Biochem. J. (1968) **109**, 482
Printed in Great Britain

Biosynthesis of Phytoquinones: Utilization of Homogentisic Acid by Maize Shoots for the Biosynthesis of Plastoquinone

By G. R. WHISTANCE and D. R. THRELFALL
Department of Biochemistry and Agricultural Biochemistry, University College of Wales, Aberystwyth

(*Received* 12 *July* 1968)

Whistance & Threlfall (1967) demonstrated that the aromatic carbon atoms and β-carbon atom of exogenous tyrosine can be utilized by maize shoots for the biosynthesis of the nucleus and one nuclear methyl group respectively of the following compounds: plastoquinone, γ-tocopherol, α-tocopherol and α-tocopherolquinone. Threlfall, Whistance & Goodwin (1968) have shown that the remaining nuclear methyl groups of these compounds are formed by the transfer of intact methyl groups from methionine.

On the basis of the observed incorporation of the β-carbon atom of exogenous tyrosine into plastoquinone, tocopherols and α-tocopherolquinone, it was postulated that one of the normal biosynthetic steps involved in the formation of these quinones and chromanols must be an intramolecular rearrangement of *p*-hydroxyphenylpyruvic acid (Whistance & Threlfall, 1967). This postulate, when considered in conjunction with reports of the occurrence in higher-plant tissues of homogentisic acid (Bertel, 1903) and homoarbutin (Inoue, Arai & Takano, 1958), led to the proposal that in higher plants these compounds might be synthesized by the following pathway: prephenic acid→*p*-hydroxyphenylpyruvic acid→homogentisic acid (possibly as its β-glucoside)→homoarbutin (β-glucoside of toluquinol)→plastoquinone, tocopherols and α-tocopherolquinone (Whistance & Threlfall, 1967).

In this communication we present direct evidence to support the proposal that homogentisic acid is an intermediate in the biosynthesis of plastoquinone in maize shoots.

Experimental and results. [U-^{14}C]Homogentisic acid and [α-^{14}C]homogentisic acid were synthesized enzymically by incubating *p*-hydroxy[U-^{14}C]-phenylpyruvic acid and *p*-hydroxyphenyl[β-^{14}C]-pyruvic acid respectively with αα′-bipyridyl-inhibited rat liver homogenates. The procedure followed was similar to that described by LaDuc & Zannoni (1955). After their isolation from the incubation medium, the [^{14}C]homogentisic acid species were purified by repeated chromatography on thin layers of Kieselgel G developed with benzene–methanol–acetic acid (45:8:4, by vol.) (R_F 0·35).

Etiolated 7-day-old maize shoots (var. Rhodesian White Horse Tooth) (200/experiment) were excised at the node and the cut ends dipped into water (50ml./100 shoots) containing 5μc of the appropriate [^{14}C]homogentisic acid species together with ascorbic acid (25mg./100 shoots) (required to keep the homogentisic acid reduced). The shoots were then exposed to continuous illumination (300 lumens/ft.^{2}) for 24hr. At the end of this period the compounds under investigation were isolated from the shoots and assayed for radioactivity by our usual procedures (Whistance, Threlfall & Goodwin, 1967). Table 1 summarizes the results obtained for the incorporation of radioactivity from [U-^{14}C]-homogentisic acid and [α-^{14}C]homogentisic acid into plastoquinone, β-carotene, 3β-hydroxy sterols and fatty acids.

It was established by routine degradation procedures (Whistance & Threlfall, 1968) that in plastoquinone labelled from [U-^{14}C]homogentisic acid the radioactivity was almost totally confined to, and uniformly distributed between, the carbon atoms of the *p*-benzoquinone nucleus and one of the nuclear methyl groups. The radioactivity in the plastoquinone labelled from [α-^{14}C]homogentisic acid was located almost entirely in one of the nuclear methyl groups.

Discussion. The results obtained in these experiments provide conclusive evidence that in maize shoots the *p*-benzoquinone nucleus and one nuclear methyl group of plastoquinone can be derived from the aromatic carbon atoms and α-carbon atom respectively of homogentisic acid. In accordance with our previous proposals (Whistance & Threlfall, 1967) it is suggested that the α-carbon atom of homogentisic acid will give rise to the nuclear methyl group of plastoquinone *meta* to the nonaprenyl side chain.

All the available experimental evidence suggests that in maize shoots homogentisic acid is formed directly from *p*-hydroxyphenylpyruvic acid. Thus the specific radioactivity of plastoquinone (121600counts/min./μmole) isolated from maize shoots that had been incubated with [α-^{14}C]-homogentisic acid (Table 1) is found to be some 30 times the specific radioactivity of plastoquinone (4190 counts/min./μmole) isolated from maize shoots incubated under the same conditions with

Table 1. *Incorporation of* [U-^{14}C]*homogentisic acid and* [α-^{14}C]*homogentisic acid into plastoquinone, β-carotene, 3β-hydroxy sterols and fatty acids*

Etiolated 7-day-old maize shoots were excised and exposed with continuous illumination to the appropriate ^{14}C-labelled homogentisic acid species for 24 hr.

	Specific radioactivity (counts/min./μmole)	
Terpenoid	[U-^{14}C]Homogentisic acid (4·9 mc/m-mole): 200 shoots incubated with 5 μc	[α-^{14}C]Homogentisic acid (6·85 mc/m-mole): 200 shoots incubated with 5 μc
Plastoquinone	89700	121600
β-Carotene	450	615
3β-Hydroxy sterols	390	1724
Fatty acids*	0	0

* Obtained by saponification of sterol esters.

p-hydroxyphenyl[β-^{14}C]pyruvic acid (Threlfall & Whistance, 1968). In addition, on administration of unlabelled homogentisic acid to maize shoots metabolizing *p*-hydroxyphenyl[β-^{14}C]pyruvic acid, the incorporation of radioactivity into plastoquinone is markedly decreased (D. R. Threlfall & G. R. Whistance, unpublished work).

The route by which homogentisic acid is incorporated into plastoquinone remains to be determined. However, the proposal that homoarbutin is a key intermediate in this pathway (Whistance & Threlfall, 1967) would, in the light of more recent experimental evidence (Whistance & Threlfall, 1968), appear to be incorrect. Indeed, the results obtained from isotope competition experiments in which a range of phenolic compounds were administered to maize shoots metabolizing [β-^{14}C]tyrosine (Whistance & Threlfall, 1968) lead us to suggest that consideration should be given to the possibility that C_6–C_1 compounds are not involved in the biosynthesis of plastoquinone.

The finding that radioactivity from [U-^{14}C]-homogentisic acid and [α-^{14}C]homogentisic acid is incorporated into β-carotene and 3β-hydroxy sterols (Table 1) indicates that in higher-plant tissues ring-opening of homogentisic acid can occur. The fact that radioactivity is incorporated into terpenoids but not into fatty acids suggests that, as in animal tissues (Meister, 1965), ring-opening of homogentisic acid gives rise to acetoacetic acid and fumaric acid, the former compound being an effective biosynthetic precursor of terpenoids but not of fatty acids.

Although in this communication we have only concerned ourselves with studies on the biosynthesis of plastoquinone, preliminary observations have indicated that in maize shoots homogentisic acid, as predicted (Whistance & Threlfall, 1967), is also involved in the formation of α-tocopherol, γ-tocopherol and α-tocopherolquinone.

This work was supported by the Science Research Council. G. R. W. was in receipt of an Agricultural Research Council Scholarship. We thank Miss Marian E. Williams for technical assistance.

Bertel, R. (1903). *Chem. Zbl.* **1**, 178.
Inoue, H., Arai, T. & Takano, Y. (1958). *Chem. pharm. Bull., Tokyo*, **6**, 653.
LaDuc, B. N. & Zannoni, V. G. (1955). *J. biol. Chem.* **217**, 777.
Meister, A. (1965). In *Biochemistry of Amino Acids*, vol. 2, p. 894. New York and London: Academic Press Inc.
Threlfall, D. R. & Whistance, G. R. (1968). *Biochem. J.* **108**, 24 P.
Threlfall, D. R., Whistance, G. R. & Goodwin, T. W. (1968). *Biochem. J.* **106**, 107.
Whistance, G. R. & Threlfall, D. R. (1967). *Biochem. biophys. Res. Commun.* **28**, 295.
Whistance, G. R. & Threlfall, D. R. (1968). *Biochem. J.* (in the Press).
Whistance, G. R., Threlfall, D. R. & Goodwin, T. W. (1967). *Biochem. J.* **105**, 145.