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ACCUMULATION PATTERN OF FLAVONOIDS: QUANTITATIVE PHENOMENOLOGY AND SOME SPECULATIONS

Introduction

Although considerable success has been achieved in decoding the pathway of flavonoid biosynthesis, intracellular mechanisms responsible for the control of flavonoid accumulation have still remained comparatively vague. One of the reasons is that in most studies so far carried out mainly correlative criteria have been used as a basis of judgements, whereas specific quantitative aspects of flavonoid metabolism have received only slight attention.

The aim of this paper is to point out some quantitative regularities which can be observed in the accumulation of flavonoids in vegetative plant tissues capable of synthesizing two or more different derivatives of flavonoid nature. The regularities were found to be particularly clear-cut in buckwheat seedlings, yet they can be revealed, more or less clearly, also in other plant species. Certain generalizations, therefore, seem to be justified although possible mechanisms operating can be envisaged only speculatively. Some of the general principles of the control of accumulation of flavonoids and related phenylpropanoid compounds have been discussed by the author earlier (Margna, 1977).

Discussion

The flavonoids — a family of biogenetically related compounds responding similarly to the influence of modifying factors

At present there has remained no doubt that phenylalanine — an aromatic amino acid synthesized via the shikimic acid pathway — is the common precursor of flavonoids and most of the other polyphenolic compounds a higher plant is capable of forming in its cells (Fig. 1).

After removal of the amino group by phenylalanine ammonia-lyase (PAL), the amino acid is transformed into trans-cinnamic acid which subsequently combines with three moieties of malonate to give rise to a chalcone — the first and the simplest 15-carbon compound of characteristic phenolic structure already considered to be a flavonoid. Chalcone or, by other suggestions, its isomeric form, flavanone (Wong, 1968; Wong, Grisebach, 1969), fulfills the role of a parent compound from which, through independent biosynthetic routes, flavones, flavonols, anthocyanins, or any other group of flavonoids are formed (Harborne, 1962, 1967; Grisebach, 1965, 1967; Grisebach, Barz, 1969; Pacheco, 1969; Hahlbrock,

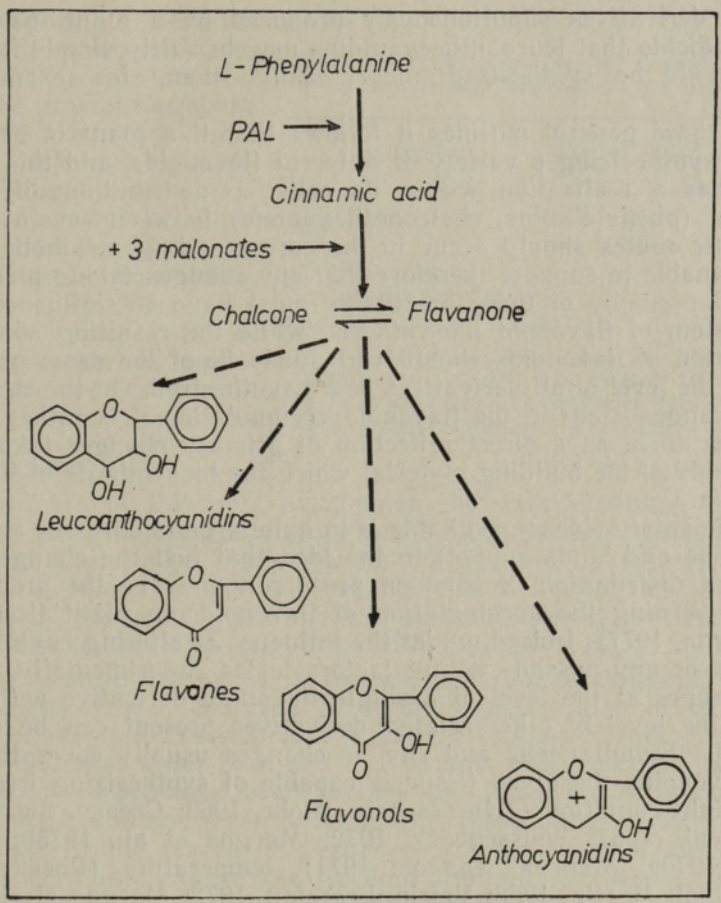

Fig. 1. Pathway of flavonoid biosynthesis (an outline). Broken arrows leading from the parent chalcone/flavanone to the flavonoid end-products symbolize the presumed relative length of the biosynthetic routes involved in the building of flavonoids of different classes.

Grisebach, 1975). The nature of intermediate biochemical transformations and the number of individual steps along these separate pathways is as yet largely unknown. Various genetical and biochemical data allow, however, to suggest that flavones belong to the simplest members of the flavonoid "family", and require a comparatively short biosynthetic pathway for their building. More intermediate steps are needed for building flavonols, while anthocyanidins, having a rather complicated basic skeleton, are obviously formed via a still more extended biosynthetic route.

The sequence of biochemical reactions involved in the biosynthesis of leucoanthocyanidins (flavan-3,4-diols), the fourth class of flavonoids commonly occurring in plants, is fully hypothetical at present. Circumstancial evidence, such as the presence of leucoanthocyanidins as a primitive character of dicotyledons (Bate-Smith, Lerner, 1954; Sporne, 1975), similar oxidation level of the central heterocyclic ring of leucoanthocyanidins and flavanones (Seshadri, 1962), and abundant production of leucoanthocyanidins as compared with the accumulation of the other

flavonoid derivatives simultaneously produced by a plant tissue (see below), indicate that leucoanthocyanidins may be fairly simple flavonoids which should be synthesized more easily than, for example, the flavonols.

From these general outlines it follows that if a plant is genetically potent of synthesizing a variety of different flavonoids, and this potency, in fact, has a realization within its cells, a distribution of common precursors (phenylalanine, chalcone/flavanone) between several parallel biosynthetic routes should occur in the corresponding metabolic centres. It is reasonable to suppose therefore that any changes taking place in the intracellular supply of these precursors must have an influence on the **whole** system of flavonoid biosynthesis, while the resulting shift in the accumulation of flavonoids should very likely be of the same qualitative nature at the level of all derivatives being synthesized. On the other hand, if such changes occur in the flavonoid accumulation it is rather justified to consider them as a direct reflection of primary changes taking place in the supply of the building material which the biosynthesis of flavonoids starts from.

Experimental evidence available is in quite a good harmony with these assumptions and lends support to the idea that just the changes in the supply and distribution of common precursors may be the predominant factors governing the accumulation of flavonoids in plant tissues (see also Margna, 1977). Indeed, under the influence of altering environmental conditions or any possible acting factors tested experimentally, accumulation changes at the level of a single flavonoid derivative without any effect at the level of other related derivatives present can be observed very rarely. Simultaneous and similar changes usually cover the whole set of flavonoids the given tissue is capable of synthesizing irrespective whether light conditions (Harraschain, Mohr, 1963; Creasy, Swain, 1966; Scherf, Zenk, 1967; Weissenböck, 1972; Margna et al., 1973b; Халлоп, Маргна, 1970a; Маргна, Халлоп, 1971), temperature (Rossiter, Beck, 1966; Paynot, Martin, 1968; Voirin, Lebreton, 1972; Margna et al., 1973a; Creasy, 1974), mineral nutrition (Rossiter, 1969; Krause, Reznik, 1972; Hilton et al., 1973; Абышева, 1972; Школьник, Абышева, 1975), level of feeding exogenous nutritives (Margna et al., 1974a, b) or any other conditions (Маргна и др., 1969; Волынец, 1969) are varying. This similarity holds also in senescent leaves: autumnal reddening of leaves so typical of certain plant species does not mark only a sharp increase in the accumulation of anthocyanin pigments but is also accompanied by a considerable rise in the content of other flavonoids (Ishikura, 1972; Creasy, 1974; Маргна и др., 1974).

Normal quantitative parameters of flavonoid accumulation

It needs to be pointed out, however, that with complete qualitative resemblance of responses at the level of all flavonoid derivatives there are always marked and rather characteristic quantitative differences between the range of changes in the accumulation of separate flavonoids. Before examining this aspect it is appropriate to point out some general quantitative regularities which can be normally observed in the accumulation of flavonoids.

When more than one derivative are simultaneously formed in a vegetative plant tissue, the accumulation of separate derivatives is typically manifested in the following quantitative manner: the content of the simplest forms is the highest, much less relatively is the amount of flavonoids having a more complex basic structure, while the

most complicated derivatives show the lowest accumulation rate. This correlation can be exemplified by the accumulation data of different flavonoids in 5-days-old buckwheat seedling organs (excised) incubated in distilled water (Table 1). As can be evaluated from the table, under these conditions the approximate molar ratio of the accumulation of anthocyanins, rutin and leucoanthocyanidins in the hypocotyls proved to be 1 : 3 : 38, while in cotyledons having a more complicated set of flavonoid compounds the corresponding ratio was 1 : 6 : 27 : 44 for anthocyanins, rutin, glycoflavones, and leucoanthocyanidins, respectively. Under other circumstances the numerical expression of these ratios may be somewhat different, yet the general arrangement of separate flavonoids by the relative rate of their accumulation always remains the same in these tissues (Margna et al., 1973a, b, 1974a, b).

Table 1

The content of flavonoids in excised buckwheat hypocotyls and cotyledons, nmol/seedling*

Flavonoid	Hypocotyls	Cotyledons
Anthocyanins	8.2	12.7
Rutin	23.8	79.7
Glycoflavones	—	344
Leucoanthocyanidins	311	556

* Extrapolated from data in (Margna et al., 1974b); the material was excised from 80-hr-old etiolated seedlings and incubated for 40 hr (16 hr light + 24 hr darkness) in distilled water.

Similar quantitative ratios in the accumulation of different flavonoid compounds have been observed to occur normally also in other plant species. For example, in strawberry leaves the content of anthocyanins was about 40—90 times lower than that of flavonols while leucoanthocyanidins (flavolans) were produced even in 130—180 times larger amounts than was the accumulation of anthocyanins (Creasy, Swain, 1966; Creasy, 1968, 1974). In the leaves of *Periploca graeca* the approximate quantitative ratio of anthocyanin and flavonol accumulation was 1 : 8 (Melin, 1975a). The average level of leucoanthocyanidins measured in this tissue was of about the same order that the level of flavonols (Melin, 1975b), yet as none of the assay methods so far elaborated for leucoanthocyanidin measurements allows to quantify the presence of these compounds completely, the actual level of that group of flavonoids in *Periploca* leaves could be at least 3 times higher than the level of flavonols and, consequently, about 20—25 times higher than the level of anthocyanins. Flavonol glycosides were found in substantially less amounts than leucoanthocyanidins (and flavan-3-ols resp. catechins) also in the leaves and vegetative shoots of tea plant (Forrest, Bendall, 1969), in the leaves and other vegetative parts of *Rhododendron* species (Шалашвили, 1970), in *Phaseolus* hypocotyls (Rathmell, Bendall, 1971), in *Euonymus* leaves (Creasy, 1974), and in a number of other tissues (Paech, Eberhardt, 1952; Feucht, 1975).

Preferential accumulation of simpler forms was observed also in the case of some other combinations of flavonoids. In *Spirodela intermedia* grown under varying light conditions, the amount of glycoflavones produced (the sum of vitexin and orientin) always exceeded the level of a cyanidin-glucoside; the maximal quantitative ratio of their accumulation was about 20 : 1 (McClure, 1968). In light-treated first internodes of *Sorghum* the content of primitive apigeninidin and luteolinidin anthocyanins lacking 3-OH group in their molecules was about 4 times higher than the content of a cyanidin derivative having molecular structure typical of common anthocyanidins (Stafford, 1966). An approximate

evaluation of experimental data obtained in our laboratory suggests that in senescent leaves showing autumnal reddening flavonol glycosides always accumulate much more abundantly than anthocyanins (Маргна и др., 1974).

Although exceptions from the general rule are not excluded (Weissenböck, 1972; Ламан, Волынец, 1974), these data do support the regularity pointed out: the more complicated the suggested biosynthetic pathway of a flavonoid is relatively, the less tends to be the rate of its accumulation, relatively, in comparison with the accumulation of simpler flavonoids simultaneously synthesized by the same vegetative tissue.

It is noteworthy that the same correlation tends to be valid not only with respect to the flavonoids having different basic structure, but seems to fit also, within certain groups of flavonoids, with the accumulation pattern of closely related derivatives differing from one another by the number of substitute groups attached to the basic skeleton. This point is in the best way illustrated by the accumulation of glycoflavones in buckwheat cotyledons. The four-membered group of glycoflavones of that tissue consists of two pairs of related compounds, one of them representing the derivatives of 5,7,4′-trihydroxyflavone or apigenin (vitexin and iso-vitexin), whereas the other pair of compounds (orientin and iso-orientin) has the structure of 5,7,3′,4′-tetrahydroxyflavone or luteolin (Margna et al., 1967; Тохвер и др., 1967). Within both pairs one derivative has its glucose moiety attached in the position 8 (vitexin and orientin) while in the corresponding twin-compounds (iso-vitexin and iso-orientin) the 6th carbon atom of the basic skeleton is involved in the building of C-glycosidic linkage (Fig. 2). As

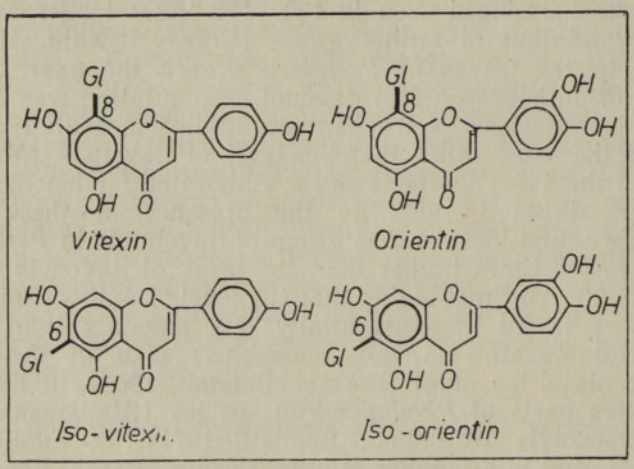

Fig. 2. Glycoflavones of buckwheat cotyledons.

can be seen from Fig. 3, apigeninic glycoflavones are accumulating at a rate about twice as high as that of the corresponding luteolinic derivatives having one more OH-group in their molecules. Again the ratio of accumulation rates of tri- and tetrahydroxyflavone derivatives needs not necessarily be the same under all circumstances, yet in all cases the amount of luteolinic glycoflavones (which require at least one more step for their building) remains considerably lower than the amount of the respective apigeninic derivatives (Margna et al., 1973a, 1974a).

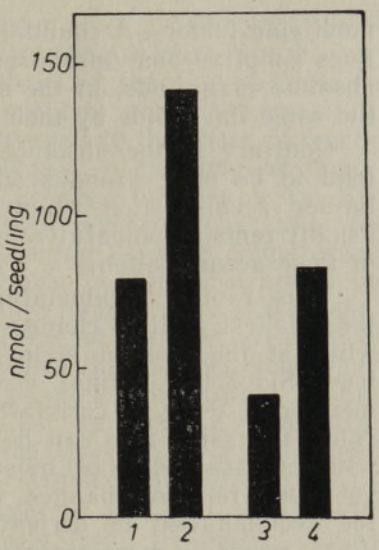

Fig. 3. The content of separate glycoflavones in excised buckwheat cotyledons, nmol/seedling (extrapolated from data in Margna et al., 1974b; see footnote in Table 1). *1* — vitexin, *2* — isovitexin, *3* — orientin, *4* — iso-orientin.

Similar quantitative differences in the accumulation of apigeninic and luteolinin derivatives were found to occur also in the other glycoflavone-synthesizing plant tissues: in barley seedlings (Carlin, McClure, 1973), in *Spirodela* species (Wallace, Alston, 1966; McClure, 1968; Wallace, Grisebach, 1973; Wallace, 1975), and, in a less pronounced form, also in flax seedlings (Thakur, Ibrahim, 1974). This regularity is in a good agreement with the facts which indicate that the B-ring oxidation pattern of C-glycosylflavones is determined at an early stage of their biosynthesis, probably at the flavanone or C-glycosylflavanone level (Wallace et al., 1969; Wallace, 1975). Under these circumstances a later oxidative conversion of 4′-OH-intermediates into their 3′,4′-OH-analogues is excluded resulting in that biosynthetic pathways of apigeninic and luteolinic glycoflavones remain fully independent from each other.

In the biosynthesis of most other classes of flavonoids the stage at which introduction of an additional OH-group into the B-ring can be accomplished, seems not to be so strictly determined, and a secondary transformation of less oxidised intermediates into more substituted ones may occur at later stages of biosynthesis (Grisebach, 1967; Hahlbrock, Grisebach, 1975). That may be the reason why higher accumulation rates of 4′-monohydroxylated derivatives as compared with the rate of accumulation of the corresponding 3′-,4′-di- or 3′-,4′-,5′-trihydroxylated ones are not typical of flavonols, flavones and anthocyanins, although examples of such a prevalence of simpler forms can also be found for these classes of flavonoids (Bottomley et al., 1965; Pachlich, 1969; Wildanger, Herrmann, 1973; Lawanson et al., 1975).

Quantitative characteristics of accumulation changes

Multiple experimental evidence obtained in our laboratory with using buckwheat seedlings unequivocally shows that the general quantitative tendencies just outlined form the background of any changes taking place in the formation of flavonoids and play an essential role in determining the level of increase or decrease in the accumulation of separate derivatives. First of all it must be noted that there are great differences depending on whether absolute or relative changes are concerned, although definite regularities can be observed in both cases.

The range of a b s o l u t e c h a n g e s in the accumulation of separate derivatives generally coincides with the initial range of accumulation of the same derivatives. That is, flavonoid compounds most abundantly synthesized in the tissue are tending to show also the highest absolute increases or decreases in their accumulation under the influence of

modifying factors. Accumulation changes at the level of any other derivatives simultaneously produced are smaller and can be arranged, by their absolute magnitude, in the same order as would be the arrangement of the same flavonoids by their normal range of accumulation.

Contrary to the absolute changes the relative ones always tend to be most pronounced in the case of those derivatives showing limited accumulation while major components of the complex present usually remain comparatively inert and show only small relative changes in their accumulation.

Thus, rapidly accumulating derivatives show the largest absolute, yet the smallest relative changes when a shift in their accumulation occurs, while at the level of minor components just the opposite is generally true. Since the normal accumulation rate of flavonoids, as already mentioned, tends to correlate with the level of complexity of their molecules, the same rule can be formulated also as follows: simpler forms with comparatively short biosynthetic pathway usually show large absolute yet small relative changes when their accumulation is modified, while the accumulation of derivatives having a more complicated structure (resp. requiring more biosynthetic steps for their building) typically show large relative changes, although in absolute terms the changes may remain rather small.

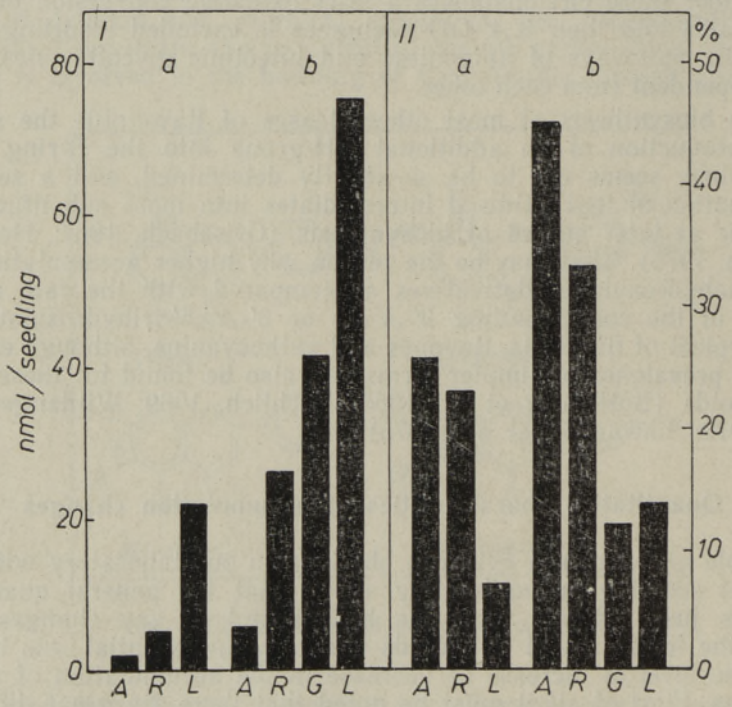

Fig. 4. Azote-induced decrease in the accumulation of flavonoids in excised buckwheat hypocotyls and cotyledons (extrapolated from data in Margna et al., 1974b). The 80-hr-old material excised from etiolated seedlings was incubated for 40 hr (16 hr light + 24 hr darkness) in a 0.1 per cent solution of NH_4NO_3. I — absolute decrease, nmol/seedling; II — relative decrease, %. *a* — hypocotyls, *b* — cotyledons; *A* — anthocyanins, *R* — rutin, *G* — glycoflavones, *L* — leucoanthocyanidins.

The validity of this rule may be illustrated by the following buckwheat data. A 40-hour incubation of isolated seedling organs in a 0.1 per cent solution of ammonium nitrate brought about an absolute decrease in total flavonoids of about 30 nmols in hypocotyls and 150 nmols in cotyledons. The most part of this total decrease fell to the share of leucoanthocyanidins and glycoflavones (in cotyledons) — the simplest flavonoid derivatives abundantly accumulating in these tissues (Fig. 4). Absolute decrease in the content of rutin was several times lower in both organs, while the share of anthocyanins having the most complicated molecule did not exceed 6 nmols in cotyledons and about 2 nmols in hypocotyls (about 4 and 7 per cent of the total absolute decrease, respectively). Contrary to that, the relative decrease was the greatest in the case of anthocyanins (up to 45 per cent below the control), somewhat less pronounced in the case of rutin, yet remained the lowest in the accumulation of glycoflavones and leucoanthocyanidins not exceeding, within the latter two groups of flavonoids, the level of 12 or 7—14 per cent below the control values, respectively (Margna et al., 1974b).

Similar were the quantitative characteristics of changes promoted in the accumulation of buckwheat flavonoids by light treatment. Again leucoanthocyanidins and glycoflavones typically showed the highest and anthocyanins the lowest absolute levels of responses (stimulation in that case), while rutin in both hypocotyls and cotyledons occupied an intermediate position. Relative light-induced changes could be arranged in the reverse order (Table 2). The same rule held generally also in other experiments with buckwheat seedlings, although its manifestation, depending upon conditions, remained sometimes less clear-cut.

Table 2

Light-induced increase in the content of flavonoids in 5-days-old buckwheat seedlings continuously illuminated during the final 48-hr period of development

Flavonoid	Absolute increase, nmol/seedling*		Relative magnitude of the increase as compared with the dark control, %**	
	Hypocotyls	Cotyledons	Hypocotyls	Cotyledons
Anthocyanins	5.6	4.0	∞	1200
Rutin	31	69	500	125
Glycoflavones	—	125	—	45
Leucoanthocyanidins	107	144	70	40

* Extrapolated from data in (Margna et al., 1973b).

** Approximate estimates calculated from data in (Hallop, Margna, 1968; Халлоп, Маргна, 1970а) for anthocyanins, in (Hallop, Margna, 1969; Халлоп, Маргна, 1970а) for rutin, in (Халлоп, Маргна, 1970б) for glycoflavones, and in (Margna et al., 1973b) for leucoanthocyanidins.

Special investigations of that aspect in other plant species are lacking at present and it is difficult to say therefore which is the generalizing power of the buckwheat data reviewed here. However, an analysis of several other data which allow such comparison (Bottomley et al., 1965; Carlin, McClure, 1973; Weissenböck, Effertz, 1974) seems to support the general idea of this section and suggests that the rule formulated may be valid more widely.

Possible mechanisms operating

If it is true that the quantitative regularities described are a reflection of the conditions in substrate supply, it seems inavoidable to conclude that a mechanism is operating in plant cells which in quite a specific manner controls the distribution of common precursors between the independent biosynthetic pathways of different flavonoids.

The way of operation of this mechanism seems to be determined by the following principles:

i) the rate at which the precursors can be utilized for the biosynthesis of different flavonoids is inversely related to the number of intermediate steps required for the completion of their building, so that the precursors are most effectively channelled through the simpler pathways and less effectively through the more complicated ones;

ii) the distribution ratio of precursors between different flavonoid pathways does not remain constant under all circumstances but depends either upon the rate at which the precursors are supplied to the metabolic centres involved or upon the level the content of separate flavonoid derivatives has reached in the cells up to that time.

In any case, at enhanced levels of substrate supply (resp., at higher existing levels of flavonoids in the cells) the portion of precursors which can be utilized for the building of more complex flavonoids seems to be increased relatively, while the portion which can be consumed for the building of simpler flavonoids shows a relative decrease. By contrast, at reduced levels of substrate supply (resp., at lower initial content of flavonoids) relatively lesser amounts of common precursors can be channelled for the building of more complicated flavonoids, whereas their use for the building of simpler ones becomes relatively still more dominating.

Confirmation of these principles may be found in the following isotope experiment carried out in our laboratory.

80-hr-old excised cotyledons of etiolated buckwheat seedlings were incubated under continuous illumination for 40 hr in a 10^{-2} M solution of 1-C^{14}-phenylalanine. Every 4 hours the content and the radioactivity of anthocyanins, rutin, and glycoflavones were measured in the material, and the absolute increase in the amount of each of these flavonoids was determined as compared with the initial level of their content in the material at the beginning of the incubation. On the grounds of these data the share of exogenous phenylalanine in the formation of separate derivatives was calculated.

The results showed that during the experimental period (40 hr) about 600 nmols of L-phenylalanine entered a pair of cotyledons with about 12—13 per cent of that amount incorporated into the flavonoids. The pattern of total radioactivity of different flavonoids approximately resembled the general accumulation pattern of flavonoids typical of buckwheat cotyledons, yet the figural expressions of the quantitative ratios were rather different (Fig. 5). While the normal ratio of the accumulation of anthocyanins, rutin and glycoflavones was approximately 1 : 6 : 27 in buckwheat cotyledons, the distribution ratio of total radioactivity between the same flavonoids was found to be 1 : 2.5 : 7.4 under these experimental conditions. Since the overall production of flavonoids was continuously increased during the experimental period, there remained no doubt that at the enhanced level of phenylalanine supply resulting from feeding

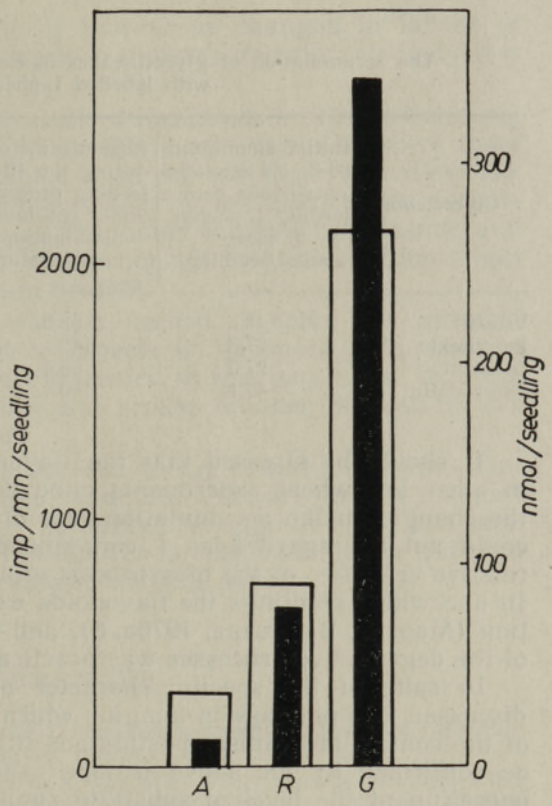

Fig. 5. Comparison of the distribution of radioactivity in different flavonoids, after feeding labelled L-phenylalanine, with the normal accumulation pattern of the same flavonoids in buckwheat cotyledons. White bars — content of flavonoids, nmol/seedling (extrapolated from data in Margna et al., 1974b); black bars — radioactivity, imp/min/seedling (details in the text). *A* — anthocyanins, *R* — rutin, *G* — glycoflavones.

amino acid exogenously, the distribution of that precursor between different flavonoid pathways had changed in favour of building more complex derivatives — anthocyanins and rutin. In other words, at that level of phenylalanine supply its utilization for the formation of anthocyanins and rutin was considerably increased relatively, as compared with its utilization in these biosyntheses at the normal level of phenylalanine supplied from endogenous pools.

Analogical were the results within the group of glycoflavones. As was shown above (Fig. 3), in buckwheat cotyledons apigeninic glycoflavones are normally accumulating at a rate about twice as high as that of the corresponding luteolinic derivatives, while within both pairs the 6-C-isomers (iso-vitexin and iso-orientin) are formed in much larger amounts than their 8-C-analogues (vitexin and orientin). In accordance with that, similar were the relative amounts of these glycoflavones also before the onset of the feeding program (Table 3), reflecting normal distribution ratio of precursors in their biosynthesis in buckwheat cotyledons. Under conditions of enhanced phenylalanine supply, the relative rate of forming luteolinic glycoflavones, however, gradually increased and that of apigeninic ones decreased, resulting in that the total production of orientin and iso-orientin during the whole 40-hr experimental period of feeding phenylalanine was even somewhat greater than the production of vitexin and iso-vitexin during the same period. A comparison of radioactivites showed that the total incorporation of L-phenylalanine into luteolinic glycoflavones was, under these conditions, markedly greater than into the apigeninic ones, thus contrasting the situation which occurred in the cotyledons at the endogenous level of precursor supply, up to the transfer of the material into the phenylalanine solution. It was not surprising therefore that the original large quantitative differences in the amount of apigeninic and luteolinic glycoflavones, so typical of buckwheat cotyledons normally, were practically smoothed out by the end of the experimental period (Table 3).

Table 3

The accumulation of glycoflavones in excised buckwheat cotyledons fed with labelled L-phenylalanine

Glycoflavone	Initial amount in 80-hr-old etiolated cotyledons before feeding L-phenylalanine, nmol/seedling	Absolute increase during the 40-hr feeding program under continuous illumination, nmol/seedling	Final amount at the end of the feeding program, nmol/seedling	Total radioactivity resulting from feeding labelled L-phenylalanine, imp/min/seedling
Vitexin	43.3	53.9	97.2	492
Iso-vitexin	77.3	142	219	668
Orientin	27.0	65.4	92.4	616
Iso-orientin	49.1	149	198	911

It should be stressed that the flavonoid changes found in that and in other buckwheat experiments cited here, were wholly conditioned by the changes in the accumulation rate of separate derivatives, while they could not be regarded as a consequence of shifts taking place in the relative velocities of the biosynthesis and degradation of these flavonoids. In buckwheat seedlings the flavonoids were not subject to rapid degradation (Маргна, Вайнъярв, 1976а, б), and the possible interfering influence of the degradative processes was practically ruled out.

In spite of the specific character of the quantitative regularities discussed it is not easy to imagine which might be the biochemical nature of the control mechanism postulated, although there are all probabilities, as confirmed by the above isotope experiment, that the mechanism is operating at the level of substrate supply. One of the possibilities may be that the enzymes responsible for the biosynthesis of every different class of flavonoids are organized into separate multienzyme or multienzyme-like complexes which all start from one and the same parent precursor (chalcone/flavanone?). If to believe that a further molecule of that initial substrate, most likely, can be captured by such complexes after the preceding one has passed through all the intermediate steps and the flavonoid end-product has been released, a satisfactory basis for interpretations would be gained. Enzyme complexes consisting of few links should be able to consume, during a time unit, much more substrate molecules than do the related complexes having a more complicated enzymic structure. As a result, the consumption of common precursors proceeds with the highest efficiency by the enzyme complexes responsible for the building of simpler flavonoids. This provides a good explanation why just the latter are usually accumulating much more rapidly than the flavonoids having a more complex basic skeleton.

It is rather difficult to explain, however, why the distribution of common precursors between separate flavonoid pathways tends to be changed by altered levels of precursor supply/increased content of flavonoid compounds in the tissue. One might assume that the flavonoid end-products are able to act as weak feedback inhibitors of the relevant enzyme complexes and that the activity of these complexes is dependent upon the concentration of flavonoids in the cells. Hence, according to how the content of a particular class of flavonoids increases in the cells, the catalytic activity of the enzymes involved should become progressively more suppressed, resulting in that the enzymes lose a certain part of their initial capacity of reacting with the substrate. As a consequence, the accumulation rate of these flavonoids should be decreased and the relative

amounts of all flavonoid products in that tissue changed in favour of other related flavonoids whose enzyme complexes remain relatively less suppressed by that time.

The difficulty however is that the bulk of flavonoids formed is obviously rapidly removed from the sites of biosynthesis into vacuoles where these compounds become practically isolated from cell metabolic activities. The mechanism suggested may thus work only provided that certain proportionality exists in the distribution of flavonoids between cytoplasmic and vacuolar compartments of the cell. Whether or not that precondition might be fulfilled normally is not known at present.

In conclusion, much further work is needed to solve the intricate problems related to the quantitative aspects of flavonoid accumulation. It is hoped that the facts and views presented in this paper will facilitate the development of new approaches and arouse broader interest in this field, so far practically unexplored.

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FLAVONOIDIDE AKUMULATSIOONI KVANTITATIIVSED SEADUSPÄRASUSED VEGETATIIVSETES TAIMEKUDEDES

Resümee

Artiklis on antud ülevaade andmetest, mis näitavad, et erinevused eri tüüpi flavonoidide akumulatsiooni kiiruses olenevad nende ühendite põhistruktuuri keerukusest ning biosünteesitee suhtelisest pikkusest. Kui taimekude on võimeline üheaegselt sünteesima mitmeid flavonoidderivaate, siis kõige lihtsamaid neist moodustub tavaliselt kõige rohkem. Keerulisemate vormide biosüntees toimub aeglasemalt, ehituselt kõige komplitseeritumate derivaatide sisaldus aga jääb kõige madalamaks. Sama tüüpi korrelatsioonid ilmnevad ka siis, kui flavonoidide moodustumise intensiivsus vaadeldavas koes mingi modifitseeriva teguri toimel muutub. Lihtsama ehitusega *resp.* kiiremini sünteesitavate derivaatide puhul on muutused tavaliselt absoluutväärtuselt suured, kuid suhtelise ulatuse poolest väikesed. Keerulisema struktuuriga *resp.* raskemini sünteesitavate flavonoidide puhul on pilt teistsugune: absoluutselt võttes jäävad muutused reeglina väikesteks, kuid suhtelises plaanis ulatuvad märksa suuremate väärtusteni, kui seda täheldatakse lihtsama ehitusega flavonoidderivaatide puhul. Oletatakse, et niisuguste seaduspärasuste kujunemise määravad erinevused ühiste lähtesubstraatide jaotumises eri struktuuriga flavonoidide biosünteesiteede vahel.

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КОЛИЧЕСТВЕННАЯ ХАРАКТЕРИСТИКА НАКОПЛЕНИЯ ФЛАВОНОИДОВ В ТКАНЯХ ВЕГЕТАТИВНЫХ ОРГАНОВ РАСТЕНИЙ

Резюме

Рассматриваются экспериментальные данные, свидетельствующие о том, что различия в скорости накопления флавоноидов в вегетативных тканях растений зависят от степени сложности их основной структуры и относительной длины путей их биосинтеза. При одновременном формировании в тканях различных флавоноидов в наибольших количествах обычно синтезируются самые простые производные, менее интенсивно идет биосинтез более сложных форм, а меньше всего накопляются самые сложные по строению флавоноидные вещества. Корреляции такого же характера обнаруживаются и при возникновении изменений в ходе накопления флавоноидов, независимо от того, каким фактором эти изменения вызваны. У более простых форм с относительно коротким путем биосинтеза (быстро аккумулирующие производные) изменения обычно весьма значительные в абсолютном и незначительные в относительном плане. В накоплении же флавоноидов более сложного строения (медленно синтезирующиеся производные) картина иная: изменения, как правило, не очень большие по абсолютным размерам, но весьма существенные процентно, если сравнить с исходным содержанием этих производных до воздействия модифицирующими факторами. Предполагается, что проявление указанных количественных закономерностей определяется различиями в распределении единых предшественников между параллельными путями биосинтеза различных по структуре флавоноидов.

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