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THE STRUCTURE OF THE AZA-ANTHRAQUINONE PHOMAZARIN

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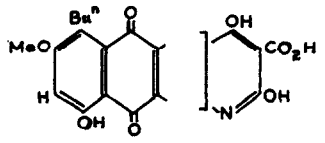
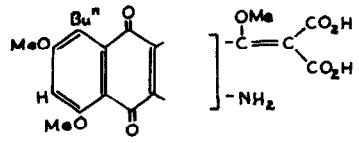
Phoma terrestris Hansen, the fungus responsible for the "pinkroot disease" of onions,[1] produces an orange pigment, phomazarin, $C_{19}H_{17}NO_8$, when grown on Czapek-Dox medium containing starch.[2] This metabolite was suggested by Kögl and his co-workers[2,3,4] to have the unique aza-anthraquinone structure (I), in which the orientation of the heterocyclic ring was not established. Redox properties and colour reactions indicated the presence of a quinonoid system, necessarily a p-quinone in view of the pigment's extreme stability towards alkali. The substitution pattern of the benzenoid ring followed from the isolation[3] of 3-n-butyl-6-hydroxy-4-methoxyphthalic acid[4] after oxidation of the acetylated pigment with chromic acid. Mild treatment[3] of tri-O-methylphomazarin methyl ester, $C_{23}H_{25}NO_8$, with ethanolic alkali gave "dimethylphomazarin hydrate", $C_{21}H_{23}NO_9$. On melting, this latter compound lost the elements of water and carbon dioxide to afford di-O-methyldecarboxyphomazarin, $C_{20}H_{21}NO_6$, identical with material obtained (together with the corresponding tri-O-methyl derivative) by methylation of decarboxyphomazarin with methyl iodide in the presence of



(I)



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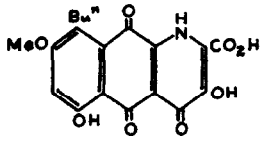

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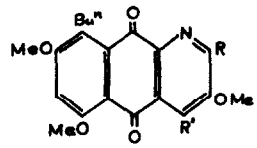
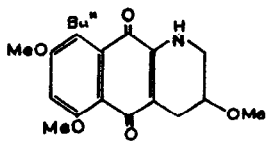


(IV; R = CO_2Me, R' = OMe)

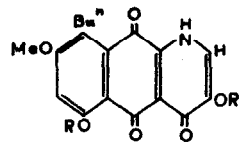
(V; R = H, R' = Cl)

(VI; R = H, R' = OMe)

(VII; R = H, R' = H)

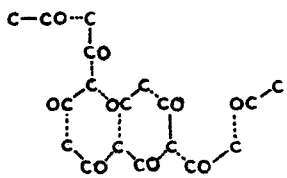


(X)



(VIII; R = Me)

(IX; R = H)



(XI)

silver oxide. Kögl[3] accordingly assigned to "dimethylphomazarin hydrate" the ring-opened malonic acid structure (II), which would cyclise and decarboxylate on heating, and upon this based his formulation of phomazarin (I) as a 2,4-dihydroxy-3-carboxy-1-aza-anthraquinone.[4]

We find that crystallisation of "dimethylphomazarin hydrate" from non-aqueous solvents gives material m.p. 123°, analysing for $C_{21}H_{21}NO_8$. Consequently, Kögl's preparation, m.p. 116° from aqueous methanol, is probably a hydrate of di-O-methylphomazarin, and his evidence[3,4] for the structure (I) of the heterocyclic ring of phomazarin is invalid.* We present here evidence leading to the revised structure (III) for phomazarin.

Hydrolysis and decarboxylation of tri-O-methylphomazarin methyl ester (IV) with hot sulphuric acid gave directly di-O-methyldecarboxyphomazarin (VIII), which with phosphoryl chloride afforded the chlorodeoxy-compound (V), $C_{20}H_{20}ClNO_5$, m.p. 160-161°. Displacement of the introduced halogen function by hydroxide or methoxide ion gave respectively di- or tri-O-methyldecarboxyphomazarin (VIII and VI). Hydrogenation of the chlorodeoxy-compound (V) over palladised charcoal yielded, after aerial re-oxidation of the quinonoid system, di-O-methyldeoxydecarboxyphomazarin (VII), $C_{20}H_{21}NO_5$, m.p. 138-140°, together with the corresponding 1,2,3,4-tetrahydro-derivative (X), $C_{20}H_{25}NO_5$, m.p. 92-93°.

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*This conclusion was reached independently by Dr.K.Schofield and Mr.D.E.Wright, whose work on phomazarin will be reported together with our own in a subsequent full publication.

The deoxydecarboxy-compound (VII) failed to react with o-phenylenediamine under forcing conditions, confirming the presence of a p-quinone nucleus, rather than an o-quinone.

In contrast to the infrared spectrum (in $CHCl_3$) of the aza-anthraquinone (VII), in which both quinonoid carbonyl groups absorbed at 1673 $cm.^{-1}$, the spectrum of the tetrahydro-compound (X) showed separate carbonyl frequencies at 1668 and 1620 $cm.^{-1}$. Such low frequency absorption is indicative of a vinylogous amide system [cf. Cromwell et al;[5] 2-amino-1,4-naphthaquinone[6] has $\nu_{max.}$ (in CCl_4) 1686, 1640 $cm.^{-1}$], and locates the nitrogen function adjacent to the quinonoid ring in (X) and in phomazarin itself (III). In agreement, the tetrahydro-compound (X) was non-basic.

The heteroaromatic methoxyl system in the deoxy-compound (VII) was stable to acid hydrolysis and did not rearrange to an N-methylpyridone on heating with methyl iodide in a sealed tube, suggesting a 1,3-relationship to the nitrogen. In comparison with the n.m.r. spectrum (in $CDCl_3$) of the methyl ester (IV), that of the decarboxy-compound (VI) showed an additional aromatic proton singlet at τ 1.33, corresponding in chemical shift to hydrogen adjacent to heteroaromatic nitrogen.[7] In the deoxydecarboxy-derivative (VII), the protons resonating at τ 1.33 (adjacent to nitrogen) and τ 2.12 were mutually spin-coupled to an extent (3 c./sec.) characteristic[7,8] of m-related aromatic protons. This leads to the arrangement (III) of heterocyclic substituents in phomazarin, which was confirmed by resonances in the n.m.r. spectrum of the tetrahydro-compound (X) at τ 6.2

(C<u>H</u>OMe), 6.56 (CHOC<u>H</u>$_3$ and COC=CNHC<u>H</u>$_2$), and 7.3 (C=CC<u>H</u>$_2$).

In the infrared spectrum (in $CHCl_3$) of di-<u>O</u>-methyldecarboxyphomazarin (VIII), mesomerism lowers to 1640 cm.$^{-1}$ the stretching frequency of the two carbonyl groups β to the amino function, the remaining quinone carbonyl absorbing at 1675 cm.$^{-1}$. Decarboxyphomazarin (IX), $\nu_{max.}$ (in $CHCl_3$) 1630, 1665 cm.$^{-1}$, also possesses a quinone carbonyl group which is involved neither in mesomerism nor in strong H-bonding, despite the presence of a <u>peri</u>-hydroxyl group. This necessitates orientation of the heterocyclic ring in this compound as in (IX), rather than the alternative in which this ring is inverted, and defines the structure (III) for phomazarin.

Phomazarin biosynthesis was shown[9] to involve at least eight acetate units, the carboxyl group arising by oxidation of the methyl carbon of an acetate unit. These observations, previously interpreted in terms of Kögl's formula (I), are in better accord with the revised structure (III), which would be formed from a branched polyketide intermediate (e.g. XI) containing nine acetate units.

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