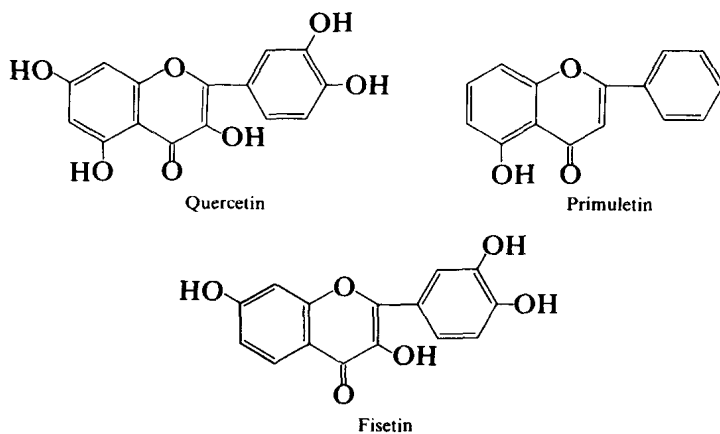


1. Flavones

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



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.

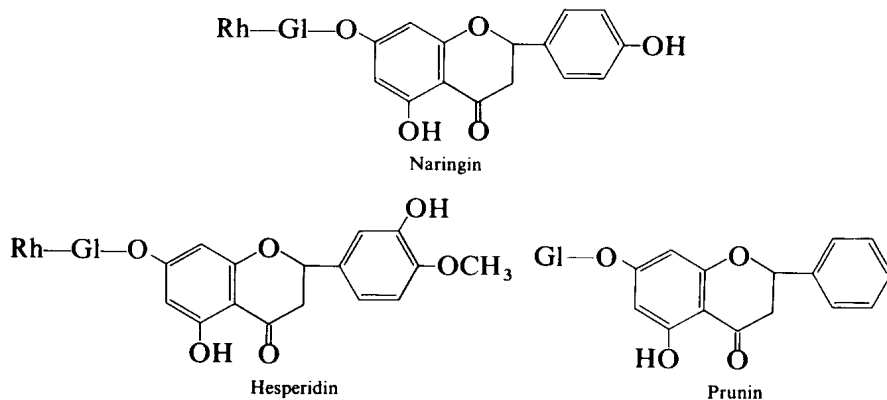


Some flavones, such as primuletin and fisetin, have only one hydroxyl group in ring A.

2. Flavanones

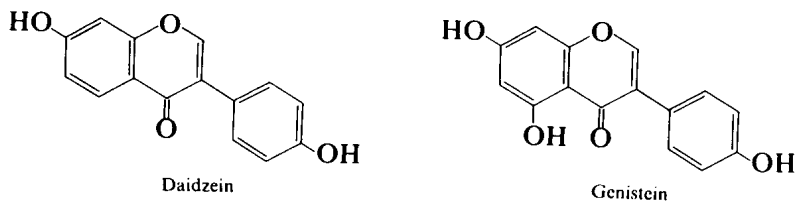
Flavanone has not yet been found in nature. Hydroxylated flavanones, however, do occur in nature, either in the free form or as glycosides. In plants they frequently coexist with the corresponding flavones, e.g., hesperidin and diosmin in the bark of *Zanthoxylum avicennae*, the rhoifolin and naringin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

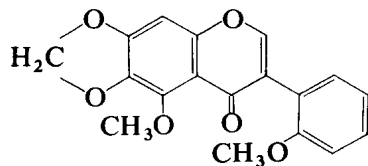
Unlike the unsaturated flavones, the saturated flavanones show reactivity of the 4-carbonyl group. The behavior of flavanones toward alkalis differs from that of flavones; the former decompose into benzaldehyde, acetic acid, and phenol under drastic conditions, whereas the latter yield phenol and cinnamic 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:



3. Isoflavones

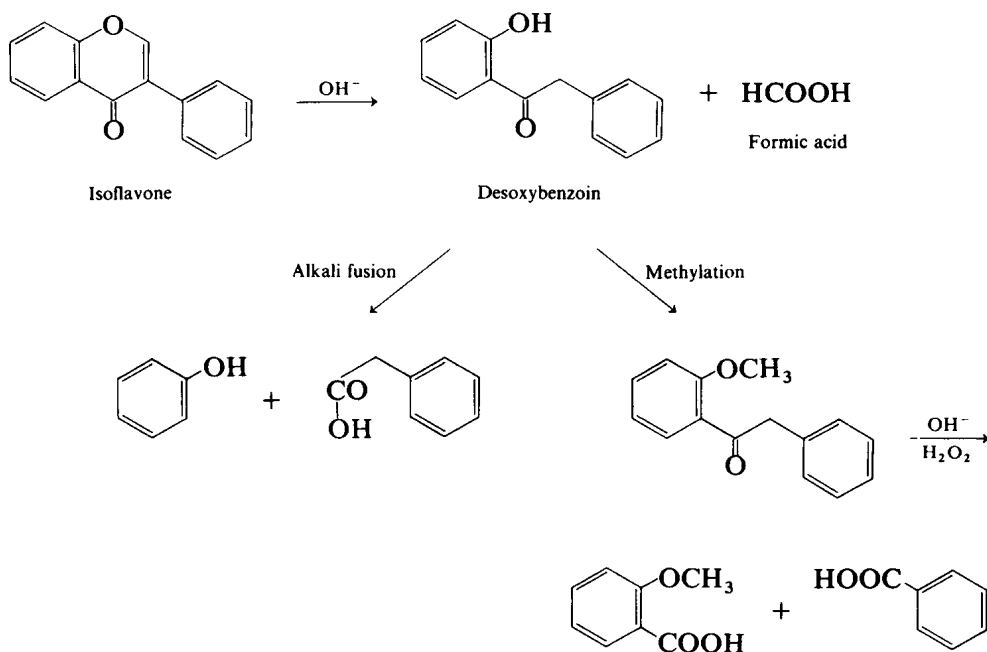
The isoflavones are 3-phenylchromones. At present about 35 isoflavones are known, of which the following are examples:





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.

