The CHEMISTRY of FLAVONOID COMPOUNDS

T. A. GEISSMAN

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PREFACE

THE phenolic compounds of the plant world comprise a body of organic substances of extraordinary variety and interest. Their occurrence in nature, the chemical and biological relationships between them, the chemistry that has been developed in the course of over half a century of study devoted to them, all present a rich field of scientific inquiry from which has come a bountiful harvest of interesting and important findings.

The flavonoid compounds occupy a prominent position among the plant phenols. They possess a close structural and chemical interrelationship that appears to reflect a similarly close relationship in the processes by which they are formed in plants. It is to be anticipated that further understanding of the manner in which flavonoid compounds are formed in nature will soon appear, and that continuing study will eventually elucidate the biological mechanisms of what are among the most common of natural synthetic processes. Much progress has already been made and the main outlines have been drawn, but much remains to be learned. It is a purpose of this book to bring together the knowledge of these compounds that has so far been gained and to present a description of the present position from which further progress can be made.

Flavonoid compounds have attracted the attention of inquiring minds for many centuries. Certain flavones are among the earliest known natural dyestuffs. The conspicuous colors that anthocyanins impart to flowers, fruits and leaves have made them objects of interest and speculation to scientists since the time of Robert Boyle. The importance of flavonoid compounds in the tanning of leather, the fermentation of tea, the manufacture of cocoa, and, more recently, in the flavor qualities of foodstuffs, have led to many recent investigations into the chemistry of derivatives of flavan. Present day studies on the synthesis, stereochemistry, physiological activity, and biosynthesis of flavonoid compounds continue to add new information to the field.

The earliest systematic investigations of the natural flavones were those of St. von Kostanecki in the period of the turn of the present century, who first established the basic structure of the flavones and synthesized a number of the natural compounds. The work of Herzig and A. G. Perkin expanded these studies and led to methods for the determination of the positions of attachment of sugar residues in the glycosides. The establishment of the structure of the anthocyanidins by Willstätter in 1913 was followed by the total synthesis of callistephin chloride by A. Robertson and R. Robinson in

1928, and of the other important natural anthocyanidin glycosides by Robinson and his collaborators in the following years. It is of considerable interest to note that many of the men whose names are among those celebrated in organic chemistry began their scientific investigations in the chemistry of flavonoid substances. The names of all those who have played important parts in the development of flavonoid chemistry cannot be mentioned in a brief introduction. Many of them are contributors to this book. They bring to their discussions a mastery of the subject developed over years of fruitful investigations into the chemistry of flavonoid compounds. The writer, as Editor of this book, is deeply indebted to his colleagues all over the world who have so generously accepted the task of providing these authoritative chapters on the subjects on which they are the recognized experts. The enthusiasm and near unanimity with which they agreed to participate in this undertaking emphasizes the need that existed for a thorough and up-to-date summary statement of the present status of the chemistry of flavonoid substances. The Editor hopes that this book will fill that need, and that it will foster the continuing development of this fascinating area of organic and biological chemistry.

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CHAPTER 1

THE OCCURRENCE OF FLAVONOID COMPOUNDS IN NATURE

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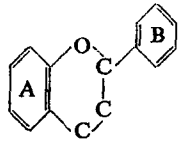
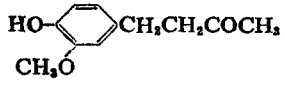
THE carbon skeletons of the flavonoid compounds can be regarded as being made up of two distinct units (I): the C_6—C_3 fragment that contains the B ring; and the C_6 fragment (A ring):



(I)

These structural entities are of different biosynthetic origins (Chap. 19), and while each can be found represented in nature in many organisms from bacteria to higher plants, their combination into the 15-carbon-atom skeleton of the flavonoid compounds is confined almost entirely to the flowering plants and ferns.

The wide occurrence in nature (with the notable exception of mammals) of the large class of phenylpropane derivatives[1] indicates that the synthesis, by the shikimic acid pathway[2], of the 1-arylpropane structure is one of the fundamental synthetic processes of nature. The combination of the C_6—C_3 unit with additional carbon atoms derived from acetic acid, in two-carbon units, can be discerned in the structures of many natural substances other than the flavonoid compounds. Zingerone (II) and shogaol (III) are probably



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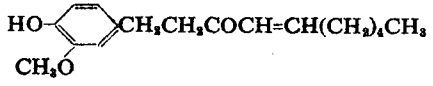
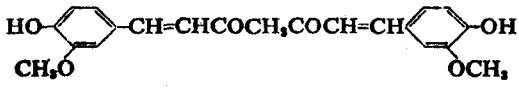


(III)

derived in this way, and curcumin (IV) may originate by a similar route. The substituted 6-styrylpyrones, kawain (V), methysticin and yangonin can be regarded as representing a stage of synthetic elaboration just short of that found in the flavonoid compounds. These pyrones appear to be cyclized

forms of a C_6—C_3—(C_2 + C_2) precursor, the last four carbon atoms of which possess the characteristic "acetate" pattern of oxygenation. Finally, the flavonoid compounds derive from a precursor C_6—C_3—(C_2 + C_2 + C_2), the last six carbon atoms of which are found in the (usually) aromatic A ring.



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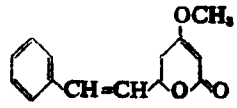


(V)

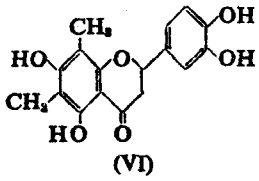
Flavonoid compounds are not found in micro-organisms, which, although capable of synthesizing the phenylpropane unit found in such compounds as phenylalanine and tyrosine, lack the necessary synthetic apparatus for the extension of the C_6—C_3 unit by the addition of two-carbon units. Extensive searches have been made for the presence of flavonoid substances in bacteria and algae; but except for some unconfirmed reports of the occurrence of flavones and anthocyanidins in algae, none has been found.

Mosses have not been thoroughly examined. They have been reported[3] to contain anthocyanins but no other well characterized flavonoid compounds have been isolated. The report[4] that sphagnum moss contains a glucoside of a flavone is not supported by the composition $C_{16}H_{26}O_{13}$ given for the aglucon. Other studies on mosses have given evidence for the presence of aromatic compounds: *p*-anisic acid has been formed by the oxidation of methylated sphagnum[5], and *p*-hydroxybenzaldehyde is formed by the alkaline nitrobenzene oxidation of sphagnum[6]. It has been suggested that this moss may contain a unique lignin built up of *p*-hydroxyphenyl units.

Fungi and lichens have not been found to produce flavonoid compounds. Lichens are characterized by an active and versatile synthetic mechanism for the formation of acetate-derived phenols such as the depsides and anthraquinones, and polyporic acid and its hydroxylated derivatives appear to be derived from a C_6—C_3 precursor. Fungi also utilize the shikimic acid pathway; the formation of *p*-methoxycinnamic acid by *Lentinus lepideus* has been studied in detail by Nord and his associates[7]. Thus, though fungi and lichens possess the mechanisms for the synthesis of the flavonoid building units, their ability to combine these in the manner characteristic of higher plants is lacking.

Ferns contain many flavonoid compounds of the types found in the flowering plants[8, 9]. Anthocyanins, flavones and other C_6—C_3—C_6 compounds have been discovered. It is interesting to note that carbon-methylated compounds are of common occurrence in ferns. Cyrtominetin (VI) and cyrtopterinetin (VII) are examples. Another of particular interest is ceroptene (VIII), an O-methyl ether of the 3,3-dimethyl derivative of the triketo form of cinnamoylphloroglucinol[10].

Flavonoid compounds occur in all parts of the higher plants: roots, stems, leaves, flowers, pollen, fruit, seeds, wood and bark. However, certain kinds of compounds are more characteristic of some tissues than of others. Anthocyanins are typically the pigments of fruits, flowers and leaves. While they do occur in other parts of the plant they are often confined to, or occur in highest concentration in one kind of tissue. Deeply colored flowers may



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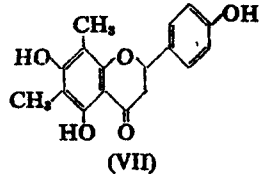

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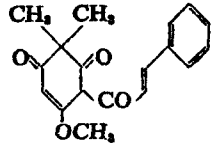

(VIII)

be borne on plants with essentially anthocyanin-free stems and leaves. In general, however, the capacity of a plant to synthesize anthocyanin at all results in the formation of at least traces of the pigment in the green parts of the plant. Occasionally, heavy anthocyanin pigmentation causes plant leaves and stems to take on red or brown colors; examples are found in the conspicuous coloration of many autumn leaves, and in the red colors of young leaves of some plants.

While other kinds of flavonoid substances are often found in one kind of plant tissue more frequently than in others, there are few cases in which the occurrence of a given compound or type of compound is restricted to flowers or leaves or to any other single location in the plant. While catechins and flavan-3,4-diols ("leucoanthocyanidins") have in recent years been isolated from woods and barks more often than from other plant parts, compounds of these classes do occur in such non-woody tissues as tea leaves, cacao beans, and fruit pulps. Chalcones and aurones are largely found in flower petals, but some species of *Coreopsis* contain these pigments in stems and leaves. Flavones, flavonols and flavanones occur in many parts of plants and cannot be said to be characteristic components of any one kind of tissue.

An exception to the above comments is to be found in the case of the complex, polymeric flavonoid tannins and phlobaphenes. These are largely confined to wood and bark, and are regarded as the end-products of the condensation of monomers (C_{15}-compounds) that arise in the actively

metabolizing zones of the stem and are subsequently transformed into immobile polymers and deposited in the woody tissues. It has been found that the proportion of monomeric tannin precursors decreases, and the molecular weight of the condensed tannin increases from the outer heartwood to the inner heartwood[11].

While representatives of the numerous classes of flavonoid compounds are to be found throughout the plant world, certain plant families and genera are characterized by an unusually high degree of occurrence of some specialized structural types.

Chalcones and aurones are not widely distributed in nature. Those in which the A ring are 2,4-dihydroxylated (resorcinol type) are nearly confined to a restricted group of the Compositae. The occurrence of a butein glycoside in *Butea frondosa*, and of the chalcone plathymenin in *Plathymenia reticulosa* (Leguminosae) are notable exceptions. Ceroptene[10] is a chalcone derivative in which the A ring has been modified by nuclear methylation to a cyclohexanetrione derivative. Aurones bearing the phloroglucinol hydroxylation pattern in the A ring form an interesting contrast in their distribution as compared with the resorcinol–A ring aurones. They are not found in the Compositae, nor is their occurrence characteristic of any one family. Representatives of this class of aurones have been found in the Scrophulariaceae and Oxalidaceae.

A notable example of the capacity for a closely allied group of plants to perform a single kind of synthetic reaction is found in the wide occurrence of O-methylated flavonoid compounds in the Rutaceae. Various citrus species contain the highly substituted polymethoxy flavones nobiletin, tangeretin, auranetin, as well as others with less extensive alkylation; and the rutaceous *Melicope ternata* contains several closely related O-alkylated flavones. Other examples will be found in Chapter 13.

The carbon-alkylation of the A ring of flavonoid and related compounds is not common, but occurs with unusual frequency in ferns and in coniferous plants. The ability of ferns to carbon-methylate rings derived from acetate condensation is especially noteworthy, since it is observed in flavonoid compounds as well as in such non-flavonoid substances as flavaspidic acid and albaspidin. Ceroptene represents a link between the non-flavonoid cyclohexanetriones of ferns and the flavonoid compounds, and is clearly the product of the superimposition of a second biosynthetic mechanism (the union of C_9 with C_6) upon a more general one (the formation of a C-methylated C_6).

One can thus discern in the distribution of flavonoid and related compounds in nature an underlying basic synthetic theme, the production of a nine-carbon building block that may finally yield any of numerous phenylpropane-derived end-products, or may be attached to further fragments, usually acetate units, to lead to more complex final products, of

which the flavonoid compounds are the most widely distributed and closely interrelated class. The addition or removal (probably at an early stage) of hydroxyl groups, oxygen- or carbon-alkylation, the attachment of sugar residues, and the wide range of oxidation stages of the central three-carbon atom unit are changes more limited in occurrence, and are often the structural hallmarks of restricted genera or familles of plants.

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CHAPTER 2

ISOLATION OF FLAVONOID COMPOUNDS FROM PLANT MATERIALS

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CONTENTS

RANGE OF CHARACTERISTICS

FLAVONOIDS, in their occurrence, represent a very large number of types with different properties. The majority of these occur as glycosides. Robinin is an example of triglycoside, others like butrin and rutin are diglycosides and a large number are monoglycosides. In several cases instead of glucose, glucuronic acid is present. Complex anthocyanin glycosides contain acids like *p*-hydroxybenzoic acid in combination with the sugar residues and this feature influences the properties of the compounds. The aglycons also occur free. They vary considerably in the number of hydroxyl groups; for example, flavone itself occurs in nature, whereas hibiscetin has seven hydroxyl groups in the molecule. Further, there are several cases of partial methyl ethers and some in which all the hydroxyl groups are methylated or methylenated; examples having all hydroxyl groups protected are nobiletin, tangeretin, kanugin and meliternatin. Some have extra furan rings also. Consequently there is a large variation in the solubility characteristics of the compounds as they occur in plant products.

The polyglycosides and diglycosides are definitely soluble in water and sparingly soluble in most organic solvents, e.g. butrin and isobutrin. The position which the sugar units occupy also plays an important part. Depending upon the position of the sugar group a glycoside exhibits different properties particularly in relation to solubility and capacity to form sparingly soluble metallic lakes or precipitates. Quercimeritrin is a 7-glycoside of quercetin whereas rutin, isoquercitrin and quercitrin are 3-glycosides. The former is sparingly soluble in water and gives a red precipitate with neutral lead acetate, whereas the latter are more easily soluble in water and form yellow precipitate with basic lead acetate. They can therefore be separated successfully in the form of their lead salts by using this difference.

Gossypetin glycosides also provide good examples. The 7-glucoside (gossypitrin) is sparingly soluble in water and almost insoluble in organic solvents, has a light yellow colour and yields a red lead salt, whereas the 3-glucoside (gossytrin) is far more soluble in water, is less coloured and yields an orange lead salt. A third glucoside of gossypetin (gossypin) is known in which the sugar group is present in the 8-position. It is soluble in water but sparingly soluble in alcohol and has a deep yellow colour. Its lead salt is more soluble and comes down slowly as a brown precipitate.

Among the aglycons there is a large range in solubility and stability. Flavones and flavonols are sparingly soluble in water and dihydroflavonols are more soluble; this difference can be used for their separation. Catechins (3-hydroxyflavans), and leucoanthocyanidins (flavan-3:4-diols) are soluble in water. The former can be extracted from aqueous solution by means of ether, and the latter can be extracted mainly by ethyl acetate. The anthocyanins are somewhat exceptional in that they are stable only as salts. Consequently they have to be extracted by acid solvents and processed under acidic conditions and isolated and preserved as chlorides. Normally they are red substances; in certain blue flowers they exist as complexes which are responsible for the special colour. Chalkones and flavanones are frequently interconvertible and hence unless special care is taken interconversion takes place and a mixture results. A typical example is that of isobutrin, a chalkone diglucoside present in the petals of *Butea frondosa*. Unless the fresh flowers are extracted it undergoes considerable conversion into butrin (a flavanone diglucoside). A parallel case is that of isoliquiritin (chalkone glucoside) and liquiritin (flavanone glucoside) in liquorice roots.

NATURE OF PLANT MATERIALS

Flavonoids have been found to occur practically in all parts of plants. Typical examples are given in later paragraphs. The methods of extraction differ depending upon the characteristics of the plant source and particularly upon the impurities present; for example seeds are frequently rich in oils, waxes and proteins, and leaves contain a great deal of resin, wax and chlorophyll. The solvents normally used are alcohol, acetone, ether and light petroleum. In special cases, water can be used but this is unusual because it brings in many other impurities. Light petroleum removes oils and waxes mostly, and only in special cases are flavonoids extracted.

TECHNIQUES

In almost all cases fractionation and separation of components have to be carried out. In several cases a choice of solvents is possible or fractionation can be effected by column chromatography. A more convenient method is to effect separation by using the difference in acidic properties of the components. This depends largely on the location of the free hydroxyl groups,

for example, a compound with a phenolic hydroxyl *para* to the carbonyl dissolves readily in sodium carbonate and in special cases sodium bicarbonate, whereas those which lack this hydroxyl or have it protected by methyl do not dissolve. A further useful method involves the formation of complexes with borates. The minimum requirements for the complex formation seem to be the existence of a 5-hydroxyl group and of a catechol unit elsewhere. Quercetin and its glycosides can be conveniently extracted by borax and can be liberated by acidification. Further, quercetin can be separated from kaempferol because the latter lacks the catechol unit and is not extracted by aqueous borax. Complex formation of a different type takes place between rotenone and carbon tetrachloride and is used in the purification of rotenone.

An important consideration in obtaining consistent analysis of plant materials is the history of the sample. It is most convenient to use fresh plant material. A good alternative is to carry out quick drying, thus preventing enzymatic changes. A wet sample deteriorates very fast, particularly in warm weather. It is most useful to follow up the progress of extraction and its efficiency by paper chromatography. Many components which were missed in earlier work have been recently detected by paper chromatography. Considerable work has been done in this line and data are available on chromatographic behaviour of the different groups of flavonoids (Chapter 3).

In the following sections the extraction of flavonoids from typical plant materials is discussed under the heads of: (1) flowers, (2) fruits, (3) seeds, (4) leaves, (5) heartwoods, roots and barks, and (6) gums and resins.

FLOWERS

Flowers display a large variety of colours and they have the largest range of components: anthocyanins, flavones, flavonols, flavanones, chalkones and aurones (benzalcoumaranones). Apart from anthocyanins which are responsible for deep and bright colours, shades of yellow can be attributed to polyhydroxyflavones and flavonols. More recently chalkones and aurones have been shown to be responsible for the bright colours of certain flowers, e.g. *Butea frondosa* and *Cosmos sulphureus*. Frequently the former are accompanied by flavanones.

Flowers are probably the most convenient for the extraction of flavonoids because they are rich sources and in general contain few extraneous impurities. But there are cases where mucilage or a high percentage of wax is present. Carotenoids form a common source of coloured impurity but they are fairly easily removed because they are sparingly soluble in aqueous alcohol and are extracted by petroleum ether. Important and typical examples are described below.

Roses

Roses, both red and yellow, are found to contain anthoxanthins; only the former contains anthocyanins.

Extraction of Anthocyanin (Cyanin)[1]

Some of the deeply red roses are very rich in pigments. The dried petals are extracted in the cold with methyl alcoholic hydrochloric acid (2 per cent) for 16 hr and filtered; the residue is washed with more solvent several times to complete the extraction and the pigment precipitated from the filtrate by adding two and a half times its volume of ether. The crude amorphous product is then allowed to stand, without drying, for 24 hr with alcoholic hydrochloric acid or better with methyl alcohol and acetic acid, when the impurities present undergo change and dissolve, but cyanin remains unchanged as a deep brown, microcrystalline residue. The product is dissolved in boiling water, mixed with an equal volume of 3 per cent ethyl alcoholic hydrochloric acid, and allowed to cool, when cyanin chloride separates as fine glistening crystals.

Extraction of Anthoxanthins (Quercetin and Kaempferol)[2]

The flower petals of red or yellow roses are extracted with boiling alcohol and the alcoholic solution concentrated under reduced pressure. The concentrate is repeatedly shaken with petroleum ether to remove impurities. The residue is boiled with aqueous or alcoholic sulphuric acid (7 per cent) for 2 hr. On extracting with ether and evaporating the extract, a mixture of aglycons is usually obtained. It generally contains quercetin and kaempferol; separation can be effected by shaking the ether extract with aqueous borax in which quercetin dissolves and can be recovered by addition of acid; kaempferol is left in the ether solution (m.p. quercetin 312°C, kaempferol 275°C).

Butea frondosa (*Butrin, Isobutrin and Palasitrin*)[3]

The air-dried flowers were repeatedly extracted with petroleum ether in the cold in order to remove waxy matter. The flowers were then repeatedly extracted with alcohol in the cold, the extract evaporated almost to dryness under reduced pressure and then treated with enough water to give a clear solution. The aqueous solution was repeatedly extracted with ether to remove the free aglycons, butin and butein, and allowed to stand in the refrigerator, saturated with ether. In a few days butrin separated as a pale yellow crystalline solid which could be recrystallized from alcohol yielding colourless needles (m.p. 194–195°C). From the mother liquor after removal of further crops of butrin, another yellow glycoside, isobutrin was obtained and also palasitrin (an aurone glycoside) as given in the following paragraph for fresh flowers.

The orange-red fresh flowers were extracted in the cold with alcohol for 2 days. The process was repeated twice with fresh alcohol. The extract was concentrated at atmospheric pressure to a small volume and set aside. A pale orange-yellow solid separated, which after two crystallizations from methanol and one from ethanol separated as colourless long needles (butrin).

The mother liquor (after removal of butrin) was further concentrated and cooled. Bright yellow crystals were obtained which crystallized from methanol as bright yellow prisms yielding isobutrin (m.p. 190–191°C).

From the mother liquor left after removing isobutrin, palasitrin (m.p. 199–200°C, d.) was isolated by following the method of Geissman *et al.* for the isolation of leptosin from *Coreopsis grandiflora* (see below).

Coreopsis grandiflora (*Leptosidin and Leptosin*)[4]

The fresh rays were covered with 95 per cent alcohol and after soaking overnight, run through a meat grinder. The resulting mixture of alcohol and petal meal was allowed to stand for 48 hr at 0°C after which time the meal was pressed dry and the extract filtered. It was evaporated under reduced pressure and the syrup was taken up in water. The solution was extracted with ether till the ether extract was no longer coloured. Extraction of the ether solution with several portions of dilute aqueous potassium carbonate yielded a deep red solution. This was acidified and extracted with ether. The ether solution was washed several times with dilute sodium acetate solution (discarded) and then with a cold concentrated solution of potassium carbonate. A copious orange-red crystalline potassium salt was obtained; on treatment with acid it gave leptosidin which could be crystallized from aqueous methanol (m.p. 252–254°C). From the aqueous potassium carbonate solution from which the salt of leptosidin had separated, 8-methoxy butin (m.p. 197°C) and luteolin (m.p. 330–331°C) could be isolated.

The aqueous solution left after the continuous ether extraction of leptosidin was saturated with ammonium sulphate and extracted with several portions of butyl alcohol. The butyl alcohol extract was diluted with light petroleum (30–60°C) and extracted with several portions of potassium carbonate solution. Acidification of the potassium carbonate extract yielded a deep red-brown solution which was shaken up with a little butyl alcohol and allowed to stand. A deep orange precipitate formed which was filtered and was found to be leptosin. This could be crystallized from aqueous methanol.

Hibiscus vitifolius (*Gossypin*)[5]

Hibiscus vitifolius forms a rich source of gossypin which is a water-soluble glycoside. When air-dried petals were employed complete extraction could not be achieved by repeated boiling with alcohol and a considerable amount of the colouring matter could be obtained from a subsequent water extract.