THE STRUCTURE OF THE PLASTOQUINONES B and C

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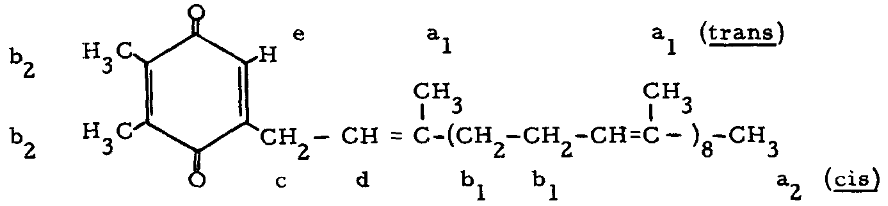
In a previous communication (Das et al., 1965) we reported the use of mass spectrometry for the determination of structural details of the plastoquinones B, C and D.



(I)

PQ A = PQ-9[***] (2,3-dimethyl-5-solanesyl benzoquinone (I) ; Kofler et al., 1959 ; Crane, 1959 ; Trenner et al., 1959) was used as a standard ; the following molecular weights were reported :

PQ A : 748, confirming the known molecular formula $C_{53}H_{80}O_2$,
PQ B : 746, which was interpreted as indicating a dehydro PQ A structure,
PQ C : 764, indicating a hydroxy PQ A structure,
PQ D : 764, showing that PQ D is an isomer of PQ C.

Mass spectra of the diacetates of the corresponding hydroquinones were in agreement with the above results ; the reduced PQ C also gave a small peak corresponding to a triacetate, thus confirming the presence of an

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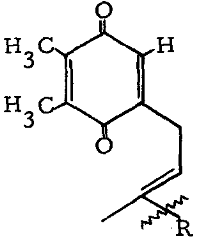
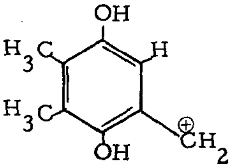
[***] For the nomenclature, see the recommandations of IUPAC-IUB, Biochim. Biophys. Acta, 107, 5 (1965).

OH group on the side chain. This has been suggested independently by infra-red measurements (Threlfall et al., 1965).

The presence of the base peak at m/e 189 in the spectra of all plastoquinones was an indication for a common structure (II) (Das et al., 1965 ; Misiti et al., 1965). Another prominent fragment at m/e 151 can be attributed to the quinol ion (III).



(II)



(III)

At a symposium in Elsinore (July 1966), Prof. R. A. Morton reported results obtained by Dr. W. T. Griffiths in his laboratory, showing that PQ B is an ester of PQ C and not a dehydro PQ A, as we had assumed. We thus had to reexamine our findings and we now wish to report results confirming the findings of Morton and Griffiths, that PQ B carries one ester group in the isoprenoid side chain ; the peak at m/e 746 is not a molecular peak, but results from the loss of the corresponding acid from the molecular ion.

We also report NMR data defining the structure of the benzoquinone ring of PQ B and PQ C and its substituents.

Plastoquinone B is an ester -

Careful determination of the mass spectrum of a purified sample of PQ B showed a series of homologous peaks in the high mass region, the peak due to the highest mass being found at m/e 1002 (homologous peaks are found at m/e 974 and 946)(Fig. 1). This corresponds to a C_{16}-acyloxy plastoquinone A ; saponification of PQ B followed by esterification of the acid fraction with diazomethane gave a product the mass spectrum of which indeed showed that it was mainly composed of methyl palmitate (m/e 270). Furthermore, the diacetate of plastohydroquinone B showed a peak at m/e 1088 (lower homologs at m/e 1060 and 1032), thus establishing the molecular formula of this PQB ($C_{69}H_{110}O_4$) corresponding to a palmitate. These results fully confirm the finding of Morton and Griffiths that PQ B is an ester.

Catalytic hydrogenation of the above diacetate gave a product, the mass spectrum of which was identical to that of the compound obtained from PQ A

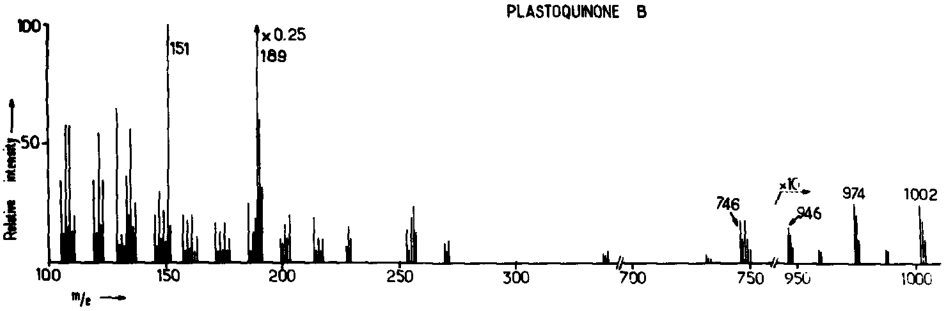
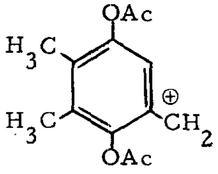


Fig. 1 -

Mass spectrum of plastoquinone B (A.E.I. MS 9 mass spectrometer, direct inlet system, 250° at 70 eV). The peak at m/e 748 is due to the corresponding quinol ion, and is similar to the M+2 ion observed in the case of plastoquinone A (Das et al., 1965).

by similar treatment. The molecular weight (M^+852) indicates that the side chain is completely hydrogenated in these compounds and that in the PQ B derivative the ester group has been lost. A peak observed at m/e 235 in these mass spectra is attributed to the ion fragment (IV). Subsequent loss of one and two acetyl groups (in the form of $CH_2=C=O$) gave peaks at m/e 193 and 151, respectively. These results suggest that the ester group in PQ B is allylic and undergoes hydrogenolysis during catalytic hydrogenation. It is therefore evident that plastoquinone B has essentially the same structure as PQ A (see also the results of NMR quoted below) with an ester substituent in the isoprenoid side chain.



(IV)

In the infrared spectra of the natural plastoquinone preparations, there is often a band around 1740 cm^{-1} which is possibly due to the C=O frequency of an ester. The values obtained with different plastoquinones are quoted below :

PQ B : 1742 cm^{-1} (very strong ; CCl_4)
PQ C : 1740 cm^{-1} (CCl_4 : Threlfall, et al., 1965)
PQ 4 : 1730 cm^{-1} (Eck and Trebst, 1963)
PQ 3 : 1730 cm^{-1} (CCl_4 : Misiti et al., 1965)

In view of the absence of this band in the infrared spectrum of synthetic plastoquinone A, it may be assumed to be due to some small contamination in the case of PQ C, PQ 4 and PQ 3.

Multiplicity of PQ B and PQ C -

Recently Griffiths et al. (1966) have reported that by using adsorption and partition thin layer chromatography they could resolve PQ B into six distinct components. They have also shown by thin layer chromatography that there are six distinct compounds eluted from a column in the PQC-PQD region (three PQ C and three PQ D compounds). These six PQ B and six PQ C + PQ D compounds are apparently isomers differing by the position of the hydroxyl (or ester) group in the side chain. Our structural deductions described above seem nevertheless valid.

Our speculations (Das et al., 1965) concerning the position of the OH group in PQ C are, however, no more tenable.

NMR spectra of the plastoquinones B and C -

Comparison of the NMR spectra of PQ B and PQ C with that of PQ A revealed close similarity in their structural features. In the following, chemical shifts are expressed in ppm with respect to tetramethylsilane as internal standard.

From the 60 mc/sec NMR spectrum, Eck and Trebst (1963) reported the following values for the different groups of protons in PQ A (I) :

a 1.63 ; b 2.03 ; c 3.15 ; d 5.14 ; e 6.44

It was mentioned by Trenner et al. (1959) that the proton (e) on the benzoquinone nucleus gave a triplet. This is in agreement with its ortho position to the side chain ($-CH_2-$) and not with a methyl (CH_3) group.

NMR results (100 mc/sec)* of PQ A, PQ B and PQ C are given below :

PQ A	a_1 1.60 ; a_2 1.65 ; b_1 1.95 ; b_2 2.05 ; c 3.05 (doublet) ; d 5.05 (broad) ; e 6.3 (triplet).
PQ B	a_1 1.58 ; a_2 1.63 ; b_1 1.95 ; b_2 2.00 ; c 3.05 (doublet) ; d 5.05 (broad) ; e 6.3 (triplet).
PQ C	a_1 1.58 ; a_2 1.63 ; b_1 1.95 ; b_2 2.00 ; c 3.05 (doublet) ; d 5.05 (broad) ; e 6.3 (triplet).

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By using spin decoupling technique, the triplet at 6.3 ppm in the NMR spectra of all the three plastoquinones A, B and C could be transformed into a singlet by irradiating the signal at 3.05 ppm. The signal at 3.05 ppm appears as a doublet proving that the β-carbon atom in the side chain bears a proton (d).

These NMR and mass spectral data clearly demonstrate that plastoquinones B and C contain grouping (II) present in PQ A.

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