NAPHTHOQUINONE BIOSYNTHESIS IN HIGHER PLANTS

II. p-HYDROXYBENZOIC ACID AS A PRECURSOR OF 2-HYDROXY 1,4-NAPHTHOQUINONE IN IMPATIENS BALSAMINA L.

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Shikimic acid has been shown to be an excellent precursor of 2-hydroxy-1,4-naphthoquinone in Impatiens balsamina L. (Chen and Bohm, 1966). Label from shikimic acid-U.L.-C^{14} was found exclusively in the quinone portion of the molecule. An excess of unlabeled p-hydroxybenzoic acid administered along with labeled shikimic acid blocked the incorporation of label into 2-hydroxy-1,4-naphthoquinone. This involvement of p-hydroxybenzoic acid is consistent with the observation that it or the corresponding aldehyde is a precursor of quinone rings in microorganisms (Rudney and Parson, 1963; Gibson and Cox, 1964; Whistance et al., 1966) and in Euglena gracilis (Powls and Hemming, 1966). This present communication records the observation that p-hydroxybenzaldehyde-U.L.-C^{14} and p-hydroxybenzoic acid-U.L.-C^{14} serve as precursors of 2-hydroxy-1,4-naphthoquinone in aerial portions of Impatiens balsamina L. This is the first report of such involvement in a vascular plant.

MATERIAL AND METHODS: Feeding experiments were performed on 6 to 7 week old Impatiens balsamina L. (Balsaminaceae) plants. The labeled precursors were administered through cut stems; the plants were allowed to metabolize for a total of 24 hours. Details of the isolation and purification procedures are to be found in our first report (Chen and Bohm, 1966). Shikimic acid-U.L.-C^{14} was purchased from New England Nuclear Corp. Hydroquinone-2,3,5,6-C^{14} was prepared by the sodium dithionate reduction of p-benzoquinone-2,3,5,6-C^{14} which

was a generous gift of Prof. G.H.N. Towers. p-Hydroxybenzaldehyde-U.L.-C^{14} and p-hydroxybenzoic acid-U.L.-C^{14} were prepared simultaneously by the alkaline nitrobenzene oxidation of tyrosine-U.L.-C^{14} which was obtained from New England Nuclear Corp. The compounds were separated by paper chromatography and purified to constant specific activity by crystallization with the corresponding unlabeled compound. The precursors were administered in pH 7.4 phosphate buffer; all label was absorbed during the metabolism period. The concentration of precursors administered in each case was 2 μmole.

RESULTS AND DISCUSSION: The results of the feeding experiments are presented in Table I. It is clear from examining these results that p-hydroxybenzoic acid is an efficient precursor of 2-hydroxy-1,4-naphthoquinone. The corresponding aldehyde is also a good precursor although somewhat less so than the acid. It is essential to point out that the concentration of naphthoquinone obtained in the shikimic acid feeding experiment is unusally high. A large concentration of product would have the effect of making the specific activity value small and the dilution value large. Had the concentration of naphthoquinone in this feeding experimont been 0.12 μmole rather than 1.21 μmoles the dilution value for this precursor would have been approximately 200. p-Hydroxybenzoic acid and p-hydroxybenzaldehyde would still have been more efficient precursors than shikimic acid. The following incomplete sequence would then appear to exist in I. balsamina: shikimic acid → p-hydroxybenzoic acid → 2-hydroxy-1,4-naphthoquinone. This is in agreement with the biosynthesis of the various quinone molecules already described in microorganisms.

Based upon the efficiency of incorporation of label from p-hydroxybenzoic acid observed in the current work and the prior observation of the inhibitory effect of the acid on the incorporation of shikimic acid into the naphthoquinone it seems highly unlikely that the p-hydroxybenzoic acid molecule would significantly label anything but the quinone ring of the naphthoquinone.

TABLE I

Results of Precursor Feeding Experiments with

Impatiens balsamina L.

	Precursor			Naphthoquinone			
	Name	Conc. Fed.[1]	Sp. Act.[2]	Conc. Isol.[1]	Activity[3]	Sp. Act.[2]	Dilution[4]
1.	Shikimic Acid-U.L.-C^{14}	2.0	1.68	1.21	1554	7.0×10^{-4}	2,400
2.	p-Hydroxybenzoic Acid-U.L.-C^{14}	2.0	0.059	0.184	6680	3.0×10^{-3}	20
3.	p-Hydroxybenzaldehyde-U.L.-C^{14}	2.0	0.13	0.084	4520	2.02×10^{-3}	64
4.	Hydroquinone-2,3,4,6-C^{14}	2.0	1.54	0.425	235	1.06×10^{-4}	14,500

1. Expressed as micromoles.

2. Expressed as microcuries per micromole.

3. Expressed as disintegrations per minute per micromole.

4. Ratio of specific activity of precursor to specific activity of product.

An attempt to check this point was made by degradation of the naphthoquinone to phthalic acid. A combination of low specific activity of the diluted naphthoquinone and low yields of recrystallized phthalic acid prevented reliable results from being obtained. The synthesis of p-hydroxybenzoic acid with high specific activity is currently underway so that this point can be studied.

It was suggested by Zenk (1964) that p-hydroxybenzoic acid underwent oxidative decarboxylation in its efficient conversion to hydroquinone in Bergenia crassifolia. Oxidative decarboxylation has also been discussed by Parson and Rudney (1965) as a step in the conversion of p-hydroxybenzoic acid to the aromatic ring in ubiquinone and rhodoquinone in Rhodospirillum rubrum. Hydroquinone-C^{14} was synthesized in the present work and examined as a possible precursor. Table I shows that this compound was by far the poorest precursor examined with an incorporation some 725 times poorer than p-hydroxybenzoic acid. Thus, free hydroquinone is not likely to be involved in the formation of the naphthoquinone. The possibility that hydroquinone is involved but only as an enzyme bound intermediate must be considered as must the possibility that, in the type of precursor administration employed here, the compound may not be transported to the site of synthesis of the naphthoquinone molecule. The metabolism of p-hydroxybenzoic acid and hydroquinone by Impatiens balsamina plants is under investigation.

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