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Biosynthesis and Metabolism of Anthraquinone Derivatives

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***Abstract*—**In review the generalized data about biosynthetic routs formation anthraquinone molecules in natural cells. The basic possibilities of various ways of biosynthesis of different quinoid substances are shown.

***Keywords*—**Anthraquinones, biochemical evolution, biosynthesis, metabolism.

I. Introduction

IN elucidating the course of biochemical evolution, it has been shown that the biosynthesis of the same substances in plants and animals occurs in much the same way. This is manifested, most of all, in similar compositions of the primary metabolites, i.e., vitally important compounds: proteins, nucleic acids, fats, carbohydrates, and some intermediate compounds of their biosynthesis.

An enormous number of other natural compounds, including quinones, are secondary metabolites, but this does not mean that these substances are insignificant or useless for the life of plants.

Secondary metabolites have played and are playing a great role in the survival of separate plant species during the progress of evolution and in the interaction of plants with the environment. Thus, the appearance of the polymer lignin at early stages of the life on the Earth allowed water plants to migrate to land and occupy it; the living nature owes the abundance of colours to the pigments of the phenolic type, in particular, to anthocyans; the protective reactions against mechanical injuries or microorganism attacks are also associated with the secondary substances - quinones. Various quinones provide for the process of respiration and the division, growth, and development of cells.

II. Results and Discussion

The biosynthesis of various substances of the secondary origin, along with the photosynthesis, is one of the characteristic features of plant organisms. Only plants and microorganisms are capable of synthesizing substances with aromatic structures. Animals do not possess this property; they obtain all necessary aromatic compounds from food, medicines, and vitamins.

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Various natural quinones: benzo-, naphtho-, benzanthreno-phenanthreno-, and anthraquinones, anthracyclinones, and fused and other quinones also belong to the group of aromatic substances of the secondary origin.

Of the quinones listed above, 9,10-anthraquinones are the most important in the life of plants. They occur in lower plants, algae, fungi, lichens, mosses, and ferns. In higher plants, the quinones are encountered in the form of polyhydroxy derivatives and are found in all parts of woody plants and bushes.

There is no consensus of opinion on the role of anthracene derivatives in plants, but it was established that, for example, hydroxymethyl anthraquinones protect plants against parasites and protect seeds against birds, promote accumulation of polysaccharides, and take part in redox processes.

All the hypotheses about the origin and formation of phenolic compounds and, in particular, hydroxyanthra-quinones in plants reduce to the following five schemes:

1) biosynthesis of aromatic nuclei proceeds simultaneously with the photosynthesis from precursors of saccharides;
2) phenols are formed through condensation of bioses and trioses with other products of saccharide decomposition;
3) phenols are produced directly from hexoses;
4) the biosynthesis of phenols occurs via acetic acid;
5) phenols are obtained from hexoses or aminoacids through shikimic acid [1].

In 1907 Colly proposed and then experimentally confirmed the possibility of obtaining aromatic substances from polyacetic acids by virtue of a «head-to-tail» type condensation [2].

Later, Birch and Donovan have shown that some other natural products can also be formed in this way, the cyclization being possible either at carbon atoms 1-6 to give ketones with a phloroglucinol ring or at carbon atoms 2-7 to give acids. To explain the absence of some OH groups, the suggestion was made that, prior to cyclizing; the molecules of polyacetic acids may be partially reduced. Whereas Colly has synthesized phenolic compounds from acetic acid, Birch has succeeded in obtaining the first experimental data supporting the acetate theory in relation to *Penicillium griseofulvum* cultures [2].

In 1935 Fischer elucidated the role of shikimic and quinic acids in the formation of gallic acid. In W. Margn's opinion, the shikimate pathway implies the participation of L-phenylalanine in the biosynthesis of phenolic compounds [2].

In spite of the common nature of the biosyntheses of phenols, different plants can produce the same compounds through different biosynthetic pathways. It is not accidental

that, along with simple amino acids and carbohydrates, the majority of plants contain phenols, phenolic acids, flavonoids, naphthoquinones, and anthraquinones. The number of the individual compounds, whose biosynthetic pathway can be regarded as proven, constitutes only a small portion of known natural compounds with established structures.

Since the anthraquinones are oxidized aromatic compounds, it would make sense to assume that their biosynthesis is similar to that of other aromatic structures - phenols, phenolic acids, benzo- and naphthoquinones, catechols, flavonoids, chalcones, aromatic amino acids, and so on.

According to numerous studies [3], [4], the origin of naphtha- quinones and anthraquinones, unlike that of, for instance, hydroxycinnamic acids, coumarins, and flavonoids, is based on several biosynthetic pathways. This accounts for the variety of structures of natural anthraquinones. As a rule, to produce the main structure, only one (the principal) biosynthetic pathway is realized, while related compounds are formed during subsequent transformations of the main structure. According to E. Leistner's classification [5], the following biosynthetic pathways are possible: acetate-malonate route; from L-tyrosine via toluhydroquinone; from L-phenylalanine via trans-cinnamic, p-coumarinic, and p-hydroxybenzoic acids; via shikimic, isochorismic, and o-succinylbenzoic acids; via mevalonic acid.

The first and the fourth biosynthetic pathways have been confirmed experimentally; however, virtually all the above-mentioned compounds have been detected among the intermediates, which suggests that a combination of several principal pathways is realized.

According to the acetate theory developed by Colly, Birch, and Donovan [6], which has been accepted as the most probable hypothesis, the acetic acid in plants undergoes condensation giving a polyketomethylene chain.

The polyketomethylene chain may also be produced by condensation of acetate and malonate fragments.

The resulting polyketide chain can bend in different ways, depending on the number of-CH_2-CO units, with the subsequent cyclization or aromatization. The simplest way of cyclization yields derivatives of salicylic acid.

Endocrocin, emodin, and clavorubin are formed via cyclization of an octaacetylpolyketide chain. Such distribution of oxygen-containing functions can serve as a distinctive feature of molecules formed according to this scheme [7].

The formation of quinones and anthraquinones by the polyketide route was confirmed by experiments with incorporation of acetate and malonate fragments labelled by radioactive isotopes, in which the way of bending of a polyketide chain was determined based on the products of cleavage of labelled molecules [7]. When the l-^{14}C-acetate was introduced into plants, the prevailing formation of aloe-emodin was observed, but in the case of introduction of the 2-^{l4}C-acetate, emodin, aloe-emodin, and small amounts of chrysophanic acid were produced [8].

The structural modifications that occur after the formation of an aromatic skeleton including O-methylation, oxidation, hydroxylation, dimerization, glycosidation, etc. can proceed in various combinations, both consecutively and simultaneously.

About half of the anthraquinone compounds in higher plants have substituents in one of the benzene rings, some of them are devoid of a side carbon chain (for example, alizarin) or hydroxy groups (for example, 2-methylanthraquinone). The majority of such compounds have been found in plants of the *Rubiaceae*, *Bignoniaceae*, and *Verbinaceae* families; in the latter two families, anthraquinones and naphthoquinones are represented virtually equally. The production of non-substituted anthraquinone in the *Quebrachia lorentzii*, many *Acacia* species of the *Leguminosae* family, and in the tobacco leaves as well as the formation of 10,10'-bianthrone in the *Ustilago Zeal* culture are the least understood [9].

Analysis of the structural variety of the anthracene glycosides isolated at different stages of plant growth has shown that the O-addition of a carbohydrate substituent is characteristic of the glycosided forms of anthraquinones, while the C-addition is a distinctive property of anthrones and dianthrones. This points to a single predominant pathway of their biosynthesis, although O- and C-glycoside bonds can sometimes be found simultaneously in the same plant and even in the structure of a single compound, for example, in cascaroside A, which is an 8-β-D-glucopyranosyloxy-10-β-D-glucopyranoside of aloe-emodin-9-anthrone.

As a rule, a freshly gathered plant material contains a small amount of aglycones, which increases upon drying and tissue destruction, because the glycosides or the reduced forms of glycosides present in tissues in vivo are easily cleaved to sugars and the corresponding aglycones, indicating that two pathways predominate in the biosynthesis of anthracene glycosides.

Naptho- and anthraquinones differ from other secondary metabolites in that they are accumulated in significant quantities in suspension cultures and callus tissues of plants, for example, in a suspension cell culture of bedstraws, in callus tissues of catalpa, puccoon, foxglove, and so on, anthraquinones being sometimes found only in suspension cell cultures and callus tissues but not in the plant itself or its individual parts. For example, 3-methylalizarin and 3-methylpurpurin have been found only in the callus tissue of the foxglove *Digitalis lanata* [9].

When the freshly gathered buckthorn bark has been studied under conditions ruling out the possibility of oxidation, it has been discovered that both aglycones and glycosides of this plant are present in it in a reduced form. Studies of the same bark under ordinary conditions and of the bark that had been stored for several years revealed only oxidized forms of aglycones and glycosides. Hence, the primary form for the biosynthesis of anthraquinones is an anthrone, that is, the reduced state of tissues in vivo, which then transforms into the oxidized state, slowly or rapidly, depending on the conditions, during storage, drying, and other processes.

This statement was confirmed by investigations of the chemical composition of Chinese rhubarb. Only anthrones were found in freshly gathered seedlings of this plant, dianthrones and anthraquinones were found in the three-month old plants, and glycosided forms of anthrones, dianthrones,

and anthraquinones appeared and accumulated in the two- and three-year old plant; the 8-10 year old plants did not contain any monoanthrones or their glycosided forms, but still did contain anthraquinone glycosides; the amount of anthraquinones and glycosides based on them gradually increased with time [10]. Similar results were observed in a study of tangut rhubarb and various types of buckthorn; investigation of *Gupericum luttiferae* experimentally demonstrated the possibility of formation of various types of bonds between the emodin-anthrone monomeric fragments during their oxidation.

In higher plants, the dimers are apparently not primary products, but in the metabolic products of moulds and lower fungi, the dimers are present initially being formed by condensation of acetate fragments followed by reduction into dimers of the rubroskirin, penicilliopsin, and other types [11], i.e., via the ordinary acetate pathway discussed above, which is realized up to dimeric forms. This fact suggests that in some families of plants, the anthrones are also present as dimers, whereas bonding of various types is a secondary process or a result of several parallel or consecutive pathways accomplished not only in different plants, but in different parts of the same plant as well [2].

For example, the biosynthesis of pachibasin, a metabolite of *Phoma fovkata*, can occur in two ways: by the acetate-malonate route and through mevalonic acid. This is also true for the biosynthesis of chrysophanol in *Rumex alpinus*, *Rhamnus frangulae*, and *Rumex obthusifolia* [9], but in *Rheum rhaponticum* only the mevalonate pathway for the biosynthesis of a chrysophanol was proved.

As noted above, the different structures of the carbon skeleton formed may be attributed to alternative ways of bending of the main polyketide chain prior to the cyclization, as in the case of a pigment of lichens - solorinic acid, versicolorins A and B, and rhodocomatulins. The presence of functional groups and their arrangement are determined by the sequence of alkylation, dehydration, oxidation, condensation, hydroxylation, and glycosidation reactions. For example, in the structures of nalgiovensin and ptilometric acid, the p-methyl group is replaced by a propyl substituent upon condensation of C_{18}-polyketide, whereas madagascin is an example of a prenylation of the main structure to the β-position.

It is noteworthy that madagascin is encountered in the plants together with its 9-anthrone and polyprenylated analogues [11].

The isolation from *Cassia torosa* of a torosachrysone-8-O-β-D-gentiobioside, having a tetrahydroanthracene structure, instead of expected physcion-8-O-β-D-gentiobioside [3], can serve as evidence for dehydration and oxidation occurring during the period of seed maturation; this points to the biosynthetic relationship between tetrahydroanthracenes and anthraquinones in plants.

In a study of 19 species of sorrel, it has been found that all of them contained the chrysophanol, emodin, and physcion triad, their highest content being found in roots. Aloe-emodin and rhein have been found in the fruits, green mass, and flowers of some species of sorrel. Such a set of compounds and the place of their localization are evidence supporting the acetate-malonate scheme of biosynthesis, according to which emodin and its anthrone are formed primarily, whereas the other anthraquinones are produced from them upon oxidation of the β-methyl group or upon alkylation and hydroxylation, for example, $CH_3 \rightarrow CH_2OH \rightarrow CHO \rightarrow COOH$ (the transition from chrysophanol to aloe-emodin, emodinic aldehyde, fallasinal, rhein, emodinic acid) or $OH \rightarrow OCH_3$ (from emodin to physcion).

Similar transitions and interconversions, apparently, occur as well in lichens in which physcion and paric tic acid, resulting from its oxidation, have been found; however, many types of lichens contain intermediate products of this oxidation chain: fallasinol and fallasinal. Thus, it has been established that in fungi and animal organisms, quinones are mainly formed via the polyketide route, whereas in higher plants they can result from several biosynthetic pathways [12].

Emodin has not been found in lichens until recently, when not only this compound but also the above-mentioned products formed via the biosynthetic chain ($CH_3 \rightarrow CH_2OH \rightarrow CHO \rightarrow COOH$), namely, citreorosein and emodinic acid, were detected in *X. parietina*; this means that the oxidation occurs both as hydroxylation of the rings and as oxidation of the methyl group [7].

Nevertheless, by the present time, the islandicin and cynodontin have not yet been found in lichens; they are known only as the products of mould metabolism [13].

The results of a number of works, in which labelled ^{14}C-precursors (l-^{14}C-acetate, 2-^{l4}C-acetate, and 2-^{l4}C-malonic, 2-^{14}C-mevalonic, 7-^{l4}C-shikimic, and 1,2-^{l4}C- shikimic acids) have been introduced into the *Rhamnus frangula* and *Rumex alpinus* folios, and the resulting anthraquinones have been isolated and cleaved, provided evidence for the advantage of the mixed acetate-malonate pathway for the biosyntheses of emodin, chrysophanol, alizarin, and morindone [13]. Analogous results have been obtained for *Rumex obthusiafolia*, *Aloe saponaria*, and *Eriococus coriaceas* [4].

The second principal pathway of the biosynthesis of aromatic compounds, in particular anthraquinones, is connected with the formation of shikimic acid. The initial substances in the shikimate route are products of carbohydrate metabolism, phosphoenol pyruvate (PEP) and erythroso 4-phosphate, the condensation of which with participation of DAP-synthetase yields 3-dehydroxy-D-arabinoheptulosonate 7-phosphate (DAHP) and then 3-dehydroquinic and shikimic acids.

The latter undergoes phosphorylation to give shikimate 3-phosphate, which condenses with PEP affording 5-enolpyruvylshikimate 3-phosphate (EPSP), with participation of EPSP-synthetase, and then chorismic acid.

The chorismic acid corresponds to a point of branching of biosynthetic processes; it can be converted into prephenic acid, which is the basis for producing the majority of natural compounds including anthraquinones [14].

Quinones can be formed both via prephenic acid and via shikimic and chorismic acids.

Since naphtho- and anthraquinones are encountered in the same plants of numerous families, it has been suggested that naphthoquinones and naphthols are either biogenetic precursors of anthraquinones or intermediate products in their biosynthesis [14].

After that, prenylation occurs with participation of a dimethylallyl phosphate, resulting in the formation of l,4-dihydroxy-3-prenyl-2-naphthoic acid; subsequent cyclization of the prenyl fragment gives rise to ring C of anthraquinone. This process was proved with labelled precursors in *Rubia tinctorium* [15] and other plants of the *Rubiaceae* family; at some stages of plant growth, a combination of routes 1 and II is possible.

I: shikimic acid → chorismic acid → prephenic acid → o-succinylbenzoic acid → γ,γ-dimethylallyl phosphate;

II: glutamic acid → α-ketoglutaric acid → l,4-dihydroxy-2-naphthoic acid [13].

This is also evidenced by the results of cleavage of alizarin and its analogues substituted in ring C; the C-9 atom may result from involvement of shikimic acid. Investigations of Sunderman, Zenk, and Leistner confirmed the pathway to rings A and B in alizarin by using ^{14}C-1,4-naphthoquinone [15].

Alizarin, morindone, islandicin, and purpurincarboxylic acid can also be obtained from shikimic, glutamic, and mevalonic acids through the intermediate formation of succinylbenzoic acid and the naphthalene skeleton. Whereas 19 anthraquinones of *Rubia tinctorium* are substituted in only one ring C, which confirms the common route for their biosynthesis, *Morinda* also contains anthraquinones substituted in both rings (A and C), and this allows one to suggest either the combination of biosynthetic pathways or the formation of anthraquinones through naphthol compounds.

The biosynthesis of anthraquinones in the plants of *Rubiaceae*, *Bignoniaceae* and *Verbenaceae* families is most probably similar to the syntheses of naphthoquinones and anthraquinones in which rings A and B are formed from shikimic acid [1].

The enzymology of the anthraquinone biosynthesis in plants has also been studied insufficiently; however, certain advances have been made allowing one to determine the preferential places of localization of particular compounds in individual organs, tissues, and cells in plants and also to establish the enzymes participating in their formation. Thus, from a suspension cell culture of a bedstraw (G.Mollugo), an enzyme preparation has been isolated, which catalyzes the formation of the Co-A ester from o-succinylbenzoic acid at pH 7.2 in the presence of ATP and Mg^{2+}. For cultivated cells of cinchona (*Cinchona succirubra*), five anthraquinone-specific glycosyl transferases have been described; they had equal molecular weights and the same optimum pH; however these glycosyl transferases were found to glycoside not only anthraquinone molecules, but also some flavonoids and hydroxybenzoic acids, although emodin, anthrapurpurin, quinizarin, and 2,6- and 1,8-dihydroxyanthraquinones are their preferred substrates [16].

The presence of two intermediates and the quantitative transformation of sennoside A to glycosylrheinanthrone may be indicative of the possibility for the sennoside A to transform, depending on the conditions, into sennidine A, sennidinyl monoglycoside, or rhein, due to the easy oxidation of rhein anthrone.

Until recently, anthraquinones have been considered to be the final products of metabolism because they are accumulated by some organisms in relatively large amounts. But in some fungi they undergo further changes. For example, the pigments of ergot contain antraquinones and ergochromones resulting from cleavage of the central ring giving o-benzoylbenzoic acids and subsequent cyclization to xanthones and their dimerization products. Hence, ergochromones can be regarded as secondary products of the anthraquinone metabolism [2], [9].

Studies of other glycosides (frangulins A and B; glycofrangulins A and B) and comparison of the conditions of reactions of α- and β-glycosidases with the identical glycosides showed that each particular case requires strictly specific experimental conditions and enzymes, which should be specially selected.

The complexity and labour intensity of these experiments accounts for the small number of studies devoted to metabolism in general and, in particular, to that of anthraquinone derivatives.

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