1983, Vol. 47, pp. 131–140, © Hippokrates Verlag GmbH

Journal of Medicinal Plant Research **Planta medica**

Review Article

Chemistry, Occurrence and Biosynthesis of C-Glycosyl Compounds in Plants

Gerhard Franz, Michael Grün[1]

Lehrstuhl für Pharmazeutische Biologie, Universität Regensburg, Federal Republic of Germany

Received: August 2, 1982

Introduction

The rapid development of natural product chemistry has led to the isolation of a wide variety of secondary metabolites, which, in many cases, was shown to be of glycosidic nature. In particular our knowledge of the chemistry, occurrence and biogenesis of glycosides has grown substantially during the last two decades [1, 2]. Most of this effort has been directed toward the large group of the O-glycosides as well as the S- and N-glycosides. Far less effort has been given to the C-glycosides due to analytical difficulties.

C-Glycosides are a special type of glycoside since the aglycone is directly attached to carbon 1 of a pyranose ring of a sugar while O-glycosides possess a hemiacetal linkage. It is more correct to regard them as C-glycosyls or anhydropolyols as proposed by BANDYUKOVA and YUGIN [3]. Since the original term C-glycoside is well established, it should be maintained.

One of the main characteristics of C-glycosides is resistance towards acid hydrolysis. Even after prolonged acid treatment the sugar residue, which is attached by a carbon-carbon-bond, remains attached to the aglycone. Cleavage of the C-C-linkage is achieved only under drastic conditions. When oxidizing reagents are used partial degradation of the sugar residue is likely [4].

Classification of C-glycosides is usually based on the aglycone [5, 6]. Five aromatic ring systems are known to occur C-glycosylated (Fig. 1). The largest group, the C-glycosyl flavonoids, has been intensively studied by many authors and was the subject of two recent review articles [3, 7]. Xanthone C-glycosides appear to be biogenetically related to C-glycosyl flavonoid compounds. Another group, C-glycosylated chromone derivatives, are relatively rare. Aromatic three-ring systems, notably the anthrones, are C-linked to glucose. C-Glycosyl derivatives of gallic acid also exist. Application of such analytical tools as UV, IR, NMR and mass spectroscopy as well as optical measurements has resulted in the identification of large numbers of such compounds in recent years.

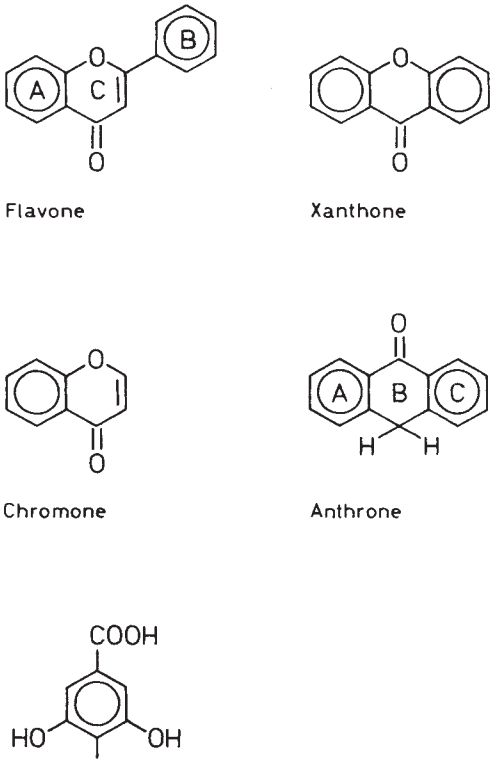


Fig. 1. Basic structures of C-glycoside aglycones.

Interestingly, C-glycosides are not restricted to plants. Carminic acid is an anthraquinone C-glycoside from the insect *Dactylopius coccus* (references given in [3]). Kidamycin and toromycin are antibiotics produced by *Streptomyces* sp. and represent a new type of polycyclic microbial metabolite with unusual C-glycosyl moieties [8, 9]. Recently, C-conjugated

[1] Present address: Institute of Biological Chemistry, Washington State University, Pullmann, Washington 99164-6340, USA.

compounds with glucuronic acid have been found in mammals, which are probably analogous to C-glycosylated compounds in plants. C-Glucuronides of drugs in man [10] and other mammals [11, 12] represent a novel type of drug metabolite.

As regards the biosynthetic pathway which leads to the formation of the carbon-carbon bond in C-glycosides, very little is known. In some cases it is thought that the attachment of the sugar moiety takes place during an early stage of the aglycone formation but definite proof is still missing and cannot be generalized for all groups of the C-glycosides.

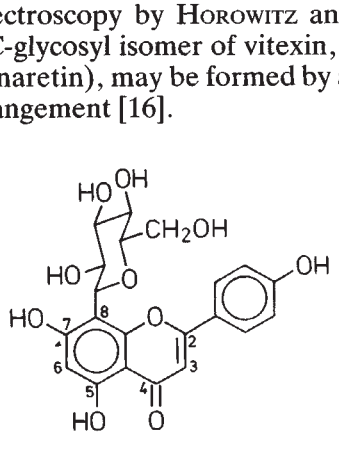
The aim of this article is to present a short review of the principal structural features and occurrence of the different classes of C-glycosides and further, to discuss the few biosynthetic findings of the C-glycosyl attachment which have been published so far.

C-Glycosyl Flavonoids

Chemistry of C-glycosyl Flavonoids

As it has been pointed out, this group of C-glycosides has been the subject of a still increasing number of reports, which mainly deal with the occurrence and structural investigation of these compounds. Since they have been the subject of several review articles [3, 6, 7, 13, 14] only the main features are given here.

At the present over one hundred C-glycosyl flavonoids have been shown to exist in the plant kingdom. The model compound and one of the first to be isolated was vitexin (8-C-β-D-glucopyranosylapigenin) (Fig. 2), the structure of which was established by NMR spectroscopy by Horowitz and Gentilini [15]. The 6-C-glycosyl isomer of vitexin, named isovitexin (saponaretin), may be formed by an intramolecular rearrangement [16].



Vitexin

Fig. 2. Vitexin as a representative C-glycosyl flavonoid.

A free ortho-position to one or two phenolic hydroxyl groups appears to be a common feature that is prerequisite for the formation of a C-glycosidic linkage in flavonoids. Therefore, the glycosyl residue is linked to carbon 6 or 8 of ring A (Fig. 1) according to its specific substitution pattern. The same aglycone may possess a second C-linked sugar or O-linked sugar residues attached either to phenolic OH-groups or alcoholic OH-groups of the C-linked sugar. In the latter case, C,O-diglycosides and the C,O-triglycosides are formed. It is interesting to note that in most cases D-glucose serves as C-linked sugar but in the case of flavonoid derivatives the only C-glycosyl compounds currently known contain other C-linked sugar residues, i.e. galactose, rhamnose, xylose and arabinose [3]. A wide variety of O-linked sugars may be present as well. As for the aglycone moiety five types of flavonoid structures have been demonstrated, i.e., flavones, flavonols, flavanones, isoflavones and dihydrochalcones [7]. No anthocyanidines have been identified as C-glycosides. The most widespread aglycone structures which have been reported in literature and which show different glycosidic variations are apigenin and luteolin [3]. More than 30 derivatives of these aglycones have been reported so far.

Occurrence of C-Glycosyl Flavonoids

The review of Alston [13] on C-glycosyl flavonoids mentions about 20 Angiosperm families where these compounds occur. There are no reports of C-glycosyl flavonoids in Gymnosperms and only a few of mosses and algae. Recently Bandyukova and Yugin [3] listed 54 Angiosperm families with a varying number of species that contain C-glycosyl flavones in significant quantity. Gymnosperms and several lower plants also possess these "unusual" flavonoid derivatives. One can conclude that the C-glycosyl flavonoids are widespread among plants, but with restricted chemotaxonomic significance as Chopin and Bouillant have pointed out [7]. The most abundant examples seem to be vitexin, isovitexin and their derivatives.

Biosynthesis of C-Glycosyl Flavonoids

Most types of substituted flavonoid aglycones occur as the O-glycoside with different sugars attached to alcoholic or phenolic groups. Biosynthesis of "normal" flavonoids and their glycosylated derivatives involves three acetate units (ring A) and phenylalanine (ring B and the C_3 segment) as shown by *in vivo* and *in vitro* tracer studies (see reviews by Hahlbrock and Grisebach [17], Hahlbrock [18]). In the case of C-glycosyl flavonoid aglycones, participation of phenylalanine and p-coumaric acid in ring B and C formation has been established [19, 20].

The mechanism of flavonoid O-glycosylation, whereby the sugar moiety of a nucleotide sugar is transferred via more or less specific transferases to the aglycone acceptor is well understood and is the subject of recent reviews [2, 21]. The second type of glycoside formation, i.e., the biosynthesis of the C-glycosidic linkage, has never been explained in detail. A few reports, however, give some indication about the biosynthetic stage of the aglycone at which

the C-glycosidic linkage might be formed. WALLACE et al. [22] fed different radioactive precursors such as the flavone aglycones, apigenin and luteolin, to *Spirodela* sp. or *Lemna* sp. (Lemnaceae) and detected radioactivity on O-glycosylated and O-methylated flavones. Radioactivity was not incorporated in flavones containing C-glycosyl linkages, indicating that O-glycosylation of the flavones occurs readily *in vivo*, but C-glycosylation does not occur under conditions in which both compounds are known to be synthesized in the plant. WALLACE and GRISEBACH [23], on the other hand, showed that C-glycosylation was only possible at the flavanone level using *in vitro* cultured *Spirodela polyrhiza* (Lemnaceae) clones. Again Wallace [24] stated that formation of the C-glycosidic bond is always prior to O-glycosylation. Pre-existing O-glycosyl flavonoids are not C-glycosylated. C-Glycosyl flavonoids, however, might be O-glycosylated in a later step of biosynthesis (Fig. 3). INOUE and FUJITA [25] and Fujita and Inoue [20] supported these findings by feeding possible ^{14}C-labeled precursors to *Pueraria lobata* (Fabaceae) and *Swertia japonica* (Gentianaceae) plants. C-Glycosylation again occurred at the chalcone of flavanone level.

Radioactive phenylalanine is rapidly incorporated into C-glycosyl flavones of the primary leaf of oat plants (*Avena sativa*, Poaceae). Two vitexin-derived O-rhamnosides and one isovitexin O-arabinoside are formed and show a slow turnover during the experimental period of ten days, thus indicating that the C-glycosides are not necessarily metabolic end products [26, 27]. However, MARGNA and VAINJÄRV [28] have demonstrated that amongst the major flavonoids of buckwheat seedlings (*Fagopyrum esculentum*, Fabaceae), rutin and the C-glycosyl flavonoids do not undergo appreciable turnover during a seven-day period.

Genetic control of flavonoid biosynthesis and mainly O-glycosylation has been extensively studied. Vitexin and isovitexin glycosylation in *Melandrium* sp. and *Silene* sp. (Caryophyllaceae) are the subject of a series of publications (see review by HÖSEL [21] and [29, 30, 31, 32]). Nothing, however, is shown for the control of the C-glycosylation process.

Besides these *in vivo* studies concerning the formation of C-glycosyl flavonoids essentially nothing is known about the enzymatic mechanisms which lead to the formation of the C-glycosidic bond.

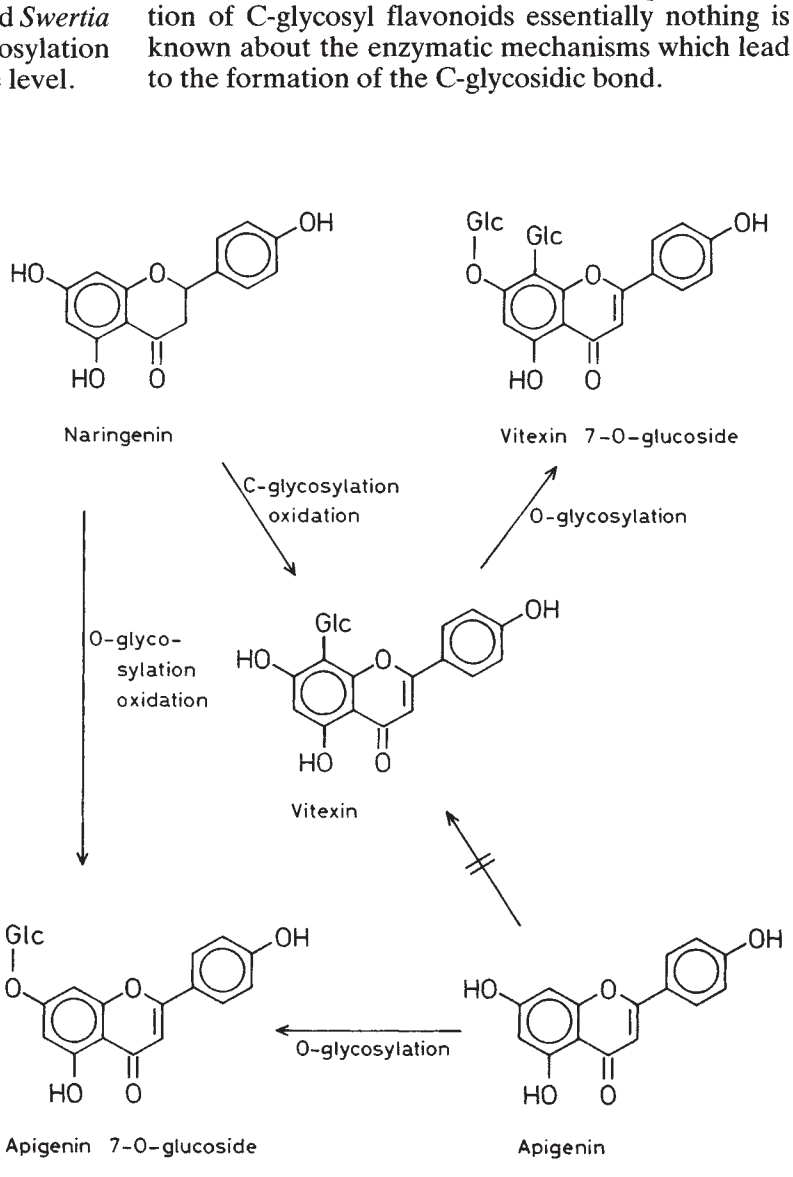


Fig. 3. Sequence of glycosylation and oxidation reactions involved in the biosynthesis of flavonoid O- and C-glycosides according to [22, 23].

Xanthone C-Glycosides

Chemistry of Xanthone C-Glycosides

More than ten glycoxanthones from plant sources are now known. Some information about the chemotaxonomic distribution, isolation and structural determination, synthesis and pharmacology of most compounds is given by HOSTETTMANN and WAGNER in a review on xanthone glycosides [33].

The first compound with a C-glucosyl residue linked to a polyoxygenated xanthone nucleus was obtained by WIECHOWSKI in 1908 [34] but the final structure of mangiferin (Fig. 4) as 2-C-β-D-glucopyranosyl-1,3,6,7-tetrahydroxyxanthone was not established until 60 years later [35–41]. Mangiferin, being the best known C-glucosyl xanthone, represents the most important features of all other hitherto known related substances: the glucosyl residue is either attached to carbon 2 or 4 of the xanthone nucleus which is oxygenated in 1, 3, 6 and 5 or 7 positions, respectively.

Isomangiferin (Fig. 4), the 4-C-glucosyl isomer of mangiferin, has been isolated and characterized by ARITOMI and KAWASAKI [42]. Interestingly, there is a similarity between these compounds and other pairs of position isomers like the flavonoid 8-C- and 6-C-glucosides orientin and isoorientin, or vitexin (Fig. 2) and isovitexin, respectively. The 3-monomethyl ether of mangiferin, homomangiferin (Fig. 4), is the second known xanthone C-glucoside [43]. Mangiferin derivatives, acetylated or benzoylated in the glucose moiety of the molecule, have been reported by MARKHAM and WALLACE in 1980 [44]. ^{13}C-NMR studies indicate that the glucose 6-hydroxyl is acetylated whereas benzoylation occurred at 2'-O-4'-O- and 6'-O-atoms of mangiferin. However, those mangiferin derivatives were not obtained pure but could be separated by paper chromatography as a mixture from another component which represented a mangiferin esterified with two mols of benzoic acid.

SMITH and HARBORNE [45] and GLYZIN et al. [46] found hydrolyzable derivatives of mangiferin and isomangiferin which yielded glucose and the two isomers on acid treatment. Spectral data suggest that the glucosyl residue is attached to a hydroxy group of the C-glucosyl moiety. The characteristics of those C,O-glucosides are analogous to that of O-glucosides of 6- or 8-C-flavone glycosides. Two C-glucosyl xanthones with a substitution pattern different from that of mangiferin have been found. 2-C-β-D-Glucopyranosyl-1,3,5,6-tetrahydroxyxanthone (Fig. 4) was isolated by GHOSAL and CHAUDHURI [47]. The structure of its 5-monomethyl ether (Fig. 4) has been determined by chemical and spectral studies and has been named irisxanthone [48]. Recently, two highly methylated compounds, 2-C-β-D-glucopyranosyl-1-hydroxy-3,5,6-trimethoxyxanthone and 2-C-β-D-glucopyranosyl-1-hydroxy-3,5,6,7-tetramethoxyxanthone were described [49].

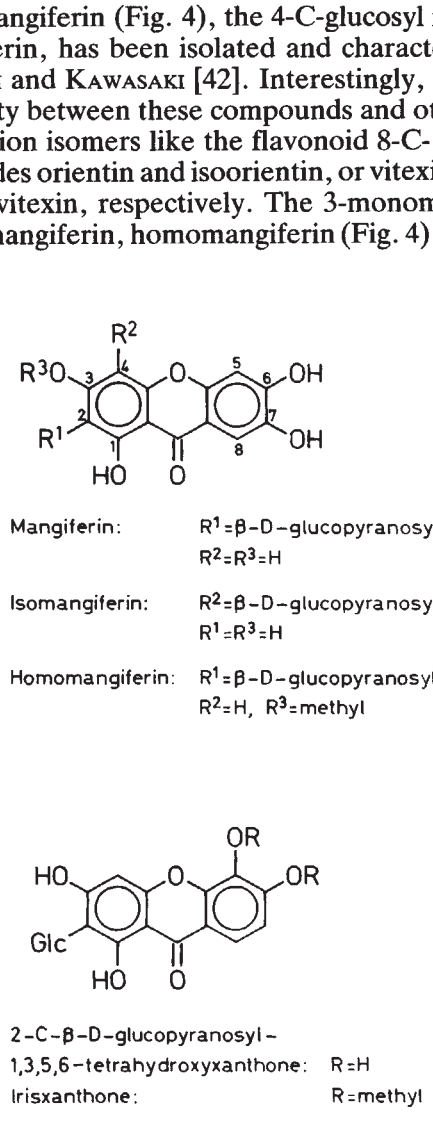


Fig. 4. Xanthone C-glycosides.

Occurrence of Xanthone C-Glycosides

Mangiferin is the most wide-spread glycoxanthone in higher plants [50]. First isolated from *Mangifera indica* (Anacardiaceae) [34], it has been detected later in many other families. In addition ot that reported by CARPENTER et al. [50] it has been found in the Orchidaceae [51] and some ferns of the Aspleniaceae [45] and the Hymenophyllaceae [44]. A more detailed view about the distribution of mangiferin among a number of *Gentiana* and *Swertia* sp. (Gentianaceae) is given in [33]. Although of little taxonomic significance in the broad sense, its presence or absence at the species level may be significant.

Its isomer, isomangiferin, has been identified together with mangiferin in *Anemarrhena asphodeloides* (Liliaceae) [42], *Mangifera indica* [52], *Hedysarum flavescens* (Fabaceae) [46], Orchidaceae [51] and Aspleniaceae [45]. The fern genus *Asplenium* (Aspleniaceae) and *Hedysarum flavescens* are until now the sole source of O-glycosylated xanthone C-glycosides [45, 46]. Homomangiferin, shown to accompany mangiferin in *Mangifera indica* [53], was also detected in *Hoppea dichotoma* (Gentianaceae) together with tri- and tetramethoxy-xanthone C-glycosides [49].

Acetylated and benzoylated mangiferins have been obtained only from some ferns of the Hymeno-

phyllaceae [44] so far. 2-C-β-D-Glucopyranosyl-1,3,5,6-tetrahydroxyxanthone was isolated from the roots of *Canscora decussata* (Gentianaceae) [47] and its methyl ether irisxanthone from *Iris florentina* (Iridaceae) [48]. Often, C-glucosylated xanthones co-occur with related C-glucosyl flavonoids [33, 49], a fact that will be discussed later.

Biosynthesis of Xanthone C-Glycosides

Xanthone derivatives are found in nature as free aglycones and O- and C-glycosides [33, 50]. It has been shown that biosynthesis of the xanthone nucleus in fungi proceeds via an intermediate with a benzophenone structure derived from acetate [54] and from shikimate-acetate in higher plants [55, 56]. *Gentiana* xanthones appear to be formed from a C_6-C_1 precursor derived from phenylalanine by the loss of two carbon fragments and from three acetate units yielding a benzophenone as intermediate. Subsequently, the xanthone is synthesized by oxidative coupling of this intermediate. Earlier findings on the biosynthesis of xanthones are summarized by CARPENTER et al. [50].

Recently, the formation of mangiferin in the Liliaceae *Anemarrhena asphodeloides* has been studied by FUJITA and INOUE [57, 58]. These authors have provided strong evidence that the 1,3,6,7-tetrahydroxyxanthone moiety of mangiferin is derived from a C_6-C_3 compound and two acetate units. When [1-^{14}C]-, [2-^{14}C]- and [3-^{14}C]phenylalanine are fed to excised aerial parts of the plant, the intact C_6-C_3 unit is incorporated into the aglycone moiety of mangiferin. [2-^{14}C]Malonic acid is also incorporated into mangiferin with high efficiency. By feeding [1-^{14}C]- and [2-^{14}C]phenylalanine and [2-^{14}C]malonic acid, the radioactivity of mangiferin is localized in the phloroglucinol ring of the aglycone. Furthermore, [3-^{14}C]cinnamic acid and p-[2-^{14}C]coumaric acid are also utilized for mangiferin and isomangiferin formation. Administration of C_6-C_1 compounds (benzoic acid, p-hydroxybenzoic acid or protocatechuic acid, ^{14}C-labeled in the carboxyl carbon) did not produce labeled C-glycosides. These results suggest that the postulated benzophenone intermediate of mangiferin biosynthesis might be formed by condensation of p-coumarate with two malonates (Fig. 5). Here, the biosynthetic relationship of mangiferin with the flavonoids becomes evident [59].

In further studies of C-glycosylation of mangiferin in the Liliaceae Fujita and Inoue fed radioactive

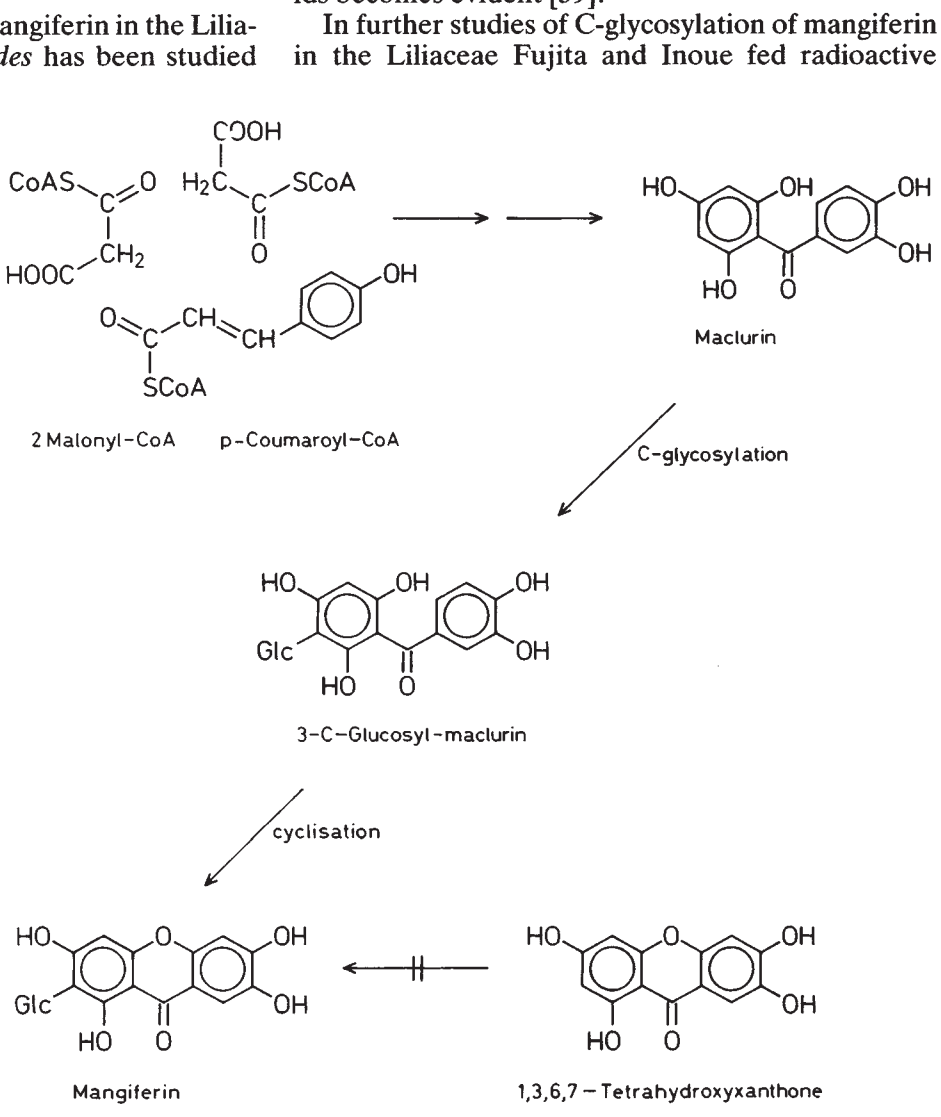


Fig. 5. Proposed pathway for the biosynthesis of mangiferin via maclurin after [19, 25].

1,3,6,7-tetrahydroxyxanthone and the benzophenone maclurin to *Anemarrhena asphodeloides* and found that only maclurin was efficiently incorporated into mangiferin and isomangiferin [60] (Fig. 5). These results indicate that C-glucosylation of mangiferin and isomangiferin occurs at the stage of maclurin, prior to the formation of the xanthone nucleus, and that both isomers may be biosynthesized via 3-C-glycosyl maclurin. When this compound is converted to C-glucosyl xanthone by ring closure, four isomeric C-glucosyl xanthones may be expected, three of which have been reported.

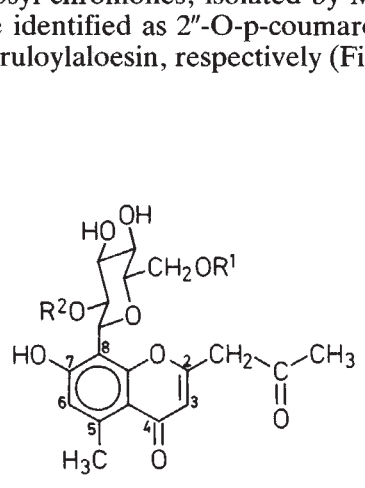
How the C-glycosidic linkage in glycoxanthones is formed is a problem yet to be solved.

C-Glycosyl Chromones

Chemistry of C-Glycosyl Chromones

Chromones are derivatives of benzopyran-4-one. They are usually colourless and produce a strong blue fluorescence under UV light. This characteristic fluorescence led to the detection of the first C-glycosyl chromones [61].

The isolation and structural elucidation of aloesin, identical with aloesin B or aloeresin B (Fig. 6) was reported by Haynes et al. [62] and, independently, by Rattenberger [63]. By means of spectroscopic and degradative studies these authors established its structure to be 2-acetonyl-8-C-β-D-glucopyranosyl-7-hydroxy-5-methylchromone. All other known chromone C-glycosides ar derived from aloesin by esterification with acid products of phenylpropane metabolism. Aloesin A (Fig. 6) was identified as 6″-O-p-coumaroylaloesin [63]. Two new 2″-O-acylated C-glycosyl chromones, isolated by Makino et al. [64], were identified as 2″-O-p-coumaroylaloesin and 2″-O-feruloylaloesin, respectively (Fig. 6).



Aloesin (B)	: $R^1=R^2=H$
Aloesin A	: R^1=p-coumaroyl, $R^2=H$
2″-O-p-Coumaroyl-aloesin	: $R^1=H$, R^2=p-coumaroyl
2″-O-Feruloyl-aloesin	: $R^1=H$, R^2=feruloyl

Fig. 6. C-glycosyl chromones.

Occurrence of C-Glycosyl Chromones

Simple chromones which contain no fused ring system on the chromone nucleus are rare as natural compounds. This is especially true for C-glycosylated benzopyrones.

Aloesin, the first compound of this type, and its 6″-O-p-coumaroylester were detected in pharmaceutically-used aloes. Subsequently, aloesin has been found to be present in more than thirty *Aloe* species (Liliaceae). McCarthy and Haynes [65], McCarthy [66] and Holdsworth [67] investigated the distribution of aloesin and other C-glycosyl compounds in more than one hundred *Aloe* species. The two 2″-O-acylated derivatives (Fig. 6) were obtained from *Aloe arborescens* Mill. var. *natalensis* (Liliaceae) [64]. Thus, the C-glycosyl chromones presently known are found only in *Aloe* species, an observation of unique chemotaxonomic significance for this species of the Liliaceae.

Biosynthesis of C-Glycosyl Chromones

Very little is known about the biosynthesis of chromones in general (confer [68]). Virtually no experiments have been reported dealing with the formation on C-glycosylation of the chromone nucleus. Aloesin and related compounds differ in their substitution pattern from most other chromones which have a 2-C-methyl group and a 5-C-phenolic group [68] or are unsubstituted in the pyrone ring [69, 70]. Thus, it is difficult to decide whether the aglycones of these compounds are derived from acetate or synthesized via a "mixed pathway". It is unlikely that they are degradation products of flavonoids [69,70] since their substitution pattern differs from that of flavonoids and no related flavonoids from *Aloe* have been reported.

Anthrone C-Glycosides

Chemistry of Anthrone C-Glycosides

Naturally occurring anthracene C-glycosides are derived from 9(10H)-anthracenone with a C-glucosyl residue linked to carbon 10. These normally yellow substances have been obtained through chemical synthesis by condensation of tetra-O-acetyl-α-D-glucopyranosyl bromide with the appropriate anthrone derivative [71, 72]. Mass spectra of a series of anthrone glycosides were studied and compared by Prox [73] and Evans et al. [74]. Aloin (= barbaloin), the first crystalline C-glycosyl compound to be isolated as early as in 1851 [5], was characterized structurally in 1952 by Mühlemann [71] and shown by chemical synthesis to be 10-C-β-D-glucopyranosyl-1,8-dihydroxy-3-(hydroxymethyl)-9(10H)-anthracenone (Fig. 7). The suggestion that aloin might exist in two diastereomeric forms [71, 75, 76] due to opposing configurations of the glucose moiety linked to carbon

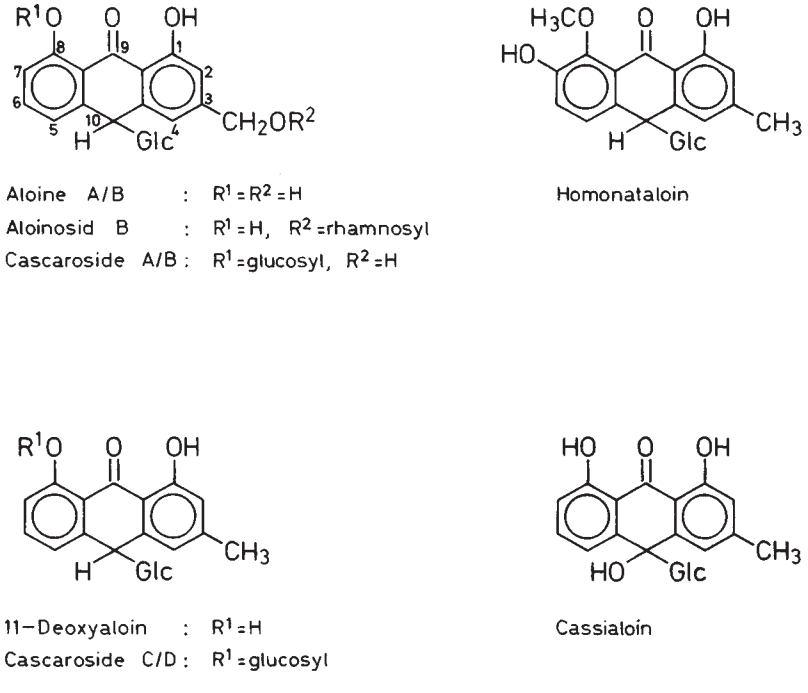


Fig. 7. Anthrone C-glycosides.

10 of the aglycone was recently confirmed by AUTERHOFF et al. [77] and GRÜN and FRANZ [78]. Genuine aloin extracted from plant material or chemically synthesized is separated by high performance liquid chromatography on reverse phase columns into aloin A and B which show differences in optical rotation and circular dichroism. The respective diastereomers are interconvertable which might explain their common anthranol form. Aloin is readily oxidized in aqueous solutions. Aloe emodine and 4-hydroxyaloin are two possible degradation products [75, 79]. Over one hundred papers deal with the isolation, chromatography, quantitative determination and degradation of aloin and some of these studies are discussed in recent dissertations [75, 80, 81].

11-Deoxyaloin (= chrysaloin, Fig. 7), first obtained by catalytic hydrogenation of aloin [82], was later isolated from plant material [83]. The structural elucidation and occurrence of a hydroxylated O-methyl derivative, homonataloin (Fig. 7), is described in [5].

Recently a simple new C-glycosylated anthrone, cassialoin, was isolated by HATA et al. [84]. It is 10-hydroxy-10-C-β-D-glucopyranosyl-3-methyl-9(10H)-anthracenone, a 10-hydroxylated 11-deoxyaloin (Fig. 7). All related compounds are O-glycosylated aloins or 11-deoxyaloins which yield glucose or rhamnose upon acid hydrolysis. Aloinosid B is the 11-α-L-rhamnoside of aloin [5] and aloinosid A presumably is the respective 10-C-glycosyl isomer. The 8-O-β-D-glucosides of the diastereomeric aloins have been named cascarosides A and B (Fig. 7), whereas the cascarosides C and D (Fig. 7) are analogs derived from the diastereomeric 11-deoxyaloins. The isolation and structural determination of those C-10-glycosides by means of partial hydrolysis, NMR, mass spectrometry and optical measurements were the subjects of several publications by FAIRBAIRN et al. [85, 86, 87] and WAGNER and DEMUTH [76, 88].

Occurrence of Anthrone C-Glycosides

The commonest C-glycosylated anthracene derivative is aloin. It is a major constituent of many bitter aloes and represents the active principle of these cathartic drugs [89]. It has been detected in the leaf juice of more than twenty *Aloe* species (Liliaceae) [66]. Homonataloin also is widely distributed in South African *Aloe* species [66]. Of the more than one hundred species examined, about one-half contain aloin and homonataloin, roughly in equal proportions. Aloesin (see above), appears almost exclusively in those species which contain aloin or homonataloin. The aloinosides are more restricted, occurring in five *Aloe* species [66]. Outside the Liliaceae, aloin has been identified in only one family, Rhamnaceae (*Rhamnus purshiana* = Cascara sagrada) where it occurs together with cascarosides A, B, C and D and 11-deoxyaloin ([83], confer [76]). Only one instance of cassialoin is known. Accompanied by 11-deoxyaloin, it was isolated from the heartwood of *Cassia garrettiana* (Caesalpiniaceae) [84].

Thus, 11-deoxyaloin, cassialoin and the cascarosides are very rare C-glycosyl anthrones which are unique to two species of the Rhamnaceae or Caesalpiniaceae, respectively, plant families known to contain anthraquinone O-glycosides.

Biosynthesis of Anthrone C-Glycosides

Biosynthesis of compounds containing the anthraquinone and anthrone nucleus were the subject of several publications which were summarized in a recent review on the biosynthesis of plant quinones by LEISTNER [90]. Anthraquinones may be synthesized via the acetate – polymalonate or the shikimic acid – O-succinyl benzoic acid – mevalonic acid pathway [90]. Derivatives with substituents in both ring A and ring C (Fig. 1) are usually acetate-derived in both higher and lower plants, while other anthraquinones, without substituents in ring A, are generally produced by the shikimic acid pathway in higher plants. It has never been established which pathway is operative during the biosynthesis of C-glycosyl anthrones of the aloe emodine – or chrysophanol anthrone-type. From their substitution pattern it appears that the aglycones of those compounds are derived from eight acetate units [90].

Recently, GRÜN and FRANZ studied the biosynthesis of aloin in *Aloe arborescens* (Liliaceae) [81, 91, 92]. Feeding experiments with $^{14}CO_2$ and quantitative determinations of both diastereomeric aloins in leaves of *Aloe arborescens* at different stages of development demonstrated that only one isomer, aloin B, is preferentially synthesized by the plant. The occurrence of the respective isomer, aloin A (see above), is explained by partial conversion of aloin B [92]. It was shown that the aloin B/aloin A equilibrium is reached non-enzymatically under physiological conditions.

Biogenesis of aloins in *Aloe arborescens* seems to be influenced by environmental conditions [81]. An earlier study on variations in the content of aloin throughout a vegetation period in several *Aloe* species gave similar results. MCCARTHY and VAN RHEEDE VAN OUDTSHOORN [93, 94] found that the content of aloin in *Aloe* leaf juices varied considerably with a maximum production during the summer period suggesting that aloin might be metabolized. Feeding experiments with ^{14}C-labeled compounds proved that leaf disks of *Aloe arborescens* preferentially incorporated [2-^{14}C]acetate into the aglycone moiety of aloins while [U-^{14}C]glucose was incorporated into both the aglycone and sugar moiety [92]. Further studies with cell-free extracts demonstrated that crude enzyme extracts from *Aloe arborescens* were able to catalyze transglycosylation of [U-^{14}C]glucose from UDP-[U-^{14}C]glucose to aloe emodine anthrone forming aloin [91]. C-Glycosylation took place only when aloe emodine anthrone was added to the reaction mixture. [U-^{14}]Glucose containing substrates such as free glucose, glucose-1-phosphate, ADP-glucose, GDP-glucose and UDP-glucose were tested. Only the latter was shown to be most effective for the transfer of the glucosyl residue to carbon 10 of aloe emodine anthrone. The crude enzyme found in the 40.000 g supernatant from a homogenate of *Aloe arborescens* leaves had a pH optimum between pH 7.0 and 7.3. These experiments were the first to show *in vitro* formation of a C-glycosidic bond thus demonstrating the similarity of C- and O-glycosylation [2, 21].

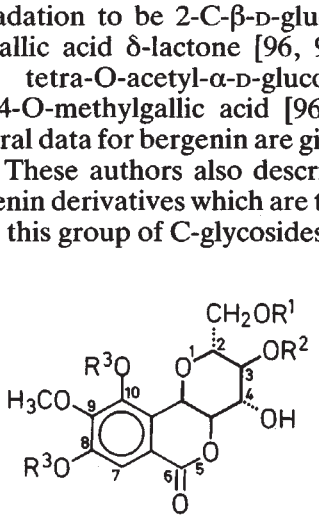
In naturally occurring C-glycosides the bond between the sugar moiety and its aglycone always involves an aglyconic carbon atom of nucleophilic character, such as the C-atom at the ortho- or para-position of relevant phenolic hydroxyl groups [5]. The general reaction mechanism of C-glycosylation may be the transfer of an activated sugar form a nucleoside-diphosphate sugar to the nucleophilic C-atom of the appropriate aglycone. It follows that formation of O- and C-glycosyl compounds in nature may utilize similar metabolic processess as HAYNES [5] has already postulated.

C-Glycosylated Gallic Acids

Chemistry of C-Glycosylated Gallic Acids

Derivatives of 3,4,5-trihydroxybenzoic acid (gallic acid, Fig. 1) may be considered as the simplest naturally occurring C-glycosides with regard to the aglycone moiety. In these compounds, a β-D-glucosyl residue is C-linked to a hydroxylated phenylcarboxylic acid ortho to the carboxyl group. In addition, the carboxyl group is esterified with the C-2 hydroxyl group of the glucosyl moiety to form a δ-lactone ring. Formally these compounds are dihydroisocoumarin derivatives [95].

Bergenin, the most prominent representative of this class of compounds (Fig. 8), is a colourless substance whose structure is confirmed by synthesis and degradation to be 2-C-β-D-glucopyranosyl-4-O-methylgallic acid δ-lactone [96, 97]. It is synthesized from tetra-O-acetyl-α-D-glucopyranosyl bromide and 4-O-methylgallic acid [96]. ^1H and ^{13}C-NMR spectral data for bergenin are given by RAMAIAH et al. [98]. These authors also describe three methylated bergenin derivatives which are the only other examples in this group of C-glycosides (Fig. 8) yet known.



Bergenin : $R^1=R^2=R^3=H$
8,10-di-O-methyl ether : $R^1=R^2=H$, R^3=methyl
Tri-O-methyl ethers : $R^1=R^3$=methyl, $R^2=H$
: $R^2=R^3$=methyl, $R^1=H$

Fig. 8.

Occurrence of C-Glycosylated Gallic Acids

Bergenin was isolated first in 1880 by GARREAU and MACHELART from rhizomes of three *Bergenia* sp. (= *Saxifraga,* Saxifragaceae) [99]. Later, it was found in several other families (confer [95], [98]), notably the Hamamelidaceae and the Saxifragaceae which include many bergenin containing sp. [95]. Of the sixteen Saxifragaceae sp. examined only those of the Saxifragoideae, i.e., *Astilbe, Bergenia, Saxifraga, Mitella, Rodgersia*, contain bergenin [100]. It is present in all plant organs but the highest content usually occurs in the rhizomes. Bergenin methyl esters were isolated from the heartwood of *Macaranga peltata* (Euphorbiaceae) accompanaied by their parent compound [98].

Biosynthesis of C-Glycosylated Gallic Acids

Biosynthetic pathways to substituted benzoic acids in plants or microorganisms involve side-chain degradation of cinnamic acids, aromatization of dehydroshikimic acid, hydroxylation and subsequent oxidation of methylphenols as well as condensation of one acetyl-CoA with three malonyl-CoA units. These biosynthetic routes are summarized by GROSS in a review on phenolic acids [101]. Aromatization of dehydroshikimic acid is probably the most important reaction which leads to gallic acid in plants ([101], confer [102]).

With regard to bergenin KINDL [103] reported in a preliminary communication on ^{14}C-tracer experiments with leaves of *Astilbe chinensis* (Saxifragaceae). He introduced [7-^{14}C]benzoic acid, DL-[3-^{14}C]phenylalanine, [3-^{14}C]cinnamic acid, [3-^{14}C]isoferulic acid and p-[3-^{14}C]-coumaric acid into bergenin and obtained the following results: hydroxylated cinnamic acids and DL-phenylalanine were incorporated more efficiently than cinnamic acid and the latter incorporated better than benzoic acid. Utilization of [1-^{14}C]acetate was negligible during short feeding periods. These findings led to the hypothesis that C-glucosylation occurs at the stage of a C_6-C_3 compound rather than that of a C_6-C_1 compound. ^{14}C-Labeled gallic acid was not included in this set of experiments.

On the other hand, TANEYAMA and YOSHIDA [104] incubated leaf disks of *Saxifraga stolonifera* (Saxifragaceae) with D-[U-^{14}C]glucose in the presence of unlabeled gallic acid, 4-O-methylgallic acid and protocatechuic acid (= 3,4-dihydroxybenzoic acid). The addition of gallic acid to the incubation medium enhanced significantly the incorporation of label from D-[U-^{14}C]glucose into bergenin. The other benzoic acid derivatives were ineffective. These results indicate that gallic acid may be a likely glucosyl acceptor in bergenin biosynthesis and that methylation may be a late step in the formation of the molecule.

Considering the fact that bergenin co-occurs with the related 2-O-galloyl-arbutin (= gallic acid ester of the hydroquinone β-D-glucosid arbutin) in *Bergenia* sp. (Saxifragaceae) some authors [105, 106] offered mechanistic schemes in which this compound is biogenetically rearranged to the C-glucosyl structure of bergenin. However, attempts to convert the galloyl ester to a cmpound of the bergenin type were unsuccessful [106].

An interesting study by MINAMIKAWA et al. [107] demonstrates the degradation of bergenin by *Erwinia herbicola*, a strain of soil bacteria isolated from the rhizosphere of *Bergenia crassifolia* by elective culture with bergenin as a sole carbon source in the growth medium. This organism degraded bergenin to yield 4-O-methylgallic acid among other minor products and apparently utilized the sugar moiety of the C-glycoside as a source of carbon and energy. Thus, this organism provides a rare example of biological cleavage of C-glycosides.

Acknowledgement

The authors wish to thank Prof. Dr. F. A. LOEWUS, Pullman, Washington, USA for the assistance in editing the english manuscript and Frau K. WOLF for the skillful preparation of the figures.

References

(1) Courtois, E., F. Percheron in: Pigman, W. and D. Horton (Eds.) The Carbohydrates, Vol. 2, pp. 213–240, New York, 1970, Academic Press.
(2) Franz, G. in: Loewus, F. A. and W. Tanner (Eds.) Plant Carbohydrates I: Intracellular Carbohydrates, Encyclopedia of Plant Physiology, New Series, Vol. 13A, p. 384, Berlin, Heidelberg, New York 1982, Springer-Verlag.
(3) Bandyukova, V. A. and V. A. Yugin: Khim. Prir. Soedin, 5 (1981).
(4) Adinarayana, D. and J. Rajasekhara Rao: Indian J. Chem. Educ. *4*, 2 (1974).
(5) Haynes, L. J.: Adv. Carbohydr. Chem. *18*, 227 (1963).
(6) Haynes, L. J.: Adv. Carbohydr. Chem. *20*, 357 (1965).
(7) Chopin, J., M. L. Bouillant in: Harborne, J. B., T. J. Mabry and H. Mabry (Eds.) The Flavonoids, p. 866, London 1975, Chapman and Hall.
(8) Furukawa, M., I. Hayakawa, G. Ohta and Y. Iitaka: Tetrahedron *31*, 2989 (1975).
(9) Horii, S., H. Fukase, E. Mizuta, K. Hatano and K. Mizuno: Chem. Pharm. Bull. 28, 3601 (1980).
(10) Richter, W. J., K. O. Alt, W. Dieterle, J. W. Faigle, H.-P. Kriemler, H. Mory and T. Winkler: Helv. Chim. Acta *58*, 2512 (1975).
(11) Levy, S., B. Yagen and R. Mechoulam: Science *200*, 1391 (1978).
(12) Abolin, C. R., T. N. Tozer, J. C. Craig and L. D. Guenke: Science *209*, 703 (1980).
(13) Alston, R. E. in: Mabry, T. J., R. E. Alston and V. C. Runeckles (Eds.) Recent Advances in Phytochemistry, Vol. 1, p. 305, New York 1968, Appleton-Century-Crofts.
(14) Chopin, J. in: Wagner, H. and L. Hörhammer (Eds.) Pharmacognosy and Phytochemistry, pp. 111–125, Heidelberg 1971, Springer Verlag.
(15) Horowitz, R. M. and B. Gentilini: Chem. Ind. (London), 498 (1964).
(16) Wallace, J. W. and T. J. Marbry: Phytochemistry *9*, 2133 (1970).
(17) Grisebach, H. and K. Hahlbrock: Recent Advances in Phytochemistry *8*, 21 (1974).
(18) Hahlbrock, K. in: Stumpf, P. K. and E. E. Conn (Eds.) The Biochemistry of Plants, Vol. 7, Secondary Plant Products, p. 425, New York, London, Toronto, Sydney, San Francisco 1981, Academic Press.
(19) Inoue, T. and M. Fujita: Chem. Pharm. Bull. *22*, 1422 (1974).
(20) Fujita, M. and T. Inoue: Yakugaku Zasshi *99*, 165 (1979).
(21) Hösel, W. in: Stumpf, P. K. and E. E. Conn (Eds.) The Biochemistry of Plants, Vol. 7, Secondary Plant Products, p. 725, New York, London, Toronto, Sydney, San Francisco 1981, Academic Press.
(22) Wallace, J. W., T. J. Mabry and R. E. Alston: Phytochemistry *8*, 93 (1969).
(23) Wallace, J. W. and H. Grisebach: Biochem. Biophys. Acta *304*, 837 (1973).
(24) Wallace, J. W.: Phytochemistry *14*, 1765 (1975).

(25) Inoue, T. and M. Fujita: Chem. Pharm. Bull. *25*, 3226 (1977).
(26) Effertz, B. and G. Weissenböck: Z. Pflanzenphysiol. *92*, 319 (1979).
(27) Effertz, B. and G. Weissenböck: Phytochemistry *19*, 1669 (1980).
(28) Margna, U. and T. Vainjärv: Biochem. Physiol. Pflanzen *176*, 44 (1981).
(29) Heinsbroek, R., J. van Brederode, G. van Nigtevecht and J. Kamsteeg: Phytochemistry *18*, 935 (1979).
(30) Brederode van, J., J. Chopin, J. Kamsteeg, G. van Nigtevecht and R. Heinsbroek: Phytochemistry *18*, 655 (1979).
(31) Besson, E., A. Besset, M. L. Bouillant, J. Chopin, J. van Brederode and G. van Nigetvecht: Phytochemistry *18*, 657 (1979).
(32) Heinsbroek, R., J. van Brederode, G. van Nigtevecht, J. Maas, J. Kamsteeg, E. Besson and J. Chopin: Phytochemistry *19*, 1935 (1980).
(33) Hostettmann, K. and H. Wagner: Phytochemistry *16*, 821 (1977).
(34) Wiechowski, W.: Lotos *56*, 61 (1908); cited in [33].
(35) Ramanathan, J. D. and T. R. Seshadri: Curr. Sci. (India) *29*, 131 (1960); cited in [6].
(36) Billet, D., J. Massicot, C. Mercier, D. Anker, A. Matschenko, C. Mentzer, M. Chaigneau, G. Valdener and H. Pacheco: Bull. Soc. Chim. France, 3006 (1965).
(37) Haynes, L. J. and D. R. Taylor: J. Chem. Soc. (C), 1685 (1966).
(38) Bhatia, V. K., J. D. Ramanathan and T. R. Seshadri: Tetrahedron *23*, 1363 (1967).
(39) Nott, P. E. and J. C. Roberts: Phytochemistry *6*, 741 (1967).
(40) Nott, P. E. and J. C. Roberts: Phytochemistry *6*, 1597 (1967).
(41) Bhatia, V. K. and T. R. Seshadri: Tetrahedron Letters, 1741 (1968).
(42) Aritomi, M. and T. Kawasaki: Chem. Pharm. Bull. *18*, 2327 (1970).
(43) Aritomi, M. and T. Kawasaki: Chem. Pharm. Bull. *16*, 760 (1968).
(44) Markham, K. R. and J. W. Wallace: Phytochemistry *19*, 415 (1980).
(45) Smith, D. M. and J. B. Harborne: Phytochemistry *10*, 2117 (1971).
(46) Glyzin, V. I., A. I. Ban'Kowskii, M. G. Pimenov and K. I. Borydev: Khim. Prir. Soedin *9*, 434 (1973).
(47) Ghosal, S. and R. K. Chaudhuri: Phytochemistry *12*, 2035 (1973).
(48) Arisawa, M., N. Morita, Y. Kondo and T. Takemoto: Chem. Pharm. Bull. *21*, 2562 (1973).
(49) Ghosal, S., D. K. Jaiswal and K. Biswas: Phytochemistry *17*, 2119 (1978).
(50) Carpenter, I., H. D. Locksley and F. Scheinmann: Phytochemistry *8*, 2013 (1969).
(51) Williams, C. A.: Phytochemistry *18*, 803 (1979).
(52) Saleh, N. A. M. and M. A. I. El-Anseri: Planta med. *28*, 124 (1975).
(53) Aritomi, M. and T. Kawasaki: Chem. Pharm. Bull. *18*, 224 (1970).
(54) Birch, A. J., J. Baldas, J. R. Hlubucek, T. J. Simpson and P. W. Westermann: J. Chem. Soc. Perkin I, 898 (1976) and references cited therein.
(55) Floss, H. G. and A. Rettig: Z. Naturforsch. *19b*, 1103 (1964).
(56) Gupta, P. and J. R. Lewis: J. Chem. Soc. (C), 629 (1971).
(57) Fujita, M. and T. Inoue: Tetrahedron Letters, 4503 (1977).
(58) Fujita, M. and T. Inoue: Chem. Pharm. Bull. *28*, 2476 (1980).
(59) Swain, T. in: Harborne, J. B., T. J. Mabry and H. Mabry (Eds.), The Flavonoids, p. 1097, London 1975, Chapman and Hall.
(60) Fujita, M. and T. Inoue: Chem. Pharm. Bull. *28*, 2482 (1980).
(61) Auterhoff, H. and B. Ball: Arzneim. Forsch. *4*, 725 (1954).
(62) Haynes, L. J., D. K. Holdsworth and R. Russell: J. Chem. Soc. (C), 2581 (1970).
(63) Rattenberger, M.: Dissertation, München (1969).
(64) Makino, K., A. Yagi and I. Nishioka: Chem. Pharm. Bull. *22*, 1565 (1974).
(65) McCarthy, T. J. and L. J. Haynes: Planta med. *15*, 342 (1967).
(66) McCarthy, T. J.: Planta med. *17*, 1 (1969).
(67) Holdsworth, D. K.: Planta med. *19*, 322 (1971).
(68) Harrison, P. G., B. K. Bailey and W. Steck: Can. J. Biochem. *49*, 964 (1971).
(69) Pendse, R., A. V. Rama Rao and K. Venkatamaran: Phytochemistry *12*, 2033 (1973).
(70) Chiji, H., T. Aiba and M. Izawa: Agric. Biol. Chem. *42*, 159 (1978).
(71) Mühlemann, H.: Pharm. Acta Helv. *27*, 17 (1952).
(72) Vogt, E. and H. Mühlemann: Pharm. Acta Helv. *46*, 657 (1971).
(73) Prox, A.: Tetrahedron *24*, 3697 (1968).
(74) Evans, F. J., M. G. Lee and D. E. Games: Biomedical Mass Spectrometry 6, 374 (1979).
(75) Widmaier, W.: Dissertation, Tübingen (1975).
(76) Wagner, H. and G. Demuth: Z. Naturforsch. *31b*, 267 (1976).
(77) Auterhoff, H., E. Graf, G. Eurisch and M. Alexa: Arch. Pharm. *313*, 113 (1980).
(78) Grün, M. and G. Franz: Pharmazie *34*, 669 (1979).
(79) Graf, E. and M. Alexa: Planta med. *38*, 121 (1980).
(80) Eurisch, G.: Dissertation, Tübingen (1979).
(81) Grün, M.: Dissertation, Regensburg (1981).
(82) Owen, L. N.: Chem. Ind. (London), 37 (1956).
(83) Baumgartner R. and K. Leupin: Pharm. Acta Helv. *36*, 445 (1961).
(84) Hata, K., M. Kozawa and K. Baba: Chem. Pharm. Bull. *24*, 1688 (1976).
(85) Fairbairn, J. W., C. A. Friedman and S. Simic: J. Pharm. Pharmacol. Suppl. *15*, 292 T (1963).
(86) Fairbairn, J. W. and S. Simic: J. Pharm. Pharmacol. *16*, 450 (1964).
(87) Fairbairn, J. W., F. J. Evans and J. D. Phillipson: J. Pharm. Sci. *66*, 1300 (1977).
(88) Wagner, H. and G. Demuth: Z. Naturforsch. *29c*, 444 (1974).
(89) Wade, A. (Ed.) Martindale: The Extra Pharmacopoeia, 27the ed., p. 1334, London 1977, The Pharmaceutical Press.
(90) Leistner, E. in: Stumpf, P. K. and E. E. Conn (Eds.) The Biochemistry of Plants, Vol. 7, Secondary Plant Products, p. 403, New York, London, Toronto, Sydney, San Francisco 1981, Academic Press.
(91) Grün, M. and G. Franz: Planta med. *152*, 562 (1981).
(92) Grün, M. and G. Franz: Arch. Pharm. *315*, 231 (1982).
(93) McCarthy, T. J. and M. C. B. van Rheede van Oudtshorn: Planta med. *14*, 62 (1966).
(94) McCarthy, T. J.: Planta med. *16*, 348 (1968).
(95) Barry, R. D.: Chem. Rev. *64*, 229 (1964).
(96) Hay, J. E. and L. J. Haynes: J. Chem. Soc., 2231 (1958).
(97) Posternak, T. and K. Dürr: Helv. Chim. Acta *41*, 1159 (1958).
(98) Ramaiah, P. A., L. R. Row, D. S. Reddy, A. S. R. Anjaneyulu, R. S. Ward and A. Pelter: J. Chem. Soc. Perin I, 2313 (1979).
(99) Garreau, Machelart: Compt. Rend. 91, 942 (1880) cited by Hegnauer, R., Chemotaxonomie der Pflanzen, Vol. 6, Basal, Stuttgart 1973, Birkhäuser.
(100) Taneyama, M. and S. Yoshida: Bot. Mag. (Tokyo) *91*, 109 (1978).
(101) Gross, G. G. in: Stumpf, P. K. and E. E. Conn (Eds.) The Biochemistry of Plants, Vol. 7, Secondary Plant Products, p. 301, New York, London, Toronto, Sydney, San Francisco 1981, Academic Press.
(102) Haslam, E. in: Stumpf, P. K. and E. E. Conn (Eds.) The Biochemistry of Plants, Vol. 7, Secondary Plant Products, p. 527, New York, London, Toronto, Sydney, San Francisco 1981, Academic Press.
(103) Kindl, H.: Monatsh. Chem. *95*, 1561 (1964).
(104) Taneyama, M. and S. Yoshida: Bot. Mag. (Tokyo) *92*, 69 (1979).
(105) Wenkert, E.: Chem. Ind. (London), 906 (1959).
(106) Haslam, E. and M. Uddin: Tetrahedron *24*, 4015 (1968).
(107) Minamikawa, T., S. Yoshida, M. Hasegawa, K. Komagata and K. Kato: Agr. Biol. Chem. *36*, 773 (1972).

Address: Prof. Dr. G. Franz,
Lehrstuhl für Pharmazeutische Biologie,
Fachbereich Chemie und Pharmazie,
Universität Regensburg,
Universitätsstraße 31,
D-8400 Regensburg