TWO NEW QUINONES FROM CHLOROPLASTS*

L. P. Kegel, M. D. Henninger and F. L. Crane

Department of Biological Sciences, Purdue University

Lafayette, Indiana

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It has previously been shown that plastoquinone is concentrated in the chloroplast fraction from spinach leaves (Crane 1959). This quinone was characterized by Trenner et al. (1959) as 2,3-dimethyl-5-solanosyl benzoquinone. There is evidence that plastoquinone (PQ) is involved in electron transport and phosphorylation in chloroplasts. Bishop (1959) demonstrated a requirement for PQ for the restoration of photoreduction of indophenol after the quinone had been extracted from lyophylized chloroplasts. We have shown that plastoquinone undergoes reduction in chloroplasts in light (Crane et al. 1960) and a similar photoreduction has been observed by Redfearn and Friend (1961). Krogmann (1961) has reported restoration of phenzine-methosulfate-coupled photophosphorylation by addition of PQ to solvent-extracted chloroplasts.

In our further investigation of the oxidation reduction changes of PQ it became apparent that several quinoid compounds are present in chloroplasts. Two new quinones have been isolated which show absorption spectra identical to the original PQ but different behavior in chromatography. We have also found a quinone which has spectral properties and chromatographic behavior identical to Vitamin K_1. This latter observation is consistent

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with the previous demonstration by Dam (1942) of Vitamin K in chloroplasts by means of a biological assay.

Since the three quinones with absorption maxima at 255 mm appear to be homologs of the original plastoquinone we have designated them as follows: plastoquinone A (PQA) is the original plastoquinone with 9 isoprenoid units in the solanosyl side chain first isolated by Kofler (1946). Plastoquinone B (PQB) is a quinone which shows a lower R_f than PQA during chromatography on silicone treated paper, and plastoquinone C (PQC) shows a much higher R_f on silicone paper that PQA. The properties of these compounds are shown in table I.

Table I

Characteristics of Chloroplast Quinones

Quinone	Melting point	R_f silicone paper	R_f vaseline paper	$E^{1\%}_{1\ cm}$	Absorption maxima
PQA	44°C	0.27	0.07	246	255
PQB	35°C	0.13	0.06	202	255
PQC	oil	0.75	0.87	66	255
Vitamin K_1	oil	0.63	-	-	242,248,261,269
Coenzyme Q_{10}*		0.29			

*Included for chromatographic comparison, coenzyme Q_{10} has not been found in chloroplasts. The silicone paper method as described by Lester and Ramasarma (1959). The vaseline paper method as described by Linn et al. (1959).

We have used both direct solvent extraction and extraction after saponification of chloroplasts to obtain these quinones and determine the relative amounts present in spinach chloroplasts.

For direct extraction a chloroplast preparation (Crane 1959) having a chlorophyll content of 1.73 mg chlorophyll/ml was mixed

with 0.2M potassium phosphate buffer pH 6.5, a mixture of 1:1 propanol-heptane and water in a V/V ratio of 1:1.2:3:2.5. The mixture was shaken on a reciprocal shaker for 3 hours, after which the upper organic phase was removed through a separatory funnel. The aqueous phase was washed several times with petroleum ether and the washes combined with the original extract. After drying over anhydrous Na_2SO_4 the organic phase was evaporated to dryness under vacuum, taken up in a small volume of n-heptane and chromatographed through a decalso column. The column was eluted with (1) n-heptane, (2) 10% ethyl ether in n-heptane and (3) absolute ethanol. The second and third eluates are each passed separately through silicic acid columns (3:1 supercell : silicic acid w/w). Each column is eluted with n-heptane followed by increasing amounts of chloroform in n-heptane. PQA and PQB are obtained from the 10% ethyl ether eluate from the decalso column and PQC is obtained from the absolute ethanol eluate from decalso. In order to obtain maximum yield of PQC the entire procedure from preparation of chloroplasts to chromatography should be carried out in dim light.

For extraction after saponification we have used the method described for isolation of coenzyme Q_{10} (Crane et al. 1959). The extract is chromatographed on a silicic acid column using elution with n-heptane followed by increasing concentration of chloroform in n-heptane. PQA can be eluted with n-heptane whereas PQB and PQC are eluted successively in 10% chloroform in heptane.

Unfortunately there is always a lower recovery of quinone following saponification as compared to the direct extraction. On the other hand, saponification provides extracts from which the quinones can be more easily purified and where chlorophyll does not interfere with identification of PQC by paper chromatography. The amount of the various quinones in spinach chloroplasts as determined by the two methods are shown in table II.

Table II

Quinone Content of Spinach Chloroplasts

Quinone	Direct extraction procedure moles quinone/mole total chlorophyll	Saponification procedure moles quinone/mole total chlorophyll
PQA	0.16	0.067
PQB	0.08	0.003
PQC	0.10	0.027
Vitamin K_1	0.008	

From the pattern of distribution of these quinones during chromatography on decalso, it is clear that our previous determination of oxidation reduction changes of PQ in the second decalso eluate (10% ethyl ether elution) involve PQA and possible PQB whereas the oxidation reduction changes observed in the third fraction (ethanol eluate) may primarily be attributed to PQC (Crane et al. 1960). From our observations it appears that PQA and PQB undergo reduction in light. PQC on the other hand displays rather unique behavior in that very little is present either as quinone or hydroquinone when chloroplasts are kept in light, but the quinone appears when the chloroplasts are put in darkness. We are continuing to investigate the nature of the conversion which PQC undergoes during transfer from dark to light.

Preliminary studies of the distribution of these compounds indicate that both PQA and PQC are found in large amounts in leaves of many species, as well as in chloroplasts from both spinach and lilac. PQB seems to be present in small amounts or not at all in some species and further careful studies will have to be carried out to determine its general distribution and significance.

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