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THE STRUCTURE OF PHOMAZARIN, A POLYKETIDE AZAANTHRAQUINONE FROM *PYRENOCHAETA TERRESTRIS* HANSEN

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Phomazarin, $C_{19}H_{17}NO_8$, from the fungus *P. terrestris* Hansen, was suggested by Kögl[1] to have one of the two structures (1). The substitution pattern of the heterocyclic ring was later modified,[2] and this together with a consideration of infra-red evidence and biogenetic studies, which confirmed a polyketide origin and the derivation of CO_2H from Me of acetate, suggested (2) as a possible structure.

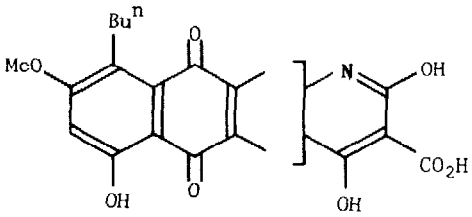
Assignment of the structure of the benzenoid nucleus depended on Kögl's conclusion[1] that (3a) is the structure of an oxidation product, which was isolated but further converted into (3b), m.p. 173-174°. The isolation of the free phthalic acid led us to question this structure since experience[3,4] with such acids substituted adjacent to both CO_2H with groups other than OH, is that the anhydride is produced spontaneously. The acid (3b) was therefore synthesised by Alder-Rickert reaction of 1,5-di-n-butyl-2,4-dimethoxycyclohexa-1,3-diene with dimethyl acetylenedicarboxylate[4] followed by hydrolysis. As expected, the free acid could not be isolated but became converted into an anhydride m.p. 134°, clearly differing from Kögl's anhydride for which he quotes m.p. 170°. Thus Kögl's evidence for the benzenoid substitution is invalid.

We now present evidence for the revised structure (4a), which is also biogenetically acceptable, for phomazarin.

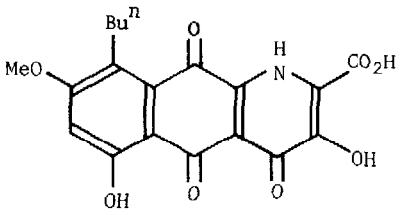
The ^1H n.m.r. resonance of the aromatic proton at the low value of 2.10 τ in trimethylphomazarin methyl ester (4b)[2] is inconsistent with structure (2) as in 1,3-dimethoxyanthraquinones, the 2-proton resonates at *ca.* 3.5 τ.[5] In anthraquinone with 2-methyl-3,4,5,7-tetramethoxy or 3-hexyl-2,4,5,7-tetramethoxy substituents, the 1-proton, peri- to a carbonyl function resonates at 2.13 τ and 2.47 τ respectively,[6,7] indicating probably a similar proton in the phomazarin derivative.

Irradiation of the benzylic methylene triplet at 7.23 τ in (4b) caused a nuclear Overhauser enhancement of 26% in the intensity of the signal at 2.10 τ, indicating an ortho-relationship between this aromatic proton and the butyl group,[8] also in accord with (4a).

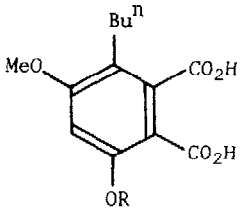
Purdie methylation of phomazarin gave, *inter alia*, a di-O-methylphomazarin methyl ester (4c), $C_{22}H_{23}NO_8$, m.p. 136-138°, which on heating with sulphuric acid produced an O-methyl-decarboxyphomazarin (5a), $C_{19}H_{19}NO_6$, m.p. 228-230°. This with $POCl_3$ gave (5b), $C_{19}H_{18}ClNO_5$, m.p. 197-199°. Similar treatment of tri-O-methylphomazarin methyl ester (4b) gave the chloro-compound (5c), $C_{20}H_{20}ClNO_5$, m.p. 160-161°, exhibiting only one absorption band at ν_{max} 1675 cm^{-1}



(1)

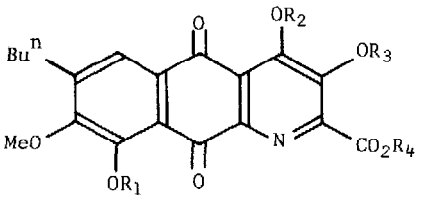


(2)



(3a) R = H

(3b) R = Me



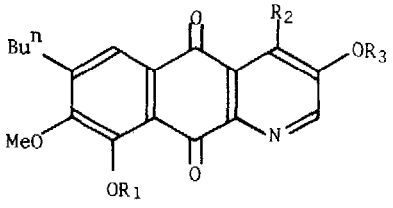
(4a) $R_1 = R_2 = R_3 = R_4 = H$

(4b) $R_1 = R_2 = R_3 = R_4 = Me$

(4c) $R_1 = H$, $R_2 = R_3 = R_4 = Me$

(4d) $R_2 = H$, $R_1 = R_3 = R_4 = Me$

(4e) $R_1 = R_2 = H$, $R_3 = R_4 = Me$



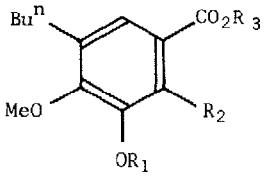
(5a) $R_1 = H$, $R_2 = OH$, $R_3 = Me$

(5b) $R_1 = H$, $R_2 = Cl$, $R_3 = Me$

(5c) $R_1 = R_3 = Me$, $R_2 = Cl$

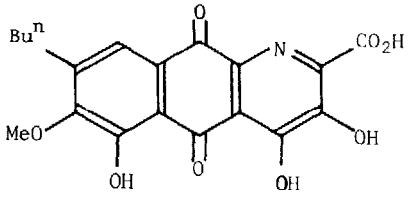
(5d) $R_1 = R_3 = Me$, $R_2 = OH$

(5e) $R_1 = R_3 = H$, $R_2 = OH$



(6a) $R_1 = CO{\cdot}Me$, $R_2 = CO_2H$, $R_3 = H$

(6b) $R_1 = R_3 = Me$, $R_2 = H$



(7)

despite the presence of two quinone carbonyls. In contrast, compound (5b) had absorptions at 1640 and 1670 cm^{-1} indicating one hydrogen-bonded and one unbonded carbonyl. The former was supported by definition of an exchangeable proton at low field, -3.6 τ, and it can only be on the peri-position of the benzenoid ring since the pyridol OH has been replaced.

Confirmation of the general conclusion was obtained by repetition of Kögl's degradation, the initial acetoxy-n-butylmethoxyphthalic acid (6a) being converted by hydrolysis and removal of CO_2H ortho to OH, followed by methylation, to produce (6b) m.p. 77°, identical with an authentic synthetic specimen.[9]

2- And 4- hydroxypyridines and quinolines usually exist as pyridone or quinolone tautomers. ^{13}C n.m.r. studies of phomazarin and its derivatives, to be discussed in detail elsehwere, suggest that the unusual 4-pyridol form predominates. Addition of $Na^{15}NO_3$ (95 atom %) as sole nitrogen source to cultures gave [^{15}N]phomazarin. The derived di-O-methylphomazarin methyl ester (4d)[2] had a low field exchangeable proton, -3.23 τ, showing no ^{15}N-^{1}H coupling (typical $J_{^{15}N-H}$ is 93Hz), indicating its presence in a pyridol OH rather than pyridone NH. A possibility that this result is due to exchange rather than lack of coupling[10] was ruled out by examination of di-O-methyldecarboxyphomazarin (5d), $C_{20}H_{21}{}^{15}NO_6$. The proton on the heterocyclic ring, resonance 1.53 τ, exhibits a two-bond ^{15}N-^{1}H coupling of 10 Hz and the ^{15}N chemical shift of 55 p.p.m. determined by double irradiation, clearly establishes the pyridol structure shown.[11]

The remaining problem is the relative orientation of the unsymmetrical heterocyclic and aromatic rings. The origin of CO_2H in Me of acetate[2] is in accord with structure (4a) only, and excludes (7) if the polyketide chain has the usual head-to-tail linkage. This structure is supported by the infra-red spectra of the O-methyldecarboxyphomazarin (5a) and the O-methylphomazarin methyl ester (4e), $C_{21}H_{21}NO_8$, m.p. 180-182° (obtainable by selective methylation of phomzarin) neither of which show absorption above 1640 cm^{-1} due to unchelated quinone carbonyl. Also in the proton-coupled ^{13}C n.m.r. spectrum of (4b) the quinone carbonyl resonance at 182 p.p.m. shows a coupling to the C-5 proton of 3.9 Hz, whereas in the proton-noise-decoupled spectrum of ^{15}N-enriched (4b) the quinone carbonyl resonance at 179 p.p.m. shows a 2 bond ^{13}C-^{15}N coupling of 7.8 Hz. A similar coupling, 8.8 Hz, is observed on the carbomethoxy resonance at 164 p.p.m. In [^{15}N]quinoline a $^{2}J_{^{13}C-^{15}N}$ coupling of 9.3 Hz is observed for the C-8 resonance. Structure (7) reversing the heterocyclic ring of (4a), would require that both of the above couplings involve the same quinone carbonyl resonance. The infra-red spectrum of decarboxyphomazarin (5a), shows absorptions at 1665 and 1630 cm^{-1}, the former suggesting the presence of a quinone carbonyl not involved in hydrogen-bonding.[2] The origin of this absorption is uncertain.

Alternative modes of biosynthesis of phomazarin (4a) would be by the condensation of two polyketide chains, or by cleavage of an anthrone or anthraquinone derived from one chain. The secalonic acids A and E known to be derived from anthraquinones by quinonoid ring-cleavage have been isolated from *P. terrestris*.[13] Further studies are in progress on phomazarin biosynthesis.

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