'Plastoquinone B' - Some Structural Studies

by

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Received November 2, 1966

A total of six plastoquinones which are thought to play an important role in photosynthesis have been isolated from plant lipids. The chemical structure of three of these compounds has been established as isoprenologues of 2:3 dimethyl-p-benzoquinone. Plastoquinone A (PQA or PQ-9) the first quinone to be isolated and which is now known to be widely distributed in plants has an isoprenoid side chain of nine C5 units in the -5 position (Trenner _et_ _al_., 1959) while PQ-4 from horsechestnut leaves has a C20 side chain (Eck and Trebst, 1963) and PQ-3 from spinach leaves has a C15 side chain (Misiti _et_ _al_., 1965).

Three other plastoquinones of unknown structure and chromatographically distinct from PQ-3, PQ-4 or PQ-9 have been described and designated PQB, PQC and PQD (Kegel _et_ _al_., 1962, Henninger and Crane, 1964). The ultraviolet absorption spectra of all these quinones are similar however in the infrared, PQC' and PQD' (≡ PQC and PQD) as isolated by Threlfall et al. (1965) showed a band in the O-H stretching frequency region at 3545 cm^{-1} which is absent from the infrared spectra of PQ-3, PQ-4 or PQ-9.

Mass spectra for PQB, PQC and PQD have been reported by Das et al. (1965). For PQB a molecular ion peak M at m/e 746 was found

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while PQC and PQD both had peaks at m/e 764 with base peaks in all cases at m/e 189. These findings and the mass spectrometry fragmentation patterns led to the suggestion that structurally PQB is similar to PQA but with an additional unsaturation probably in the second isoprene residue from the quinone ring while PQC and PQD could be considered as hydroxylated PQA or hydrated PQB.

Recent work from this laboratory (Griffiths, Wallwork and Pennock, 1966) has indicated that a quinone fraction of similar polarity to PQB could be isolated from a variety of plants and fractionated into six distinct quinone components showing the characteristic PQ ultraviolet absorption i.e. λ max. 255 and 263 mμ. These were designated PQ-B_1; -B_2; -B_3; -B_4; -B_5 and -B_6. A quinone fraction of similar chromatographic properties to PQC and PQD was also obtained in this study and this could also be fractionated into six distinct PQ type compounds described as PQ-C_1; -C_2; -C_3; -C_4; -C_5 and -C_6.

The present report gives some recent findings on the properties of the PQ-B samples. The effect of lithium aluminium hydride and the infrared and mass spectra of some of the samples are described.

Isolation and Thin Layer Chromatography (T.L.C.) of PQ-B's

Young elder (*Sambucus nigra*) leaves were used as a source of the six PQ-B samples. These were isolated from a lipid extract of the leaf material as previously described (Griffiths *et al.*, 1966) by chromatography of the lipid on alumina. The 3% diethyl ether/light petroleum (E/P) fraction was treated to adsorption T.L.C. (Kieselgel G coated plates developed with 12% di-*iso*-propyl ether in light petroleum). Three PQ-B fractions were eluted each of which on partition T.L.C. (paraffin impregnated Kieselgur chromatoplates developed with 2.5% water in acetone) yielded two PQ-B's.

Ultraviolet and Infrared Spectra

All the samples in cyclohexane and ethanol gave ultraviolet absorption spectra with maxima at 255 mμ and inflexions at *ca*.263 mμ identical with PQA. On reduction in ethanol with sodium borohydride, as with PQA, isobestic points were obtained at 232 and 276 mμ by all samples (fig. 1).

Oxidised(a) and Reduced(b) Spectra of $PQ\text{-}B_2$

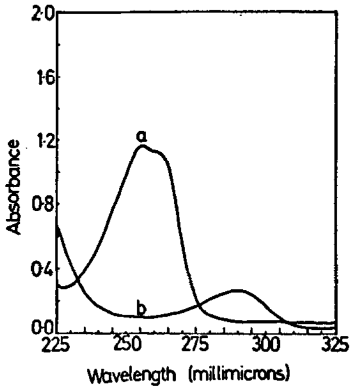

Fig. 1

Infrared spectra of the preparations were recorded as solvent-free films between rock salt plates. The spectrum of one of the samples, $PQ\text{-}B_2$, is reproduced in fig. 2. This spectrum is similar basically to that of PQA but with additional bands at 1730, 1253 (C=O and C-O-C stretch respectively of an ester grouping) and 722 cm^{-1} (CH_2 rocking of a chain of methylene groups $-(CH_2)_n-$ where $n \geq 4$). Such bands are normally found associated with fatty acid esters.

Infrared Spectrum of $PQ\text{-}B_2$

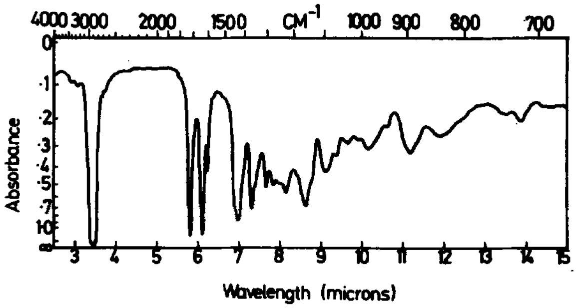

Fig. 2

The infrared spectra of all the other PQ-B's were similar suggesting the presence of an ester grouping in these compounds or alternatively the presence of an ester contaminant(s) in the preparations. It should be mentioned at this stage that repeated purification of the preparations by adsorption and partition T.L.C. failed to remove the ester absorption bands from the infrared spectra of the samples.

Mass Spectra and Effect of Lithium Aluminium Hydride.

To check the possibility of the PQ-B preparations being esters the mass spectra of three of the samples were determined at the Organic Chemistry Department, Liverpool University, on the A.E.I. MS9 instrument. The mass spectrum of PQ-B_2 showed a molecular peak at m/e 1,002. This could correspond to plastoquinone A (748) plus a palmitic acid residue (256). The base peak observed at m/e 189, corresponding to the formula $C_{12}H_{13}O_2$, also occurs in PQ 3 (Misiti et al., 1965) and in the PQA, PQB, PQC and PQD samples examined by Das et al. (1965). PQ-B_2 also showed a strong peak at m/e 746 [loss of $CH_3(CH_2)_{14}COOH$]. A similar peak described as the 'molecular peak' was observed in the mass spectrum of the PQB preparation of Das and coworkers (Das et al., 1965). The mass spectra of the other samples examined, PQ-B_5 and PQ-B_6, gave essentially the same picture as that described for PQ-B_2.

Plastoquinone-B_1, PQ-B_3 and PQ-B_4 in view of the mass spectrometry results were separately taken up in 5 ml. diethyl ether and lithium aluminium hydride/ether solution (10 mg./ml.) added till cessation of effervescence followed by 1ml. excess and allowed to stand for one minute. At the end of this period excess reagent was destroyed by the dropwise addition of ethanol followed by ice-cold 0.1N H_2SO_4. The mixtures were then extracted with ether, washed with water, dried and taken to dryness. The product so obtained from the PQ-B_1 sample was chromatographed on alumina deactivated with water to Brockmann grade III and eluted with ether/light petroleum (E/P) mixtures in the following order: 100% P; 5% E/P and 25% E/P.

The 5% E/P fraction which normally elutes the PQ-B's under these conditions contained instead a white solid transparent in the ultraviolet but showing in the infrared a peak in the O-H stretching frequency region at ~3450 cm^{-1} and two other major peaks at 1050 cm^{-1} and 722 cm^{-1}. Such a spectrum is typical of a saturated aliphatic primary alcohol. This material migrated with a similar Rf to cetyl alcohol during T.L.C. on paraffin-impregnated Kieselgur plates developed with 30% water in acetone.

The 25% E/P fraction when examined in the ultraviolet showed the typical PQ spectrum with λmax. at 255mμ and an inflexion at 263mμ. In the infrared this material gave a spectrum which was identical with that reported for PQC' and PQD' by Threlfall et al. (1965) differing from the infrared spectrum of PQA in the possession of a band in the O-H stretching region at ~3545 cm^{-1}. Furthermore, this material when chromatographed on silica gel G chromatoplates developed with 10% di-iso-propyl ether in benzene migrated with an Rf of the same order as the polar PQ-C's described previously (Griffiths et al., 1966).

Conclusions and Discussion

The results of the experiments reported briefly here have led to the proposal that some plants contain six distinct PQ-B's all of which appear to be fatty acid esters of a hydroxy-plastoquinone A type compound(s). The fact that six distinct polar (hydroxy) plastoquinones (PQ-C's) have been identified in plants (Griffiths et al., 1966) would strengthen this proposal since these may be considered as arising from the esters by hydrolysis or, alternatively, each of the six hydroxyplastoquinones may be esterified with a fatty acid thereby giving the corresponding six plastoquinone-B type compounds.

The identification of 'plastoquinone B' as esters explains why 'PQB' on adsorption T.L.C. appears more polar than PQA (migrates with a higher Rf) while on partition T.L.C. and paper chromatography appears less polar than PQA i.e. again migrates with a lower Rf than PQA (Threlfall et al., 1965, Henninger et al., 1966). During adsorption chromatography the ester grouping in 'PQB' renders it more polar than PQA whereas on reversed phase partition chromatography the long chain fatty acid residue in 'PQB' confers upon it a highly lipophilic character so that it favours the paraffinic stationary phase when chromatographed in an aqueous solvent.

It is also possible from these conclusions to reconcile the results of Das et al. (1965) on the mass spectrometry of their 'PQB' preparation. The mass peak - described as the molecular ion peak - at m/e 746 observed by this group can be accounted for by the loss of a molecule of palmitic acid (256) from the parent ester (1,002). Furthermore, a reinvestigation by Das and coworkers of their 'PQB' preparation in the light of our results has confirmed our findings to the extent that their sample also possessed a molecular ion at m/e 1,002. (Personal communication from Professor E. Lederer to Professor R. A. Morton.)

The demonstration of the occurrence in plants of six esterified plastoquinones (PQ-B's) and the possibility of the existence of a related series of six hydroxyplastoquinones (PQ-C's) serves to illustrate the complex nature of the problem of deciding upon a biochemical function or a chemical structure for the hitherto incompletely characterised plastoquinones. Work is at present in progress aimed at a more complete chemical characterisation of the plastoquinones-B and at demonstrating any structural relationships between the PQ-B's and the PQ-C's.

Acknowledgements

The author thanks Professor R. A. Morton, F.R.S., and Dr. J. F. Pennock for their advice and encouragement during this work.

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