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BIOSYNTHESIS OF PHYTOQUINONES: AN OUTLINE OF THE BIOSYNTHETIC SEQUENCES INVOLVED IN TERPENOID QUINONE AND CHROMANOL FORMATION BY HIGHER PLANTS

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Previously we have reported on the incorporation in maize shoots of [2-^{14}C]mevalonic acid (Threlfall, Griffiths & Goodwin, 1967), [methyl-^{14}C,^{3}H$_3$]-methionine (Threlfall, Whistance & Goodwin, 1967), [G-^{14}C]shikimic acid and p-hydroxy[U-^{14}C]benzoic acid (Whistance, Threlfall & Goodwin, 1966), and L-[U-^{14}C]tyrosine and L-[U-^{14}C]phenylalanine (Whistance, Threlfall & Goodwin, 1967) into phylloquinone, plastoquinone, α-tocopherol, γ-tocopherol, α-tocopherol quinone and ubiquinone (see Scheme I for the chemical structures of these compounds). The results showed that (a) shikimic acid is incorporated into the nuclei of all terpenoid quinones and chromanols examined, (b) the aromatic portions of phenylalanine and tyrosine can give rise to the nuclei of plastoquinone, α-tocopherol, γ-tocopherol, α-tocopherol quinone and ubiquinone, (c) p-hydroxybenzoic acid is incorporated only into the ubiquinone nucleus, (d) the methyl group of methionine is transferred intact to give the ring methyl of phylloquinone, the ring methyl and O-methyls of ubiquinone and one or more ring methyls of plastoquinone, α-tocopherol, γ-tocopherol and α-tocopherol quinone and (e) the prenyl portions of all terpenoid quinones and chromanols are formed from mevalonic acid.

We now present evidence which shows that in maize (Zea mays) shoots and ivy (Hedera helix) leaves the nucleus and one nuclear methyl group of plastoquinone, α-tocopherol, γ-tocopherol, δ-tocopherol and α-tocopherol quinone can be formed from the aromatic ring and β-C atom, respectively, of either phenylalanine or tyrosine. In the case of ubiquinone only the aromatic ring of the amino acids is used in the synthesis of the quinone nucleus, the nuclear methyl group coming from methionine. On the basis of these and previous findings, and from a knowledge of aromatic amino acid metabolism, a scheme is proposed to account for the biosynthesis of terpenoid quinones and chromanols by photosynthetic tissues of higher plants.

EXPERIMENTAL AND RESULTS

In the first series of experiments (I-V) etiolated maize shoots were excised and exposed for 24 hr. with continuous illumination to either [β-^{14}C]-phenylalanine, [ring-^{14}C]phenylalanine, [α-^{14}C]tyrosine, [β-^{14}C]tyrosine or [β-^{14}C]cinnamic acid. At the end of this period the shoots were examined by our usual procedures (Whistance, Threlfall & Goodwin, 1967) for the presence of ^{14}C activity in phylloquinone (K), plastoquinone (PQ), α-tocopherol (α-T), γ-tocopherol (γ-T), α-tocopherol quinone (α-TQ), ubiquinone (Q), squalene, 3β-hydroxysterols, β-carotene and fatty acids. The significance and distribution of ^{14}C radioactivity in the quinone and chromanol molecules was determined by: (a) Comparison with the ^{14}C radioactivity in other terpenoid and non-terpenoid components examined. (b) By degradation of representative molecules, usually plastoquinone, ubiquinone and a tocopherol. The ^{14}C radioactivity in the side chain was determined by ozonolytic degradation (Whistance, Threlfall & Goodwin, 1967) and that in the ring methyls by Kuhn-Roth oxidation coupled with a Schmidt degradation (Phares, 1951). Table I summarises the results obtained.

In the second series of experiments excised ivy leaves were incubated with either [methyl-^{14}C]methionine (Expt. VI) or [β-^{14}C]tyrosine (Expt. VII). The choice of this tissue was governed by the fact that it was the only readily available source of δ-tocopherol. The substrate was dissolved in water containing a few drops of Tween 80 and painted on the leaf surfaces. After 6 hr. incubation in the light the quinones and chromanols were isolated and examined for ^{14}C radioactivity. Plastoquinone, α-tocopherol, γ-tocopherol and α-tocopherol quinone were labelled from both substrates. Ubiquinone was labelled only from [methyl-^{14}C]methionine and δ-tocopherol only from [β-^{14}C]tyrosine. Degradation studies showed that [β-^{14}C]tyrosine had only labelled the ring methyl groups.

Table I

Expt. No.	Substrate	Compounds labelled[*]	Intramolecular distribution of ^{14}C
I	DL-[ring-^{14}C]phenylalanine	PQ,α-T,γ-T,α-TQ,Q-9	Nucleus
II	DL-[β-^{14}C]phenylalanine	PQ,α-T,γ-T,α-TQ	Ring methyl
III	DL-[β-^{14}C]tyrosine[†]	PQ,γ-T,α-TQ	Ring methyl
IV	DL-[α-^{14}C]tyrosine[†][‡]	All compounds	Prenyl portion
V	[β-^{14}C]cinnamic acid[§]	None	

[*] Compounds examined were K, PQ, α-T, γ-T, α-TQ, Q, β-carotene, squalene and 3β-hydroxysterols.

[†] In incubations with tyrosine α-T was never detected.

[‡] Only in the case of [α-^{14}C]tyrosine was there significant ^{14}C radioactivity in the 3β-hydroxysterols, squalene and β-carotene when compared with the phytoquinones and chromanols.

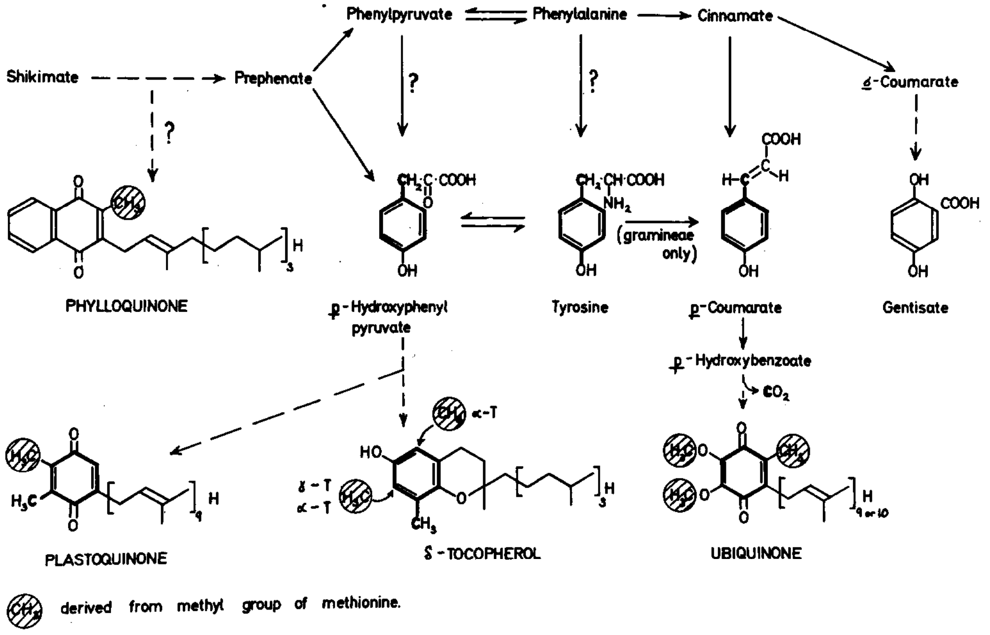
[§] C_6-C_3 and C_6-C_1 phenolic acids were labelled from this substrate

DISCUSSION

Experiments II, III and IV show that not only are the aromatic portions of phenylalanine (Expt. I) and tyrosine (Whistance, Threlfall & Goodwin, 1967) incorporated into the nuclei of plastoquinone, α-tocopherol, γ-tocopherol, α-tocopherol quinone and ubiquinone in maize shoots, but also that the β-carbon atoms give rise to a ring methyl group in each of the first four compounds. That this involves the <u>in toto</u> incorporation of a $C_6 - C_1$ unit is shown by the highly specific incorporation of ^{14}C radioactivity from [β-^{14}C]-tyrosine and [β-^{14}C]phenylalanine into the ring methyl groups of these compounds and also by the demonstration that in ivy leaves δ-tocopherol is labelled from [β-^{14}C]tyrosine but not from [methyl-^{14}C]methionine (Expts. VI and VII). As to which methyl groups of plastoquinone, α-tocopherol, γ-tocopherol or α-tocopherol quinone are labelled from the [β-^{14}C] aromatic amino acids, we would suggest on the basis of our incorporation of ^{14}C radioactivity from [β-^{14}C]-tyrosine into δ-tocopherol and the failure to find in nature monomethyl tocopherols or tocotrienols other than the 8-methyl forms, that in the case of γ-tocopherol, α-tocopherol, α-tocopherol quinone, and probably also plastoquinone, the β-C atoms of the amino acids will give rise to the methyl groups <u>meta</u> to the prenyl side chains. If δ-tocopherol, γ-tocopherol, α-tocopherol and α-tocopherol quinone are biosynthetically related then this is obviously the case.

The demonstration that ^{14}C radioactivity from [β-^{14}C]cinnamic acid is not incorporated into plastoquinone, γ-tocopherol, α-tocopherol and α-tocopherol quinone shows that the C_6 - C_1 unit incorporated into these compounds is not derived from any of the many C_6 - C_3 or C_6 - C_1 phenolic acids; for example gentisic acid (Scheme I); formed from the deamination products of either phenylalanine (cinnamic acid) or tyrosine (p-coumaric acid).

Scheme I shows the interpretation we place on the results obtained and provides a framework to account for the biosynthesis of all terpenoid

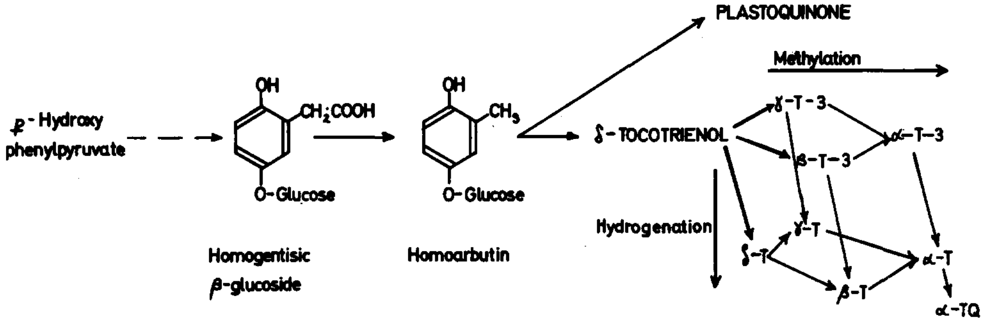


quinones and chromanols found in higher plant tissues. In the scheme we show p-hydroxyphenylpyruvate rather than tyrosine, for reasons which will become apparent shortly, as the key intermediate in plastoquinone, tocopherol and α-tocopherol quinone biosynthesis.

Threlfall (1967) has already discussed in detail the problems to be considered in phytoquinone and chromanol biosynthesis. We will only concern ourselves here with the broader implications of our proposals.

The major points emerging from Scheme I are: [i] The nuclei of plastoquinone, α-tocopherol, γ-tocopherol and α-tocopherol quinone, all of which are chloroplast components, arise from compounds which are also precursors of phenylalanine and tyrosine. In order to account for the incorporation of phenylalanine we are led to propose that this amino acid (or its α-keto acid) is converted to tyrosine (or its α-keto acid). A reaction which has not been demonstrated in plant tissues. [ii] The nucleus of ubiquinone, an extraplastidic component, is formed from p-hydroxybenzoic acid produced as a result of metabolism of phenylalanine and tyrosine (Gramineae only). The C atom(s) of the side chains of these acids make no contribution to the quinone molecule, i.e., the carboxyl carbon of p-hydroxybenzoic acid is not incorporated into the molecule. [iii] Since no ring substrate other than shikimic acid is incorporated into phylloquinone, the naphthoquinone ring probably arises early on in the shikimic acid pathway.

The route by which tyrosine (or its α-keto acid) is incorporated into plastoquinone, tocopherols and α-tocopherol quinone is still to be determined. However, from the orientations of the β-C atom and oxygen function(s) in the parent molecule (<u>para</u>) and products (<u>meta</u>) it is necessary to visualise an intramolecular shift involving one or other of these groups. A possible sequence which implicates compounds known to occur in plant tissues is: p-hydroxyphenylpyruvate ⟶ homogentisic acid (presumably as its glucoside) ⟶ homoarbutin ⟶ plastoquinone, tocopherols and α-tocopherol quinone (Scheme 2).



T-3 = TOCOTRIENOL : T = TOCOPHEROL

The first stage from p-hydroxyphenylpyruvate to homogentisic would proceed in a similar manner to that found in animal tissues, i.e., hydroxylation of the aromatic ring, shift of the side chain, and the formation of CO_2. Nonaprenylation, methylation and deglycosylation of homoarbutin would give plastoquinone. Tetraprenylation, cyclisation and deglycosylation would give δ-tocotrienol, a compound which theoretically can be regarded as the parent member to both the tocopherol and tocotrienol series. δ-Tocotrienol could then by suitable hydrogenation and methylation reactions form all members of the tocopherol and tocotrienol series. Evidence for sequential methylation comes from the finding that the specific activities of tocopherols in maize and ivy labelled from [β-^{14}C]tyrosine decrease in the order γ- and α-. δ-Tocopherol in ivy unexpectedly showed a lower specific activity than γ-; although it was still higher than that of α-. This indicates that the occurrence of non-alpha tocopherols in these tissues is possibly a consequence of side reactions rather than the accumulation of intermediates. These results, coupled with the fact that in nature all members of the tocopherol and tocotrienol series have been shown to occur, suggests that this group of compounds constitute a 'metabolic grid', the biosynthetic route to α-tocopherol depending on the plant species examined. α-Tocopherol quinone on the basis of specific activity data is probably formed by oxidation of α-tocopherol.

The intermediates and steps involved in phylloquinone and ubiquinone formation are no less obscure. Although it appears that 2-nonaprenylphenols and 2-nonaprenyl-6-methoxyphenols; compounds the decaprenyl forms of which are known to be ubiquinone-10 precursors in *Rhodospirillum rubrum* (Friis, Daves & Folkers, 1966), are not involved in the biosynthesis of ubiquinone-9 by maize tissues (Whistance, Threlfall & Goodwin, 1967).

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