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THE CHEMICAL DIVERSITY OF THE PLASTOCHROMANOLS

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Abstract—Two types of plastochromanols have been isolated and identified from leaf tissue. One type has a tertiary hydroxyl group in the isoprenoid side-chain and may be derived from plastoquinone C while the other has an esterified secondary hydroxyl function as found in plastoquinone B. These have been referred to as plastochromanol C and B respectively. In contrast to plastochromanol itself they are only found in photosynthetic tissue where they are present in only minor quantities compared with plastoquinone. These new plastochromanols are considered to be products of plastoquinone metabolism.

INTRODUCTION

Since the discovery of plastoquinone-9 (I), 2,3-dimethyl-5-nonaprenyl-1,4-benzoquinone, by Kofler[1] and Crane,[2] several related compounds have been reported. Plastoquinone-B and plastoquinone-C were described from spinach chloroplasts[3] and were later shown in each case to be mixtures of not less than six components.[4] Subsequent work by several groups[5–7] showed that the side-chain was modified to contain a hydroxyl group (plastoquinone-Cs) or an ester group (plastoquinone-Bs).

Recently Wallwork and Pennock[8] proposed a scheme for the structure and possible interrelationships of the plastoquinones. This involves epoxidation of the plastoquinone-9 side-chain, followed by isomerization to give both secondary and tertiary alcohols (plastoquinone-Cs) and then esterification of the secondary alcohols leads to the plastoquinone-Bs.

In addition to the side-chain modifications of platoquinone-9 to produce plastoquinone-C and plastoquinone-B a reduced cyclized derivative of plastoquinone-9 has been found in nature.[9] This compound, plastochromanol-8 (II), was isolated originally from *Hevea brasiliensis* and is found widely in plant tissues. This paper reports the isolation and characterization from green tissues of plastochromanols having side-chain substituents similar to those in the plastoquinones.

RESULTS

During an investigation into the lipids of the privet *Ligustrum vulgare* (Oleaceae), two previously undescribed reducing compounds were detected. After separation of the lipids by

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[1] M. Kofler, in *Festschrift fur E. C. Barell*, p. 199, F. Hoffmann-La Roche Basel (1946).

[2] F. L. Crane, *Plant Physiol.* **34**, 546 (1959).

[3] L. P. Kegel, M. D. Henninger and F. L. Crane, *Biochem. Biophys. Res. Commun.* **8**, 294 (1962).

[4] W. T. Griffiths, J. C. Wallwork and J. F. Pennock, *Nature* **211**, 1037 (1966).

[5] D. R. Threlfall, W. T. Griffiths and T. W. Goodwin, *Biochem. Biophys. Acta* **102**, 614 (1965).

[6] B. C. Das, M. Lounasmaa, C. Tendille and E. Lederer, *Biochem. Biophys. Res. Commun.* **21**, 318 (1965); **26**, 211 (1967).

[7] W. T. Griffiths, *Biochem. Biophys. Res. Commun.* **25**, 596 (1966).

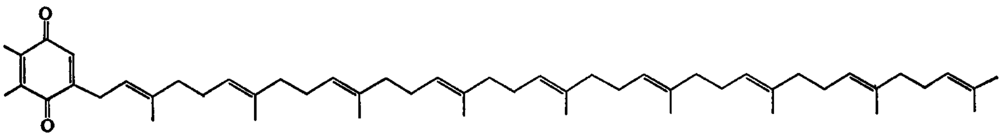
[8] J. C. Wallwork and J. F. Pennock, *Chem. Ind.* 1571 (1968).

[9] P. J. Dunphy, K. J. Whittle and J. F. Pennock, in *Biochemistry of the Chloroplasts*, Vol. 1, p. 165, Academic Press, London (1966).

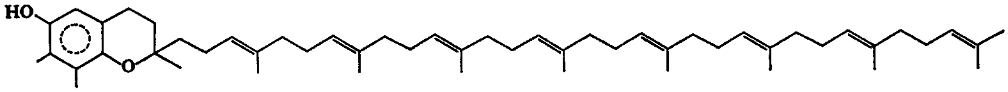
TLC, both compounds reduced $FeCl_3$ to give a red colour with 2,2′-dipyridyl, and both gave a purple colour when sprayed with diazotized-*o*-dianisidine. On adsorption chromatography one had a polarity similar to that of plastochromanol-8, while the other was more polar in nature.

Leaf tissue from *L. vulgare* and *Polygonum cuspidatum* was extracted (see Experimental) and, after purification by alumina column and preparative TLC, sufficient quantity of each was obtained for characterization. Both compounds gave qualitatively identical u.v. spectra with maxima in cyclohexane at 294 and 300·5 nm which, together with the positive diazo coupling reaction, suggested a 7,8-dimethylchromanol-type compound (e.g. γ-tocopherol). The more polar chromanol gave an $E^{1\%}_{1\text{cm}}$ (294 nm) of 54 (plastochromanol-8 $E^{1\%}_{1\text{cm}}$ 55), indicating a molecular size similar to plastochromanol-8, while the less polar chromanol gave an $E^{1\%}_{1\text{cm}}$ of 40, indicating a larger molecule. The above results, combined with physico-chemical data described below, showed the two unknowns to be plastochromanols and were given the trivial names plastochromanol-C (III), related to plastoquinone-C, and plastochromanol-B (IV), related to plastoquinone-B, the former being the more polar compound.*

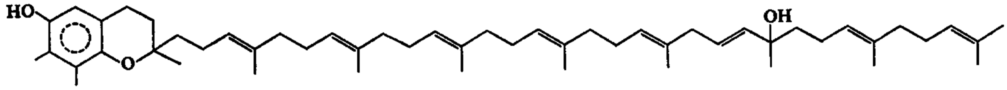
The i.r. spectrum of plastochromanol-C was similar to that of plastochromanol-8, but with evidence of extra hydroxyl absorption and bands characteristic of a *trans* symmetrically disubstituted ethylene, —CH=CH— (10·3 μ and 7·6 μ). Plastochromanol-B was also



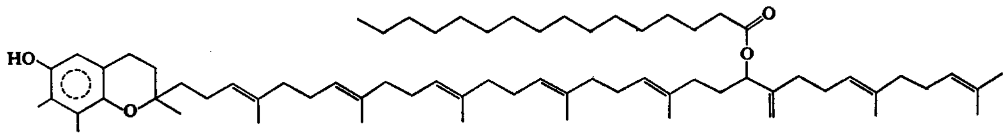
Plastoquinone-9 (I)



Plastochromanol-8 (II)



Plastochromanol-C (III)



Plastochromanol-B (IV)

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* In formulae III and IV, the position of attachment of the —OH and —OCOR groups, respectively on the isoprenoid side-chain are not known with certainty. However, it is likely that the substitutions can occur in any isoprene unit.

similar to plastochromanol-8 but in this case with ester absorption (bands at 5·8 μ C=O, and 8·6 μ —C—O—) together with evidence for an asymmetrically disubstituted ethylene $>C=CH_2$ (a band at 11·15 μ).

Mass spectra of plastochromanol-C and plastochromanol-B were in agreement with the proposed structures. Plastochromanol-C gave a molecular ion at *m/e* 766, a peak at *m/e* 748 ($—H_2O$) and the base peak at *m/e* 151 agreed with that found for plastochromanol-8.[10] Plastochromanol-B gave a weak molecular ion at *m/e* 1004 with a strong *m/e* 748 peak corresponding to the loss of palmitic acid from the molecular ion, as found in the plastoquinone-Bs.[7] Plastochromanol-B also had a peak at *m/e* 151. Both plastochromanol-C and plastochromanol-B gave fragmentation patterns similar to that reported by Mayer *et al.*[10] for plastochromanol-8.

The NMR data for plastochromanol-C are shown in Table 1 and confirm the basic plastochromanol structure. There are, however, additional bands at 4·5, 7·3 and 8·75 τ which substantiate the proposed tertiary allylic hydroxy structure. The positions of the —OH groups were not established, and furthermore the $—CH_2$ group β to the tertiary hydroxyl was masked by the olefinic methyl absorption at 8·4 τ.

TABLE 1. NMR DATA FOR PLASTOCHROMANOL-C

τ Value	Assignment	Splitting	Expected	Found
3·7	Benzenoid proton	Singlet	1	1
4·5	—*HC*=*CH*—	Multiplet	2	2
4·9	—(CH_3)C=*CH*—	Multiplet	7	7
7·3	Benzenoid—CH_2— + =CH—CH_2—CH=	Multiplet	4	4
7·9	Benzenoid—CH_3	Singlet	6	30
8·0	—CH_2—(CH_3)C=CH—CH_2—	Multiplet	24	
8·4	—(CH_3)C=CH— + │ CH_2 │ —O—C—CH_2— │ + │ HO—C—CH_2— │	Multiplet	30	30
8·75	│ CH_3—C—O—C │ + │ CH_3—C—OH │	Singlet	6	6

Chromatographic data are shown in Table 2 and it can be seen that plastochromanol-B is less polar than plastochromanol-8 or plastochromanol-C on partition chromatography, in agreement with its larger molecular size. Reduction of plastochromanol-B with $LiAlH_4$ in ether gave a product which behaved similarly to plastochromanol-C on adsorption and

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[10] H. MAYER, J. METZGER and O. ISLER, *Helv. Chim. Acta* **50**, 1376 (1967).

TABLE 2. R_f VALUES (× 100) FOR THE PLASTOCHROMANOLS

Support and solvent	Plastochromanol-8	Plastochromanol-C	Plastochromanol-B	α-Tocopherol
Adsorption TLC on silica gel G in $CHCl_3$	57	38	62	61
Adsorption TLC on silica gel G in 15% di-isopropyl ether/benzene	50	31	54	45
Reversed-phase TLC on paraffin-coated Keiselguhr G in 93% acetone–water	55	85	40, 29, 24, 21, 16, 10	72

reversed-phase TLC. Clearly, there are seven positional isomers of both plastochromanol-C and plastochromanol-B and on reversed-phase TLC plastochromanol-B separated into six reducing components confirming the plurality in this group. Plastochromanol-C did not separate into a series of isomers in any chromatographic system used.

During chromatographic purification of plastochromanol-C a small amount of reducing material was seen which, although migrating slowly on reversed-phase systems, was otherwise similar to plastochromanol-C. Although this material was not analysed further, it would seem likely that it is analogous to the fourth type of plastoquinone, the plastoquinone-Zs, which appear to contain both hydroxyl and ester functions.[8]

The concentration of these chromanols in *L. vulgare* and *P. cuspidatum* was in the order of 40 mg/kg for plastochromanol-C and 8–10 mg/kg for plastochromanol-B. Examination of several other tissues has shown these compounds to be present in all photosynthetic material analysed but were not detected in tomato parenchyma, although plastochromanol-8 was found in an amount comparable with that of α-tocopherol (Table 3).

TABLE 3. DISTRIBUTION OF PLASTOQUINONES AND CHROMANOLS

			Plastochromanol			Plastoquinone		
Plant material	Chlorophyll	α-Tocopherol	8	B	C	9	B	C
Ilex aquifolium	+	+	+	+	+	+	+	+
Ligustrum vulgare	+	+	+	+	+	+	+	+
Polygonum cuspidatum	+	+	+	+	+	+	+	+
Hevea brasiliensis	+	+	+	+	+	+	+	+
Ficus elastica	+	+	+	+	+	+	+	+
Barley (7-day)	+	+	−	−	−	+	−	?
Tomato pith	−	+	+	−	−	+	−	−

Key: + present, − absent, ? uncertain.

DISCUSSION

The finding of these two new plastochromanols with substituted side-chains poses two main questions, namely how do they arise and what is their function. Since no direct work has yet been carried out on their function and biosynthesis, we can only put forward schemes on the basis of such clues as afforded by the chemical nature and distribution of these compounds.

Structurally, the new chromanols show similarities with the hydroxyplastoquinones (PQCs) and the esterified plastoquinones (PQBs). However, whereas two varieties of plastoquinones C have been found, one type with a secondary and the other with a teritary hydroxyl, only the latter type of plastochromanol-C was found. Furthermore, whereas in the plastoquinone series many isomers of both the B and C types were isolated, separation of plastochromanols B and C into series of isomers was only achieved in the case of the former. However, the structural similarities indicate that plastochromanols B and C either are formed from plastoquinones B and C or are formed from plastochromanols in a manner identical to the formation of plastoquinones B and C from plastoquinone. It would appear that plastoquinone may undergo several reactions such as epoxidation followed by isomerization, esterfication of the secondary hydroxyls so produced and cyclization to form a chromanol (Fig. 1). Distribution studies suggest that since the side-chain-modified plastoquinones and plastochromanols are found only in photosynthetic tissue, the oxygen generated during photosynthesis may be the source of the oxygen atom of the side-chain. Cyclization does not, however, seem to be a photosynthesis-dependent reaction since plastochromanol has been found in several non-photosynthetic tissues, e.g. tomato, hevea latex, and vegetable seed oils.[9]

$$\begin{tikzcd}
\text{Plastoquinone-9} \arrow[r, "2"] \arrow[d, "1"] & \text{Plastoquinone-C} \arrow[r, "3"] \arrow[d, "1"] & \text{Plastoquinone-B} \arrow[d, "1"] \\
\text{Plastochromanol-8} \arrow[r, "2"] & \text{Plastochromanol-C} \arrow[r, "3"] & \text{Plastochromanol-B}
\end{tikzcd}$$

[1] Cyclisation
[2] Epoxidation and isomerisation
[3] Esterification

FIG. 1. POSSIBLE PLASTOQUINONE–PLASTOCHROMANOL INTERRELATIONSHIPS.

The plastochromanols reported here were found in autumnal leaves in levels comparable with plastoquinones B and C and it may be that like the plastoquinones[11] these compounds accumulate in the autumn.

Many schemes have been put forward to involve chromanols in electron transport in mitochondria, bacteria and chloroplasts[12] and therefore it is possible that the plastochromanols are involved in photosynthetic electron transport. However, so far no scheme has been substantiated and it seems more logical to assume that these compounds are by-products rather than functional components in their own right. It has been proposed that epoxides are intermediates in the hydroxylation of the terpenoid side-chain in the case of plastoquinones and plastochromanols B and C and it may be possible that the large amount of plastoquinone in the photosynthetic apparatus can be epoxidized to remove oxygen rather like the epoxide cycle suggested for certain carotenoids.[13] If this were true then the plastoquinones and plastochromanols B and C would represent further metabolites of plastoquinone epoxides and these compounds would therefore have been produced as a result of a significant reaction but would not be structurally important themselves.

[11] J. C. WALLWORK and J. F. PENNOCK, in *Progress in Photosynthesis Research*, Vol. 1, p. 315 (1969), *Int. Union of Biol. Sciences, Tübingen.*

[12] E. LEDERER and M. VILKAS, *Vitams Horm.* **24**, 409 (1966).

[13] N. I. KRINSKY, in *Biochemistry of Chloroplasts*, Vol. 1, p. 423 (1966), Academic Press, London.

EXPERIMENTAL

Extraction

Plastochromanol-C was extracted from 2 kg of *Polygonum cuspidatum* and plastochromanol-B from 1·5 kg of *Ligustrum vulgare*. Both tissues were homogenized in acetone, and then an equal volume of light petroleum was added and the acetone removed by washing with water. The petrol fraction was taken to dryness.

Purification

a. *Plastochromanol-B.* The lipid was chromatographed on Brockmann grade 3 acid-washed alumina (Woelm) and then the column was developed with three eluents: light petroleum to remove β carotene, 3% diethyl ether/light petroleum (E/P) to remove plastoquinone-9, plastoquinone-B and α-tocopherol and 10% E/P to elute the plastochromanol-B. The plastochromanol-B was isolated from the 10% fraction by preparative TLC in $CHCl_3$, and then 15% di-isopropyl ether in benzene. Final purification was by reversed-phase TLC with 93% acetone–water as solvent, followed by recrystallization from aqueous ethanol.

b. *Plastochromanol-C.* The fractions eluted from the alumina column between 25% and 30% E/P contained the majority of the plastochromanol-C. This latter fraction was purified further by preparative TLC first in 40% E/P then 20% di-isopropyl ether in benzene and finally by reversed-phase chromatography in 78% acetone–water.

Determination of Spectra

Mass spectra were obtained on an A.E.I. M.S. 12 mass spectrometer, and the NMR spectrum in a Varian A. 60 spectrometer, using $CDCl_3$ as solvent and tetramethylsilane as an internal standard. I.r. spectra were prepared as oily smears between sodium chloride discs.

Solvents

Acetone was dried over K_2CO_3 and redistilled. Light petroleum (40–60°) and diethyl ether were dried over sodium wire and distilled, the latter from reduced iron powder.

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