MASS SPECTROMETRY OF PLASTOQUINONES

THE STRUCTURE OF THE PLASTOQUINONES B, C and D *

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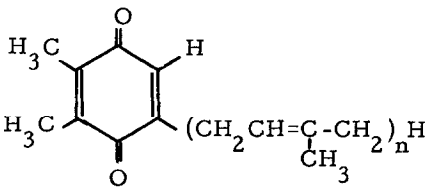
The plastoquinones are compounds which play an important role in electron transport in the chloroplasts (Bishop, 1961 ; Arnon and Crane, 1965). The structures of the following plastoquinones (PQ) have been established :

PQ A (=PQ 9 = PQ 45) (I) (Kofler et al., 1959 ; Trenner et al., 1959)

PQ 4 (=PQ 20) (II) (Eck and Trebst, 1963)

PQ 3 (=PQ 15) (III) (Misiti et al., 1965)



(I) n = 9
(II) n = 4
(III) n = 3

Kegel et al. (1962) and Henninger and Crane (1964) have discovered three other plastoquinones : PQ B, PQ C and PQ D. In the following we describe the use of mass spectrometry for the determination of structural details of these compounds.

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* Dedicated to Professor Richard KUHN on the occasion of his 65th birthday.

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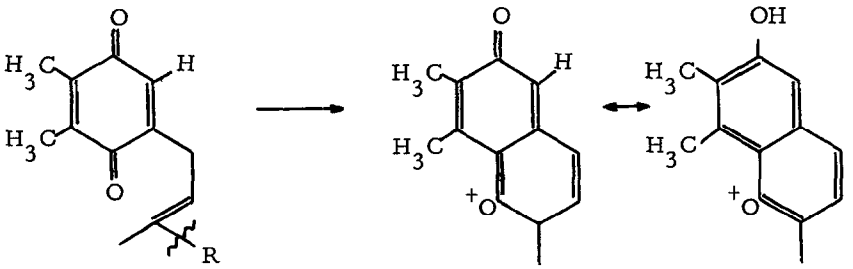
Mass spectrometry - The mass spectra of the plastoquinones were measured with an A.E.I. MS 9 mass spectrometer. The compound (about 0.1 mg) was introduced into the ion source using a ceramic direct insertion probe which was admitted through a vacuum lock. The sample is evaporated by heating the ion chamber. The spectra were recorded with the resolving power of the instrument set at about 1200 and the exact elemental composition of several peaks was accurately measured with the resolving power set at 10,000.

General features of the mass spectra of plastoquinones - In the mass spectra of PQ A and B a peak at M+2 is obtained, the intensity of which increases with time. In a personal communication, Dr. W. Vetter (Hoffmann-La Roche, Basle) has suggested that these M+2 peaks (which both he and we have also observed with compounds of the vitamin K series) are due to a dismutation reaction in the mass spectrometer, resulting in the formation of the ions of the corresponding hydroquinones.

In all the plastoquinones mentioned in this communication, the base peak is at 189 ; mass measurement shows this to correspond to the formula $C_{12}H_{13}O_2$ (V). The same base peak has also been observed by Misiti et al. (1965) for PQ 3 (=PQ 15) (III). The presence of this same base peak in all PQ mass spectra proves that they all contain the same 2,3-dimethyl 1,4-benzoquinone nucleus (IV) (or an isomeric structure) and differ mainly in the length and structure of the isoprenoid side chain.



(IV) (V) m/e 189

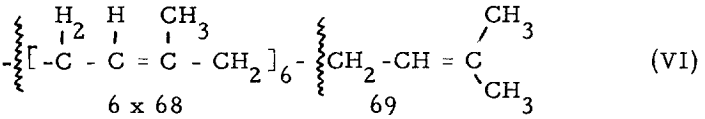
Plastoquinone A

The mass spectra of natural and synthetic PQ A (=PQ 9 = PQ 45)(I) (the latter received through the courtesy of Dr. O. Isler, Hoffmann-

La Roche, Basle) were found to be identical ; the mass peak at m/e = 748 ($C_{53}H_{80}O_2$) confirms the formula established by Trenner et al. (1959).

The diacetate of plastohydroquinone A gave a mass peak at m/e 834 ($C_{57}H_{86}O_4$) confirming the molecular formula of the parent quinone; there is no peak at M+2, in agreement with the explanation given above.

Further peaks are found at M-69-$(68)_n$ where n = 0,1,2,3,4,5 and 6 (m/e = 765, 697, 629, 561, 493, 425 and 357 respectively) due to the successive loss of seven monounsaturated isoprene units corresponding to structure (VI).



(VI)

Plastoquinone B

This compound, isolated from alfalfa, gives a molecular ion at m/e 746 ($C_{53}H_{78}O_2$) and a peak at M+2 (748) ; the base peak is again at m/e 189.

The diacetate of plastohydroquinone B gives a molecular ion at m/e 832 confirming that PQ B has two hydrogens less than PQ A. Again peaks are obtained at M-69-$(68)_n$ where n = 0,1,2,3,4,5 and 6. All these peaks are also at two mass units lower than the corresponding peaks of the spectrum of the diacetate of plastohydroquinone A.

The base peak in the mass spectrum of the quinone, at m/e 189 shows that the molecule of PQ B contains the structure (IV) (or its isomer) ; the loss of seven isoprene units shows that it also comprises the structure (VI).

It is thus apparent that the supplementary unsaturation of PQ B is located in the part of the molecule between (IV) and (VI), i.e., in the second isoprene unit starting from the quinone ring.

Plastoquinone C

PQ C (isolated from alfalfa) gives a molecular ion at m/e 764

(mass measurement confirms the molecular formula $C_{53}H_{80}O_3$) and a peak at M-18 (mass measurement shows this to correspond to $C_{53}H_{78}O_2$). There is also a peak at M-18+2 corresponding apparently to the M+2 peak mentioned above.

The infrared spectrum of PQ C shows small OH bands at 3545 and 3620 cm^{-1}. (See also the recent communication of Threlfall et al. 1965).

After reductive acetylation of PQ C, a preparation is obtained the mass spectrum of which shows a small molecular peak at m/e 892 corresponding to a triacetate of plastohydroquinone C, a stronger peak at m/e 850 corresponding to a diacetate of a triol and a very strong peak at m/e 832 corresponding to an anhydro-diacetate. These results further confirm the presence of an OH group in PQ C; the difficulty of acetylation and ease of dehydration indicate that it is secondary or tertiary.

Starting from the anhydro-diacetate peak at m/e 832 peaks are obtained at $832-69-(68)_n$, where n = 0,1,2,3,4,5 and 6 which proves that anhydro-PQ C also contains structure (VI). The base peak in the spectrum of plastoquinone C at m/e 189 shows that PQ C contains structure (IV), or its isomer, at least after dehydration.

PQ C thus could be a hydroxy-plastoquinone A, or a hydrated plastoquinone B. In both cases dehydration of PQ C could give PQ B. This point, as well as the exact location of the OH group in PQ C awaits further study.

Plastoquinone D

The mass spectrum of PQ D (prepared from alfalfa) is practically identical to that of PQ C ; thus these two compounds seem to be isomers. Due to lack of material no further analysis could be made on PQ D.

Plastoquinone-4 (=PQ 20) (II)

Thanks to the courtesy of Professor A. Trebst (Göttingen), we were able to measure the mass spectrum of a sample of PQ-4 isolated in his laboratory. The molecular peak at m/e 408 ($C_{28}H_{40}O_2$)

confirms the formula proposed by Eck and Trebst(1963).

On the identity of various PQ B, C and D preparations -

Due to kindness of Professor F. L. Crane preparations of authentic PQ B, PQ C and PQ D from his laboratory have been compared with those prepared by one of us (T. C.) and have proved to be identical (thin layer chromatography and mass spectrometry).

The PQ C' and D' preparations described by Threlfall et al. (1965) are identical respectively with authentic PQ C and PQ D. Moreover the mass spectra of PQ C' and PQ D' measured by Dr. J. W. Cornforth, are in agreement with our results. (Private communication by Professor T. W. Goodwin).

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