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Ecological Chemistry and Biochemistry of Plant Terpenoids

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6 Plant iridoids, their biosynthesis and distribution in angiosperms

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Introduction

This review will mainly be concerned with investigations in the biosynthesis of iridoid compounds from recent years. Furthermore, I will demonstrate what implications these results may have for chemical plant taxonomy. The field of iridoid biochemistry is very active at the present as seen by the large number of papers on the subject. The last comprehensive review on iridoids by El-Naggar and Beal (1980) listed 258 compounds. Looking through our files, I have calculated that to date more than 300 new compounds have been published since then. Much biosynthetic work has also been done in recent years and an excellent and comprehensive review by Inouye and Uesato (1986) has appeared.

In order to introduce the subject I will first present the parent substances, namely iridodial (1) and 8-*epi*-iridodial (2) (Fig. 6.1). The former compound was first found in, and named after, an ant genus, namely *Iridomyrmex*

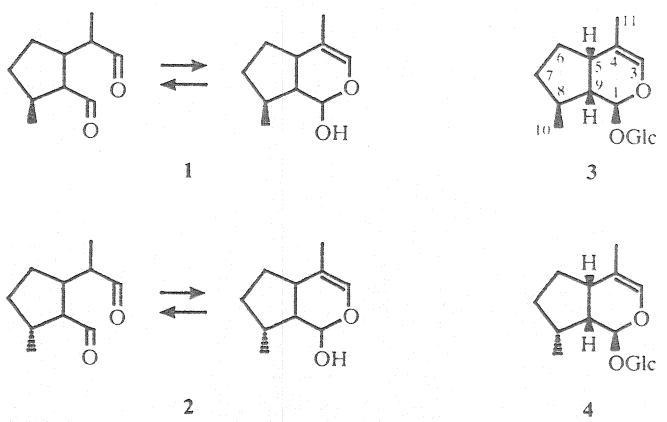


Fig. 6.1. Numbering and stereochemistry of iridoidial (1), epi-iridodial (2), and their glucosides.

(Cavill *et al.* 1956). The numbering and stereochemistry of the iridoid skeleton is given in the figure. Both compounds play an important role in the biosynthesis of iridoid glucosides in plants.

As shown in the figure, these dialdehydes may exist in equilibrium with the corresponding dihydropyran forms. The corresponding glucosides, such as (3) and (4), which occur in many plants can only exist as the dihydropyran isomer. Except for an α-L-arabinopyranoside published only this year (Morota *et al.* 1989), the compounds are consistently β-D-glucopyranosides when a sugar is present at C_1. [A compound claimed to be an iridoid rhamnoside (Purushothaman *et al.* 1988) is apparently not an iridoid at all, according to the spectral data given.] However, glycosylation may take place in other positions and then there are no restrictions on the nature of the sugar. Iridodial (1) has been isolated in trace amount from *Rauwolfia* as shown below, while the epimer (2) occurs as the glucoside (4) in *Boschniakia rossica* (Murai and Tagawa 1982).

Biosynthesis

Due to the great interest in the complex indole alkaloids, part of the biosynthetic sequence (Fig. 6.2) leading to loganin (7) and secologanin (8) has been known in some detail, for fifteen years (Cordell 1974). Note the scrambling of the radioactive labelling that takes place between C_3 and C_{11}, indicating that C_9 and C_{10} in geraniol (5) must have become equivalent during

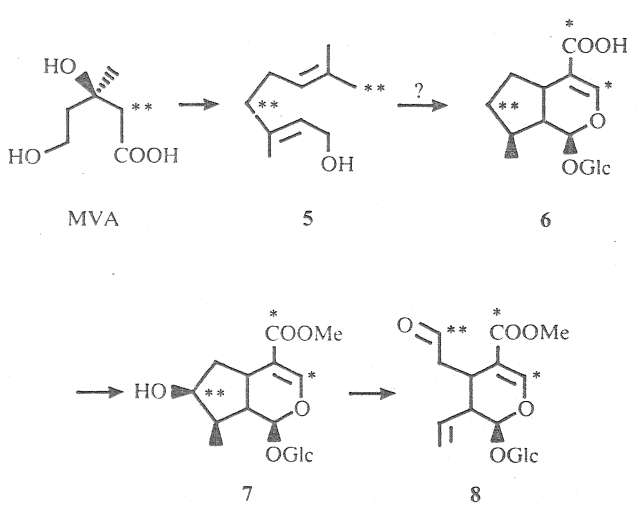


Fig. 6.2. The early established biosynthetic pathway leading to loganin (7) and secologanin (8).

biosynthesis. However, the details of what happens between geraniol and deoxyloganin (6) has remained uncertain for a long period, although a number of proposals as well as some conflicting evidence has appeared. This state lasted till 1984 when Inouye's group, partly in co-operation with Zenk, published several convincing results. By feeding compounds labelled with tritium to *Lonicera morrowii* (Caprifoliaceae) Uesato *et al.* (1984*a*) solved two main problems. Firstly, 10-hydroxycitronellol (11) was excluded as a precursor for secologanin (8). Secondly, iridodial (1) was shown to give good incorporation. On the other hand, it seemed a problem that 9, 10-dihydroxygeraniol (10) was incorporated to some degree. Similar results were found with intact *Catharanthus* plants and with a cell culture of *Rauwolfia serpentina*, both belonging to Apocynaceae (Uesato *et al.* 1984*b*, 1986*a*), where incorporations into indole alkaloids were also obtained.

In these experiments, the alcohols were considered equivalent to the corresponding aldehydes, and we will discuss the possibilities (Fig. 6.4) for the

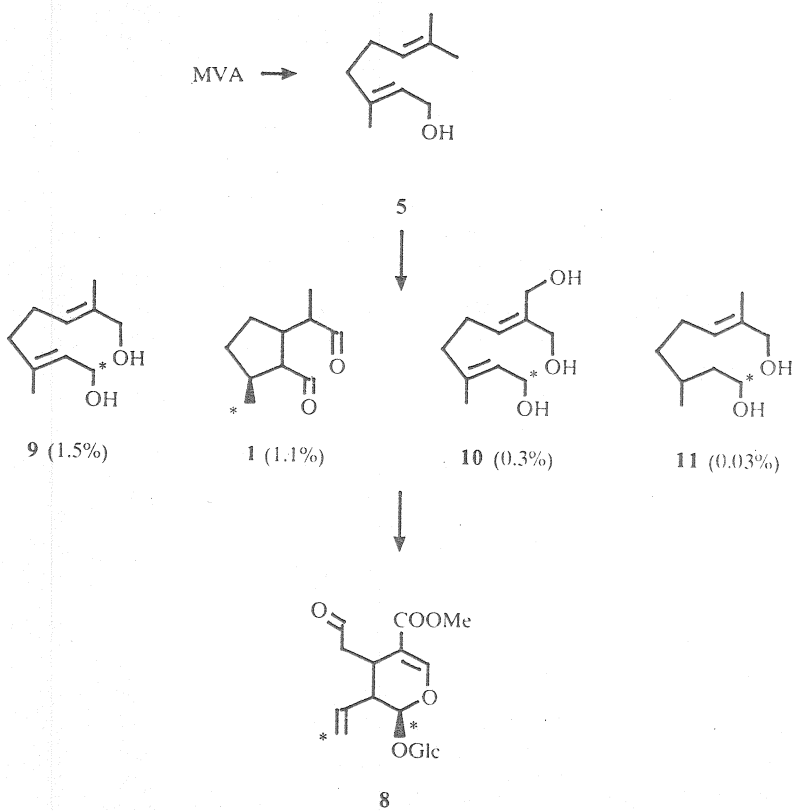


Fig. 6.3. ^{3}H-Labelled precursors and their incorporations into secologanin (8) when fed to *Lonicera morrowii*.

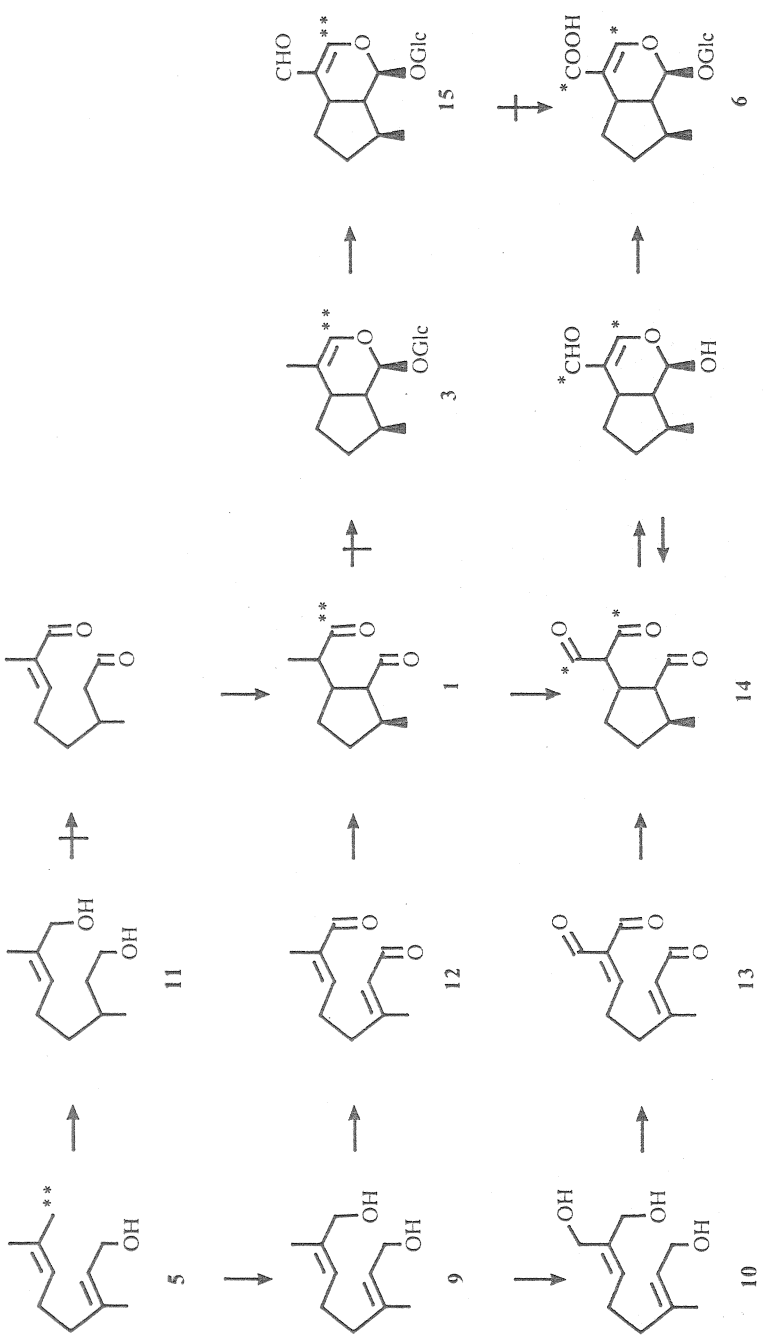
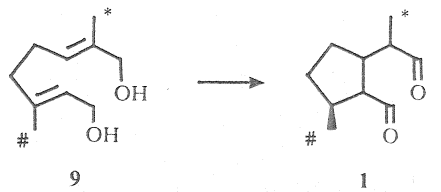


Fig. 6.4. Possible pathways leading from geraniol (5) to deoxyloganic acid (6).



9 1

Fig. 6.5. Transformation of ^{13}C-labelled 10-hydroxygeraniol (9) to iridodial in cell-free extract of cell cultures of *Rauwolfia serpentina*.

events between 10-hydroxygeraniol (9) and deoxyloganic acid (6). Firstly, 10-hydroxycitronellol (11) was excluded as an intermediate in the above experiments. Secondly, as scrambling between C_3 and C_{11} has taken place during biosynthesis, a route via iridodial glucoside (3) can be excluded, since the dihydropyran ring cannot open due to glucosidation at C_1 and this makes scrambling impossible when C_{11} reaches the same oxidation state as C_3 in iridotrial glucoside (15). The problem is whether cyclization takes place before or after oxidation of the methyl group (C_9) that eventually becomes C_{11} in the iridoid. Stated another way, the question is whether iridodial (1) or iridotrial (14) is the first cyclized intermediate.

This problem was solved very convincingly using a cell-free extract of a cell culture of *Rauwolfia* (Uesato *et al.* 1986*c*). By incubating this cell-free extract with two different ^{13}C-labelled samples of 10-hydroxygeraniol, Inouye's group together with Zenk were able to isolate a fraction that indeed contained labelled iridodial. The ^{13}C NMR signal at 20.6 ppm and the mass spectrum were used as proofs of identity when compared to an authentic specimen. Very recently Uesato (1988), continuing this work, incubated the system with tritium-labelled (9) and succeeded in isolating not only iridodial (1) and 10-oxogeranial (12), but also the two possible isomeric hydroxy-aldehyde intermediates between (9) and (12) (Fig. 6.4).

A few years earlier, our group (Damtoft *et al.* 1983) had demonstrated that 8-*epi*-deoxyloganic acid (18) was a precursor for some iridoids (see below), in conflict with results published earlier. During this work we found also that deoxyloganic acid (6) was incorporated into asperuloside (16) in *Theligonum cynocrambe* (Rubiaceae) while 18 was not. This apparently made Inouye's group (Uesato *et al.* 1986*b*) repeat some of their earlier work, using deuterium-labelled precursors. The compound asperuloside (16), a constituent of *Galium* (Rubiaceae) and many other genera and species, has a double bond at C_8. Therefore, it is not evident that it belongs to the compounds derived from iridodial. Likewise, the compound deutzioside (17) from *Deutzia* species has lost the C_{10} carbon, and either iridodial (1) or *epi*-iridodial (2) could be the precursor. The compounds tested (Fig. 6.6) in

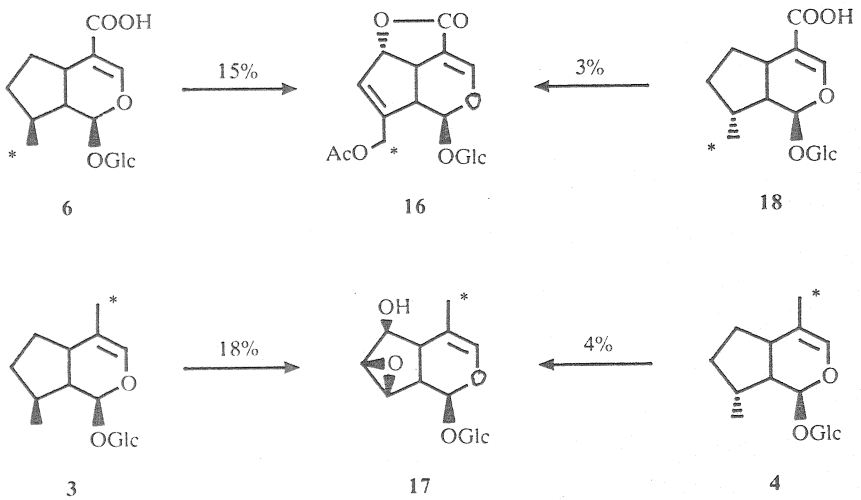


Fig. 6.6. Feeding experiments with epimeric precursors into asperuloside (16) in *Galium spurium* and into deutzioside (17) in *Deutzia crenata*.

Deutzia crenata were (3) and (4), the glucosides of (1) and (2), respectively. Only (3) was incorporated into deutzioside and no scrambling took place as was expected. Likewise, deoxyloganic acid (6) and *epi*-deoxyloganic acid (18) were both fed to *Galium spurium* and only the former was incorporated into asperuloside. The small incorporations apparently seen for (4) and (18) were due to the presence of impurities of the 8β-epimers in the compounds fed.

In the same paper (Uesato *et al.* 1986*b*), deoxyloganic acid (6) and loganic acid (19) were shown to be precursors for geniposidic acid (21), asperuloside (16), and secogalioside (22) in *Galium mollugo*, loganin (7) and secologanin (8) presumably being the intermediates leading to the latter. Neither iridotrial glucoside (3) nor *epi*-deoxyloganic acid (18) were incorporated at all. Since it has been established (Inouye *et al.* 1972) that deoxygeniposidic acid (20) is a good precursor for asperuloside, the sequence leading to this compound now seems well clarified.

Next I will refer to our own results with cornin (23) (Jensen *et al.* 1989)—or verbenalin as it has also been called. Figure 6.8 shows some 20-year-old results obtained with *Verbena officinalis* by Horodyski *et al.* (1969). They are remarkable since the positions of the incorporated label vary with the age of the plant. Feeding with 2-^{14}C-labelled mevalonic acid showed that complete scrambling took place in young plants, and this was in agreement with other workers (Hüni *et al.* 1966), but almost no scrambling was observed when old plants were used for the experiments. The interpretation

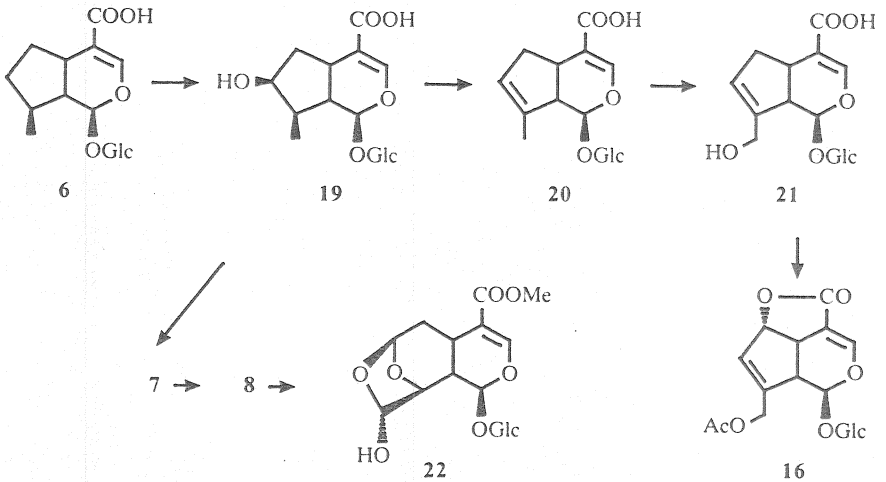


Fig. 6.7. Biosynthetic pathway from deoxyloganic acid (6) to asperuloside (16) and secogalioside (22) in *Galium mollugo*.

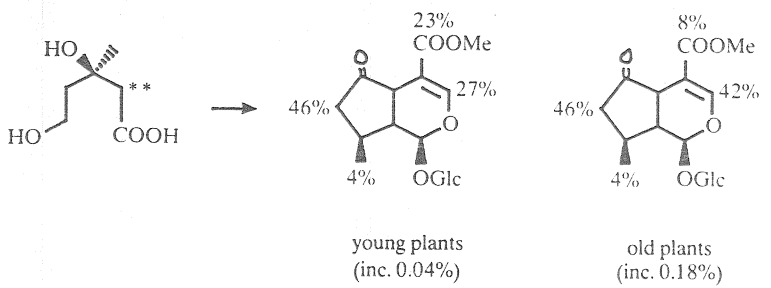


Fig. 6.8. Early biosynthetic results with cornin (23) in *Verbena officinalis*.

could be that in young plants there is the well-known (Fig. 6.4) pathway via iridodial (1) and iridotrial (14) where C_3 and C_{11} become equivalent before the dihydropyran ring is fixed by glucosylation. In old plants, the pathway should go via iridodial glucoside (3) and iridotrial glucoside (15), where no scrambling can take place when C_3 and C_{11} reach the same oxidation level.

Using plants of intermediate age in order to get sufficient incorporation, we prepared 11-^{13}C-labelled iridodial (1), iridotrial (14), and their glucosides [(3) and (15), respectively], and fed these and some other compounds (Fig. 6.9). Due to the large content of cornin in the plants, the dilution of the label in this compound was often too large for the incorporation to be measured by NMR spectroscopy. Therefore, it was measured in

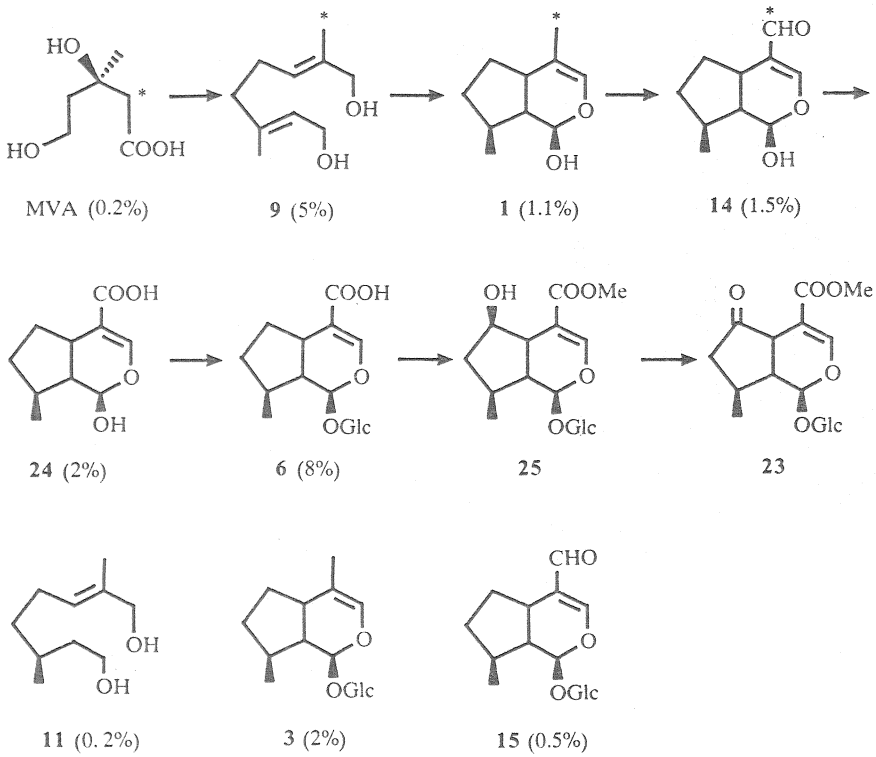


Fig. 6.9. Biosynthesis of dihydrocornin (25) and cornin (23) in *Verbena officinalis*. Precursors were labelled with $^{13}C(*)$ and incorporations (in parentheses) were measured in (25).

dihydrocornin (25) which is present in the plant in much smaller amount and which we had already shown to be an intermediate in the biosynthesis of cornin. In all cases, we found that complete scrambling of label took place. In the experiment with iridotrial glucoside (15) we could even re-isolate 14 per cent of the compound fed and an additional 58 per cent which was reduced to the 11-hydroxy compound. The incorporations seen for (3) and (15), which are not negligible, in both cases took place with complete scrambling, so the glucosides must have been hydrolysed by the plant before incorporation. Note that 10-hydroxycitronellol (11) is not a precursor. We have also succeeded in isolating a very small amount of deoxyloganic acid (6) from the intact plant. The biosynthetic pathway is remarkably similar to that of loganin and secologanin.

Up to 1980, almost all biosynthetic work on iridoids had concerned compounds with the 8β-stereochemistry derived from iridodial. However, a

paper had been published (Inouye *et al.* 1978) in which iridodial (1) and its glucoside (3) in *Lamium amplexicaule* (Lamiaceae) was shown to be incorporated into lamioside (26) and lamiide (28), albeit with very small incorporations (less that 0.01 per cent). At that time Damtoft (1981) in our laboratory fed deuterium-labelled 8-*epi*-deoxyloganin (29) and deoxyloganin (30) to *Hebenstreitia dentata* (Scrophulariaceae) containing ipolamiide (27) and lamiide (28). Satisfactory incorporations from (29) were seen in both (27) (16 per cent) and 28 (1 per cent), while (30) was not incorporated. On the contrary, this compound was hydroxylated in the 5-position by the plant to give the unnatural (31), which was isolated in undiluted form. The incorporations first found by Inouye's group were very small and could thus be explained by the above-mentioned epimeric impurities being very difficult to remove from the precursors used (cf. Inouye and Uesato 1986).

In continuation of this work (Damtoft 1983; Damtoft *et al.* 1983) we fed deuterium-labelled dexoyloganic acid (6) and the 8-epimer (18) to *Scrophularia racemosa* (Scrophulariaceae), *Plantago major* (Plantaginaceae), and *Buddleja davidii* (Buddlejaceae) all containing aucubin (32) as

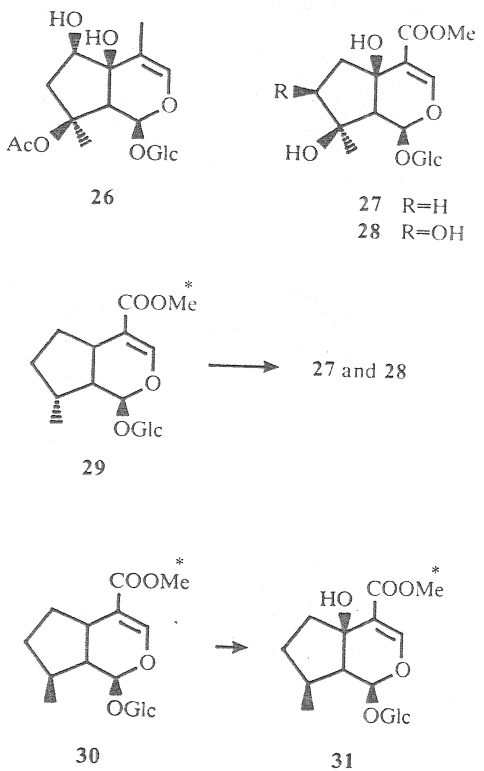


Fig. 6.10. Incoporations of *epi*-deoxyloganin (29) into ipolamiide (27) and lamiide (28) in *Hebenstreitia dentata*.

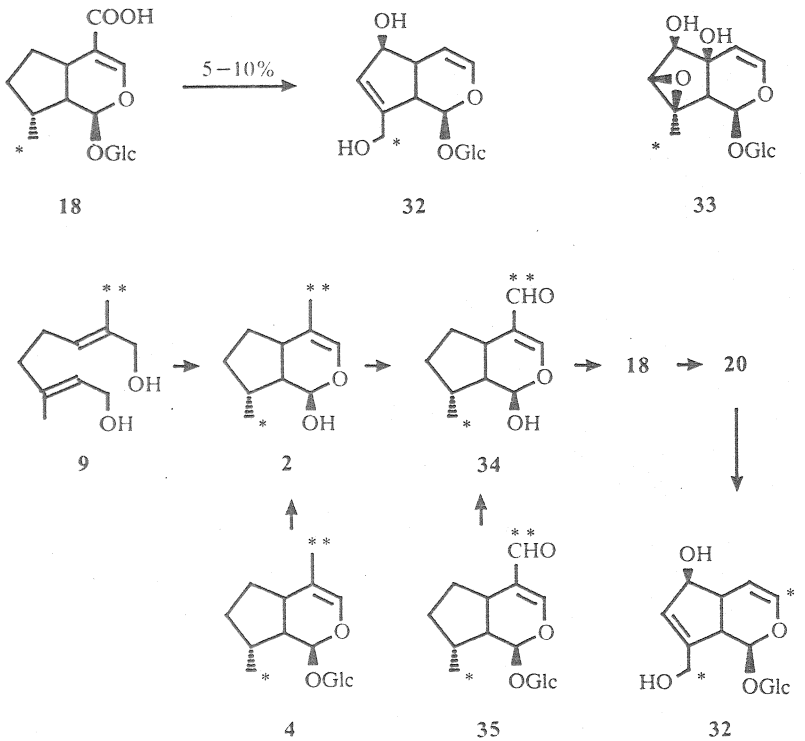


Fig. 6.11. Biosynthetic experiments with aucubin (32) and antirrhioside (33). Labelling at C_{10}:^{2}H; at C_{11}:^{13}C.

well as to *Antirrhinum major* (Scrophulariaceae) which produces antirrhinoside (33). In all cases good incorporations were obtained with (18) while (6) was not incorporated (Fig. 6.11).

We have some preliminary, unpublished results showing that the early steps leading to the compounds of the 8α-series is similar to those giving the 8β-series, except for the different stereochemistry. Thus, in *Plantago major*, [9-^{13}C]-10-hydroxygeraniol (9) gives incorporation into aucubin (32) with scrambling of the label. Furthermore, 8-*epi*-iridodial (2) and 8-*epi*-iridotrial (34) and their glucosides (4 and 35) labelled with deuterium at C_{10} and with ^{13}C at C_{11} all give good incorporations with scrambling of the C_{11} label, so that half of this is found in C_3 of aucubin. Since the glucosides (4) and (35) are both incorporated with scrambling, they are not intermediates but must have been hydrolyzed by the plant before being incorporated. Aucubin is also a constituent of *Aucuba japonica* (Aucubaceae/Cornaceae) systematically distant from the plants above. Some early results indicated that (32) might be derived from (6), but recently it has been shown by radioimmunoassay that *A. japonica* contains (18) but not (6) (cf. Inouye and Uesato 1986, p. 202).

However, not all compounds with the 8α-stereochemistry are formed via epi-deoxyloganin since gardenoside (38) is formed via a somewhat different pathway. Inouye and coworkers worked with cell cultures of *Gardenia* for many years and they finally succeeded in developing a line that produced tarennoside (37) and gardenoside (38). This culture could be used for biosynthetic experiments (Uesato *et al.* 1986*d*). When fed iridodial (1) the cultures produced iridotrial glucoside (15) which could be isolated, and no incorporation into (37) and (38) took place. Furthermore, the glucosides of *epi*-iridotrial (35) and 7, 8-dehydroiridotrial (36) were shown to be intermediates. In all the other cases discussed above, glucosylation takes place at a late stage after the final oxidation of C_{11} to a carboxyl group. The experiments were performed with deuterium or ^{13}C-labelled precursors and the incorporations were measured in tarennoside (37) by NMR. I can mention that when 10-hydroxygeraniol labelled with ^{13}C in the 9-position was fed to the culture, full scrambling was seen in the isolated *epi*-iridotrial glucoside (35).

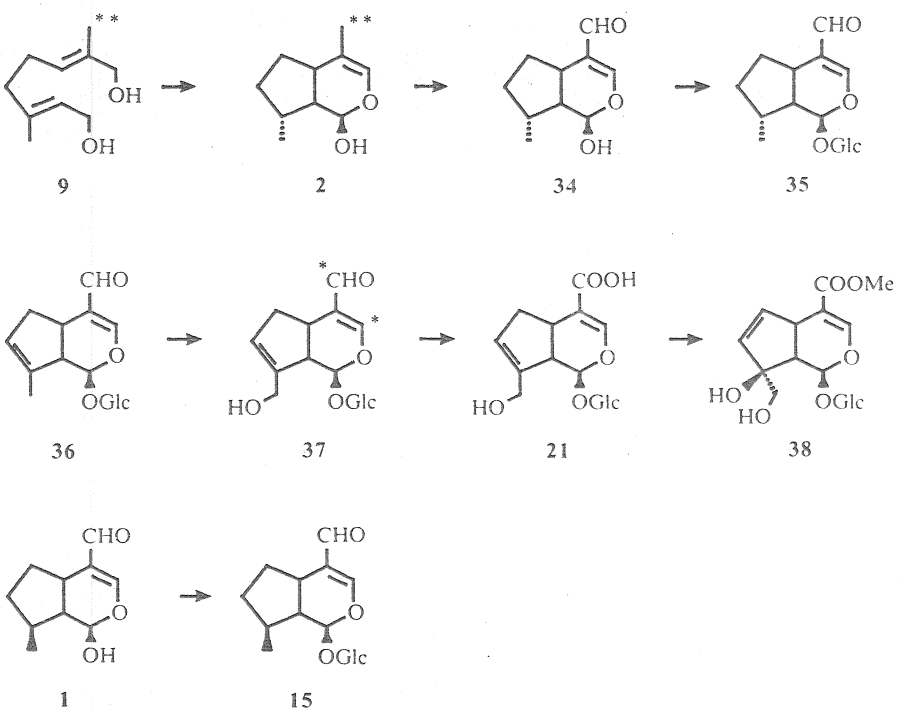


Fig. 6.12. Biosynthesis of gardenoside (38) in *Gardenia jasminoides* cell cultures.

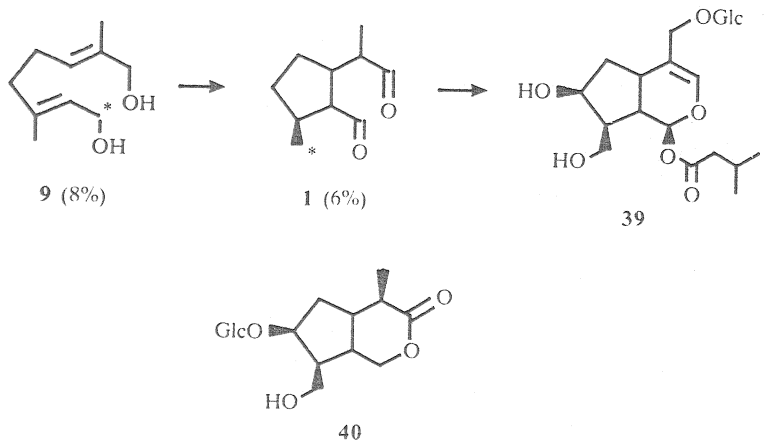
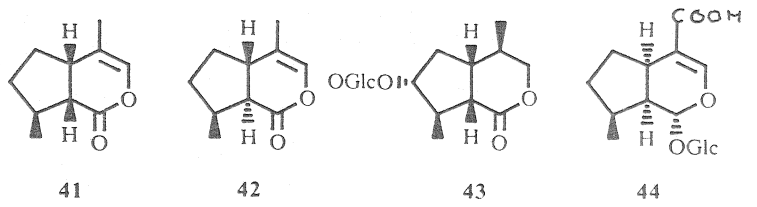


Fig. 6.13. Biosynthetic experiments in *Patrinia gibbosa*.

Note that geniposidic acid (21) is an intermediate in the formation of (38). It was in addition a precursor for (16) (Fig. 6.7), and is presumably also on the route to (32) (Fig. 6.11). Returning to the compounds with the 8β-stereochemistry, I will refer to some very recent experiments performed on *Patrinia gibbosa* (Valerianaceae) (Uesato 1988; Xie *et al.* 1989). This plant contains (Fig. 6.13) the compounds patrinoside (39) and gibboside (40), the former belonging to the so-called *Valeriana* compounds which are characterized by having an isovaleric ester at C_1 and by the oxidation state at C_{11}. When feeding the plant with tritium-labelled 10-hydroxygeraniol (9) and iridodial (1) good incorporations were obtained (8 per cent and 6 per cent, respectively) in (39), while 10-hydroxycitronellol (11) was not incorporated. No convincing incorporations into (40) were seen.

I will conclude this section on biosynthesis with some results obtained with *Nepeta cataria* and *Teucrium marum* (both Lamiaceae). In most subfamiles within Lamiaceae iridoids are commonly encountered (Hegnauer and Kooiman 1978). However, in the largest subfamily Saturejoideae, *Nepeta cataria* is the only species known to contain iridoids. The compounds found, namely nepetalactone (41), *epi*-nepetalactone (42), nepetaside (43), and 1, 5, 9,-*epi*-deoxyloganin (44) (Uesato 1988) are all unusual, particularly (44) which is unique in having three centres with a stereochemistry different from most other iridoids (Murai *et al.* 1984). Biosynthetic experiments with *N. cataria* were first done by Bellesia *et al.* (1984) and the results are shown in Fig. 6.14 (incorporations in parentheses). Feeding with 10-hydroxygeraniol (9), 10-hydroxycitronellol (11), and iridodial (1) seemed rather conclusive, since (9) was hardly incorporated at all, while (11) and (1) both gave good incorporations in nepetalactone (41). However, this work was



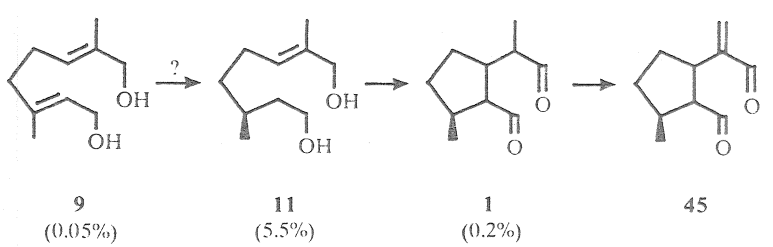
9	0.007% (0.03%)	0.014%	0.1%	0.8%
11	0.004% (2.8%)	0.016%	0.1%	0.6%
1	0.6% (1.2%)	0.03%	0.3%	1.2%

Fig. 6.14. Feeding experiments with *Nepeta cataria* leading to (41–44) (Uesato 1988) (Bellesia *et al.* 1984; incorporations in parentheses).

repeated by Uesato (1988) who used the same precursors, and in addition measured the incorporations in (42–44). In this work much smaller incorporations were obtained in nepetalactone (Fig. 6.14), but significantly, 10-hydroxycitronellol (11) gave incorporations of the same magnitude as 10-hydroxygeraniol (9).

The genus *Teucrium* is known to contain a number of iridoid glucosides (Ruhdorfer and Rimpler 1981) but *T. marum* contains dolichodial (45, the 'compound' being a mixture of epimers), the biosynthesis of which has also been investigated by Bellesia *et al.* (1983). Again in this case (11) (Fig. 6.15) was shown to be a good precursor for (45). It was a 100-fold better than (9) and 25 times better than (1). Apparently the biosynthesis of these compounds is untypical for iridoids.

If we sum up the different pathways known so far, we have three main routes to iridoids:



9 (0.05%) **11** (5.5%) **1** (0.2%) **45**

Fig. 6.15. Possible pathway to dolichodial (45) in *Teucrium marum*.

Route I derives from 10-hydroxygeraniol (9) via iridodial (1) and iridotrial (14) which with scrambling of label gives deoxyloganic acid (6), in turn being an intermediate for the group of compounds that is oxidized to the carboxyl stage at C_{11}, including all the secoiridoids. Apparently C_{11} is never lost in the compounds biosynthesized by this route. Formation of compounds like deutzioside (17) and patrinoside (39) may be considered as digressions from the main route, since plants containing such compounds often also contain glycosides which are obviously derived from this route.

Route II, which comprises the *epi*-series, is tentatively divided into two subroutes: One (IIa) starts with 10-hydroxygeraniol (9) and goes via *epi*-iridodial (2), and -trial (34), which with scrambling gives *epi*-deoxyloganic acid (18), similarly to route I. But after elaboration of the acid, decarboxylation of C_{11} often takes place. The formation of compounds like lamioside (26) may be seen as digressions from the main route. The other (IIb) is identical up to *epi*-iridotrial (34), where glucosylation takes place—and this is a few steps earlier than in the other subroute—followed by further oxidation of C_{11} to the carboxyl stage. No decarboxylation of C_{11} is known.

Route III consists of a pathway probably involving 10-hydroxycitronellol (11) and iridodial.

This classification is of course only based on the available evidence which may be insufficient at the present. It does, however, fit well with the circumstantial evidence discussed below. This is deduced partly from the distribution of the compounds from the different pathways, partly from the structural relationships between compounds of known derivation with others of unknown biosynthesis but from related plant taxa.

Iridoids and plant taxonomy

The iridoids comprise a group of compounds that shows much promise in angiosperm taxonomy, as first noted by Hegnauer (1964, 1966, 1986). Together with the late Rolf Dahlgren we have used his system of classification to demonstrate the systematic importance in the distribution of the iridoids (Jensen *et al.* 1975; Dahlgren *et al.* 1981). However, the biosynthetic knowledge was at that time too limited for using the full potential of the distribution of the individual compounds. In fact, with the present knowledge, the groupings of compounds then used were clearly insufficient.

A listing of all families reported to contain iridoids is seen in Table 6.1 together with the compounds found in each family classified according to the

Table 6.1 Superorders, orders, and families with iridoids

	Route I		Route IIa		
Taxon	Normal	Seco	Normal	Decarb.	Route IIb
RUTANAE					
Polygalales					
Malphigiaceae	x(?)				
ROSANAE					
Buxales					
Daphniphyllaceae	x(?)				
Hamamelidales					
Hamamelidaceae	x(?)				
CORNANAE					
Cornales					
Garryaceae			x	x	
Alangiaceae	x	x			
Nyssaceae		x			
Cornaceae	x	x			
Davidiaceae	x	x			
Escalloniaceae	x	x			
Toricelliaceae	x				
Aucubaceae			x	x	
Aralidiaceae	x				
Symplocaceae	x				
Icacinaceae	x	x			
Montiniaceae	x				
Hydrangeaceae	x	x			
Sambucaceae	x	x			
Viburnaceae	x	x			
Menyanthaceae	x	x			
Adoxaceae	x	x			
Eucommiales					
Eucommiaceae			x	x	
Dipsacales					
Caprifoliaceae	x	x			
Valerianaceae	x	x			
Dipsacaceae	x	x			
Calyceraceae	x	x			
LOASANAE					
Loasales					
Loasaceae	x	x			
GENTIANANAE					
Gentianales					
Desfontainiaceae	x	x			

Table 6.1 *Continued*

Taxon	Route I		Route IIa		Route IIb
	Normal	Seco	Normal	Decarb.	
Loganiaceae	x	x			
Rubiaceae	x	x			x
Theligonaceae	x				
Gentianaceae	x	x			
Apocynaceae	x	x			x(?)
Goodeniales					
Goodeniaceae	x	x			
Oleales					
Oleaceae	x	x			
LAMIANAE					
Lamiales					
Retziaceae				x	
Stilbaceae				x	
Buddlejaceae				x	
Scrophulariaceae	x(?)		x	x	
Myoporaceae				x	
Globulariaceae	x(?)			x	
Plantaginaceae				x	
Lentibulariaceae			x	x	
Pedaliaceae			x	x	
Martyniaceae				x	
Bignoniaceae	x(?)		x	x	
Acanthaceae			x	x	
Verbenaceae	x		x	x	
Lamiaceae			x	x	
Callitricaceae				x	
Hippuridales					
Hippuridaceae				x	
ERICANAE					
Fouquieriales					
Fouquieriaceae	x				
Ericales					
Actinidiaceae	x				
Ericaceae	x(?)			x	
Monotropaceae	x(?)				
Pyrolaceae	x(?)				
Stylidiales					
Stylidiaceae	x	x			
Sarraceniales					
Sarraceniaceae		x			

biosynthetic route by which they are formed (see below). Besides the superorders Ericanae, Cornanae, Loasanae, Gentiananae, and Lamianae where iridoids are common, the compounds are reported only from a few other sources. These are the genus *Stigmaphyllon* in Malphigiaceae (Rutanae) (Davioud *et al.* 1985), as well as two occurrences in Rosanae, namely *Liquidambar styraciflua* in Hamamelidaceae (Plouvier 1964) and *Daphniphyllum macropodum* in Daphniphyllaceae (Inouye *et al.* 1966). The report of the secoiridoid xylomollin from *Xylocarpus molluscensis* (Kubo *et al.* 1976) (= *X. granatum*; c.f. Chou *et al.* 1977; Ng and Fallis 1979) is dubious. We have reinvestigated the plant but found no traces of iridoids (Jensen and Nielsen, unpublished).

The distribution of the iridoids is shown in the Dahlgrenogram (Dahlgren 1989) in Fig. 6.16. The concentration of iridoid producing plants in closely connected superorders may indicate that the ability to biosynthesize the compounds has only arisen a few times. The large group of plants may even be considered monophyletic, and the few odd occurrences might be due to a secondary genetic transfer by micro-organisms.

For the purpose of classification, the use of biosynthetic pathways must inherently be better than using the individual compounds, particularly since a compound may be formed by different pathways [i.e. geniposidic acid (21) see above]. However, investigating the biosynthetic pathways in all species or genera is clearly impossible, and we must instead deduce the pathways from the compounds isolated from the plants. When determining by which route a compound is most probably formed, it is in many cases straightforward by looking at the structure and stereochemistry as well as at the compounds accompanying it in the same species (or second best, the same genus).

In Table 6.1, the compounds found in each family have been listed according to their probable biosynthetic route. With the present knowledge, secoiridoids and compounds decarboxylated at C_4 can safely be assumed to belong to *Route I* and to *Route IIa*, respectively. Furthermore, compounds with the 8β-stereochemistry are tentatively assumed to be derived from *Route I*, while compounds with the 8α-stereochemistry from *Route II*. This leaves some compounds with a double bond at C_8 and some which have lost C_{10}. In addition a problem exists in distinguishing between Route IIa and IIb. This will be discussed below.

When attempting to discriminate between the different biosynthetic routes, even at the present time with insufficient knowledge, the most promising fact is that the presence of secoiridoids (Route I) and of compounds decarboxylated at C_{11} (Route IIa) in plants are mutually exclusive up to the family level and, with a single exception, up to the ordinal level. The distribution of these two groups of compounds is shown in Fig. 6.17.

The secoiridoids (including the complex indole alkaloids), designating the

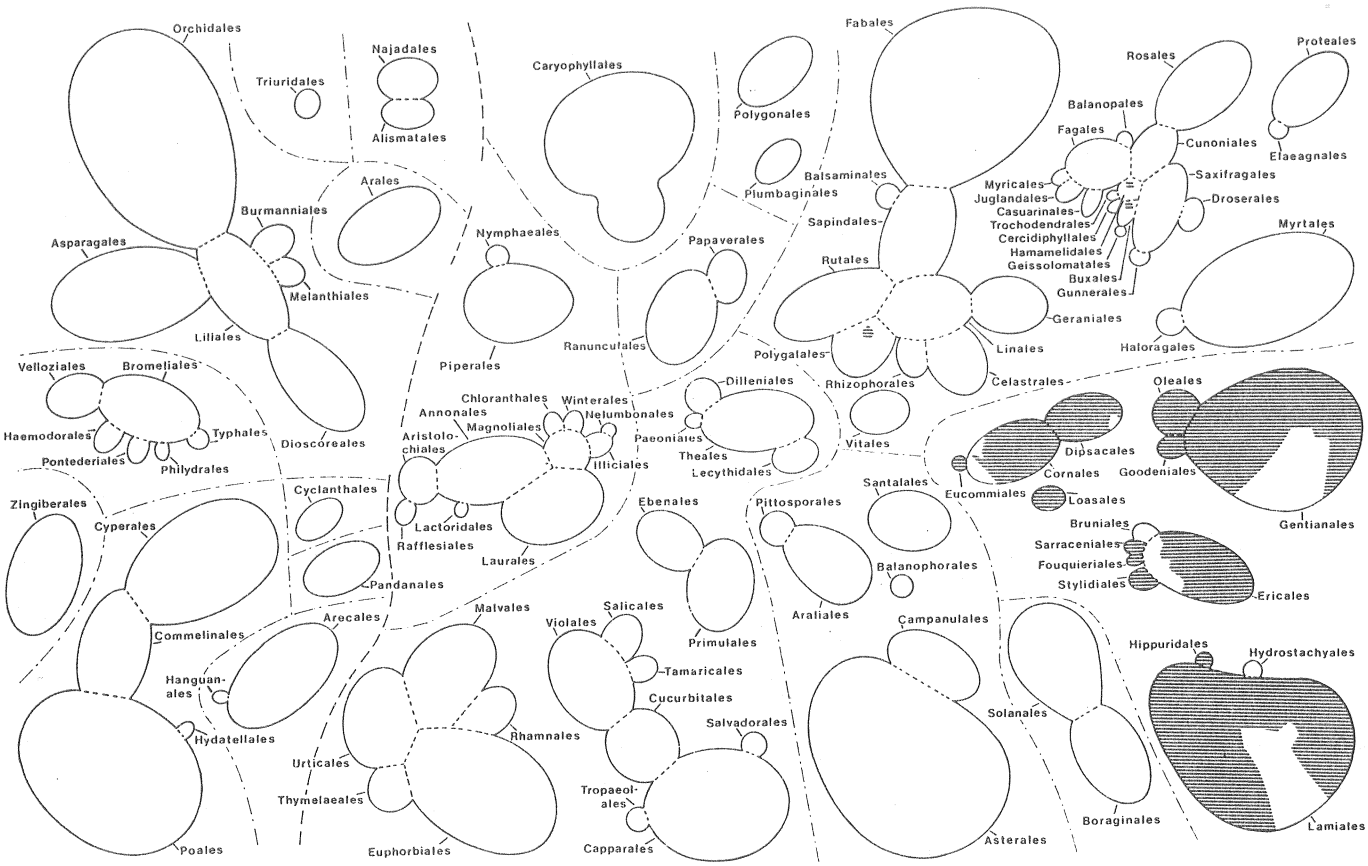


Fig. 6.16. Distribution of iridoids in Dahlgrenogram of dicotyledons.

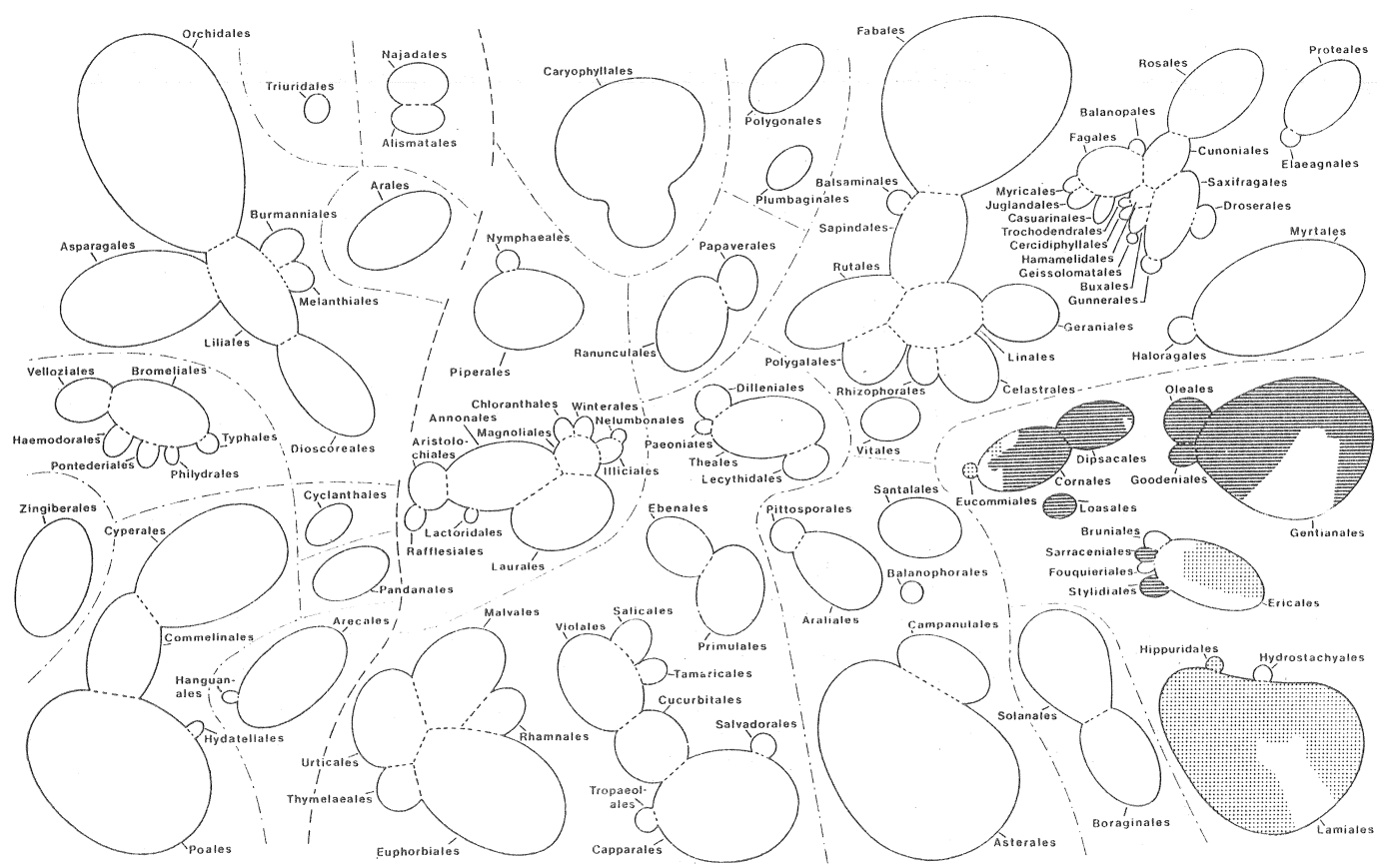


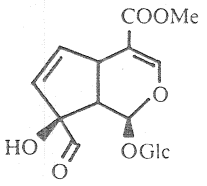
Fig. 6.17. Distribution of secoiridoids of *Route I* (hatched) and of iridoids decarboxylated at C_{11} of *Route IIa* (dots).

presence of *Route I*, are concentrated in the superorders Cornanae, Gentiananae, and Loasanae with two outlying occurrences in Ericanae, namely Sarraceniaceae (Jensen *et al.* 1975) and Stylidiaceae (S.R. Jensen, unpublished results). Conversely, the decarboxylated iridoids show the presence of *Route IIa*, and these compounds are concentrated in Lamianae with outlying occurrences in Eucommiaceae, Aucubaceae, and Garryaceae (Cornanae), as well as in Ericaceae.

When looking at Table 6.1, a good correlation can be seen between the secoiridoids and the compounds with 8β-stereochemistry on the one hand, and between the decarboxylated compounds and those with the 8α-stereochemistry on the other hand. Only few exceptions from this pattern is seen and these will now be discussed. Gentianales, Rubiaceae, and Apocynaceae contain compounds from both routes. As shown above, the compound gardenoside (38) in *Gardenia* is formed by *Route IIb*. In Fig. 6.18 is shown representatives for the compounds from the two families which are probably formed by *Route II*. Dehydrogardenoside (46) and oruwacin (47) are found in *Randia canthioides* (Uesato *et al.* 1982) and *Morinda lucida* (Adesogan 1979), respectively (both Rubiaceae). Both of these together with plumieride (48) and some related compounds (cf. Abe *et al.* 1988) from *Plumeria* and *Allamanda* species (Apocynaceae) are structurally so closely related to gardenoside, that they are very probably formed by the same route, namely *Route IIb*. Theveside (49) is also found in Apocynaceae (*Thevetia* and *Cerbera*; cf. El-Naggar and Beal 1980) and is in addition a constituent of several species of Verbenaceae (Ford and Bendall 1980; Rimpler and Sauerbier 1986). This may seem an enigma, but theveside is biosynthetically only one step removed from geniposidic acid (21), and as noted above, the latter compound is known to be an intermediate in *Route I* as well as *Route IIa* and *IIb*. Therefore, theveside could well be formed by *Route I* or *Route IIb* in Apocynaceae and by *Route IIa* in Verbenaceae.

Mussaenoside (50) and shanzhiside (51) or its methyl ester (52) are often encountered in Rubiaceae (Inouye *et al.* 1988). In *Gardenia* (51) is found together with compounds from *Route IIb*. Compounds (50–52) are also common in Lamiales (Jensen *et al.* 1988), but in these taxa they are almost consistently found together with iridoids decarboxylated at C_{11} (*Route IIa*). Again we have a situation where the probability of different pathways to the same compounds must be considered. Thus in Rubiaceae, (50–52) could well be formed by *Route IIb*, while in Lamiales by *Route IIa*. This is of course purely hypothetical and must be investigated.

In Lamiales, the overwhelming majority of compounds are formed by *Route IIa* although a few have the 8β-stereochemistry. Thus in Verbenaceae, one section of the genus *Verbena* (cf. Milz and Rimpler 1979) consistently contains these compounds, of which cornin (23) has already been shown to be formed by *Route I* (see above). (However, another section of *Verbena*



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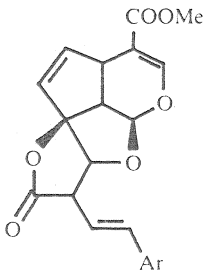

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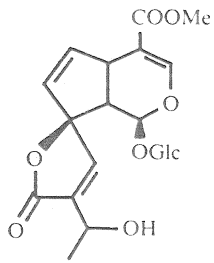

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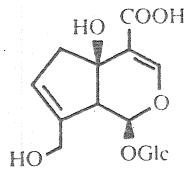

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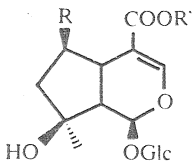
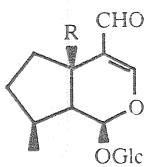


50 R=H, R'=Me

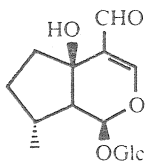
51 R=OH; R'=H

52 R=OH; R'=Me



15 R=H

53 R=OH



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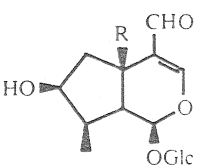
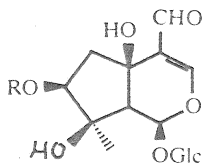


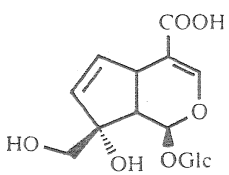
55 R=H

56 R=OH



57

R=Ester



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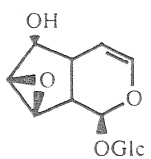

59

Fig. 6.18. Iridoid glucosides of uncertain derivation or unusual distribution.

contains compounds obviously from *Route II* only). Also such compounds have been isolated from *Nyctanthes* (cf. Rathore *et al.* 1989).

In Scrophulariaceae, some genera have been reported to contain iridoids with the 8β-stereochemistry. Thus *Euphrasia cuspidata* contains the 11-aldehydes (15), (53), (55), and (56) (Damtoft *et al.* 1981), many *Penstemon* species contain *Valeriana* compounds (cf. Gering *et al.* 1987), and *Chaenostoma foetida* contains (15) (Jensen *et al.* 1989). However, for the two former genera the typical compounds are derived from *Route IIa* (Salama and Sticher 1983; Junior 1985). Incidentally, the abnormal compounds often seen in *Penstemon* are typical *Valeriana* compounds in having an isovaleroyl group at C_1 and a glycosyl moiety at C_{11}, and the aglucone of patrinoside (39) has even been found in this genus too (Gering and Wichtl 1987). However, due to the different stereochemistry often seen for the iridoids found in *Penstemon*, these can not as a matter of course be assigned the same biosynthetic pathway as that found for (39) above (Fig. 13). Further work is necessary here.

From the monogeneric family Globulariaceae asperuloside (16) has been reported (Chaudhuri and Sticher 1981) to co-occur with aucubin (32) and a number of other compounds decarboxylated at C_{11}, these being typical *Route IIa* compounds. Asperuloside has been shown (Figs 6.6 and 6.7) to be biosynthesized by *Route I* in Rubiaceae, but the presence of this compound under these conditions definitely calls for an investigation of its biosynthesis, since an alternative route up to geniposidic acid (21) can well be imagined.

Most of the reported iridoids from Bignoniaceae are derived from *Route IIa*. However, a few genera in the family have species which produce either 8α-or 8β-methyl iridoids, while others again make both forms—consistently as 11-aldehydes (Fig. 6.18). Thus *Deplanchea speciosa* contains (54) (Davioud *et al.* 1989) and *Tecoma capensis* produces (56) (Bianco *et al.* 1983), while from *Tecoma stans* have been isolated both (53) and (54) (Bianco *et al.* 1982), and from *Campsis chinensis* among others (55) and (57) (Imakura *et al.* 1985). Since the majority of compounds in both Scrophulariaceae and Bignoniaceae are typical *Route IIa* derived iridoids, the occasional occurrence of the 8β-methyl aldehydes (15), (53), (55), and (56) presents no real taxonomic problem. They are not further transformed to true *Route I* compounds, and may merely be considered as oddities. The 8α-methyl aldehydes are more problematical since they formally belong to *Route IIb* and could be precursors for other compounds further oxidized at C_{11}. Only experiments can show whether this is the case.

The last order apparently containing compounds derived from both the main biosynthetic routes is Ericales. Only few reports of iridoids exist, but monotropein (58) has been reported from Ericaceae, Monotropaceae, and Pyrolaceae (cf. Jensen *et al.* 1975). In addition decarboxylated iridoids have been found in Ericaceae, namely unedoside (59) in *Arbutus* (cf. Jensen *et al.* 1975) and aucubin (32) in *Rhododendron* and *Menziesia* (private communication from Prof. Inouye). Monotropein is fairly often found in other families together with compounds formed by *Route I* and it has, at least formally, the 8β-stereochemistry. In Table 6.1, it is therefore tentatively considered to belong to this pathway. The biosynthesis has not been investigated, but it is presumably formed directly from geniposidic acid (21). The presence of typical *Route IIa* compounds like (32) and (59) [also with (21) as the probable intermediate] in the family, clearly make further investigations desirable. It can be added that (58) additionally is found in the neighbouring family Fouquieriaceae in company with loganin and other *Route I* compounds (Jensen and Nielsen 1982).

In conclusion, I believe that I have demonstrated that the potential for the iridoids as taxonomic markers is larger than previously believed in the superorders treated here. Part of the evidence is well substantiated, while some of it is more speculative or even circular in nature. In order to evaluate the full potential of these compounds, further work is clearly necessary, particularly on the iridoids in Rubiaceae, Scrophulariaceae (*Penstemon*) and Ericaceae.

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7 Terpenoid phytoalexins: aspects of biosynthesis, catabolism, and regulation

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Introduction

The term 'phytoalexin' was coined by Müller and Börger (1940) to denote the then hypothetical defensive substances that are produced by potato (*Solanum tuberosum*) tubers in response to infection with an avirulent race of the fungus *Phytophthora infestans* (Mont.) de Barry. In 1958, Müller redefined phytoalexins as low molecular weight antibiotics and extended the definition to include other plant-pathogen interactions. The concept of the phytoalexin response received direct experimental support in 1962 with the isolation of the isoflavonoid phytoalexin pisatin from infected pea (*Pisum sativum*) tissues (Perrin and Bottomley 1962). This was followed in 1968 with the isolation of the terpenoid phytoalexin, rishitin from infected potato tuber tissue (Tomiyama *et al.* 1968).

The most widely used working definition of the term phytoalexin is: 'Phytoalexins are low molecular weight, antimicrobial compounds that are both synthesized by and accumulated in plant cells after exposure to micro-organisms' (Paxton 1981). More than 200 phytoalexins are now known (Coxon, 1982; Ingham, 1982; Kuć, 1982; Brooks and Watson 1985) with certain chemical types being associated with particular plant families. Not unexpectedly, some plant tissues, in addition to producing phytoalexins, also accumulate small amounts of structurally related compounds. Some of these are precursors or metabolites of the actual phytoalexins while others are formed from biosynthetic intermediates as a result of side reactions. The induction of phytoalexin accumulation in a plant tissue is not dependent on

Abbreviations: DMAPP, dimethylallyl pyrophosphate (Δ^3-isopentenyl pyrophosphate); FPP, (2*E*, 6*E*)-farnesyl pyrophosphate; GGPP, (2*E* 6*E*, 10*E*)-geranylgeranyl pyrophosphate; GPP, (2*E*)-geranyl pyrophosphate; HMGR, (3*S*)-3-hydroxy-3-methylglutaryl-coenzyme A reductase; IPP, Δ^2-isopentenyl pyrophosphate; MVA, (3*R*)-mevalonic acid; PSPP, pre-squalene pyrophosphate; RNase, ribonuclease; SDW, sterile distilled water; SQS, squalene synthetase.

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