Studies in Relation to Biosynthesis. Part 47.[1] Phomazarin. Part 1. The Structure of Phomazarin, an Aza-anthraquinone produced by *Pyrenochaeta terrestris* Hansen

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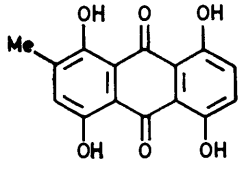
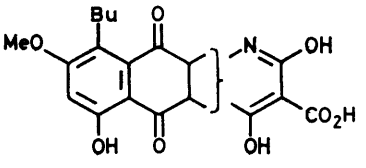
The structure (4) has been established for phomazarin, 6-n-butyl-2-carboxy-3,4,8-trihydroxy-7-methoxy-1-aza-anthraquinone, from n.m.r. and i.r. spectra, and degradative studies of phomazarin and its derivatives. Nitrogen-15 chemical shifts, and $^{15}N-^{1}H$ and $^{13}N-^{15}C$ coupling constants in derivatives of biosynthetically ^{15}N-enriched phomazarin are of particular utility in differentiating between possible alternate structures.

In 1940 Kögl and his co-workers isolated two pigments from the mycelium of *Phoma terrestris* Hansen, the fungus responsible for ' pinkroot disease ' of onions and since re-named *Pyrenochaeta terrestris* Hansen. One of these metabolites was shown to be the anthraquinone cynodontin (1), and the other, phomazarin, an orange pigment, $C_{19}H_{17}NO_8$, was suggested by Kögl to have the unique aza-anthraquinone structure (2), in which the

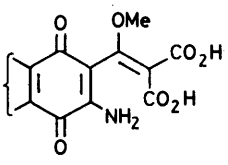


(1)

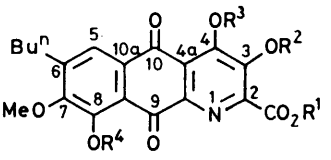


(2)

orientation of the heterocyclic ring was not established.[2-4] The redox properties and colour reactions displayed by phomazarin indicated the presence of a quinonoid system, necessarily a *para*-quinone on account of the extreme stability of phomazarin towards base. The presence of an n-butyl group was deduced from the isolation of n-butyric and valeric acids when phomazarin was oxidised with hydrogen peroxide in sulphuric acid, and larger fragments were obtained by degradation of the triacetate with chromic acid. The main product was thought to be 3-n-butyl-6-hydroxy-4-methoxyphthalic acid which established the benzenoid ring-substitution pattern in (2). The nitrogen function appeared to be tertiary but it could not be quaternised and since one derivative, the methyl ester triacetate, showed basic properties, it was concluded that the nitrogen atom must be in an aromatic ring. The structure of the hetero-aromatic ring was based on mild treatment of tri-*O*-methylphomazarin methyl ester, $C_{23}H_{25}NO_8$, with ethanolic alkali to give ' dimethylphomazarin hydrate ', $C_{21}H_{23}NO_9$, which on melting lost the elements of water and carbon dioxide to afford di-*O*-methyldecarboxy-phomazarin, $C_{20}H_{21}NO_6$, identical with material obtained (together with the corresponding tri-*O*-methyl derivative) by methylation of decarboxyphomazarin. To account for this behaviour, Kögl assigned to ' dimethylphomazarin hydrate ' the ring-opened malonic acid structure (3) which would decarboxylate and recyclise on heating, and upon this based his formulation of phomazarin as a 3-carboxy-2,4-dihydroxy-1-aza-anthraquinone. We



(3)



(4) $R^1 = R^2 = R^3 = R^4 = H$
(5) $R^1 = Me$, $R^2 = R^3 = R^4 = H$
(6) $R^1 = R^2 = Me$, $R^3 = R^4 = H$
(7) $R^1 = R^2 = R^3 = Me$, $R^4 = H$
(8) $R^1 = R^2 = R^3 = R^4 = Me$
(9) $R^1 = R^3 = H$, $R^2 = R^4 = Me$
(10) $R^1 = R^2 = R^4 = Me$, $R^3 = H$

now report studies revising the substitution pattern of both the benzenoid and heterocyclic rings and leading to structure (4) for phomazarin.[5]

RESULTS AND DISCUSSION

Initial work was hampered by the failure of the fungus to produce consistent yields of phomazarin. Previous attempts to culture *P. terrestris* as described by Kögl gave either low yields of phomazarin or the production of cynodontin only.[6] However, modifications of the medium and method of culture in these laboratories have produced, after incubation at 25 °C in shake culture, a good growth of thick filterable mycelium from which phomazarin can be isolated by chloroform extraction after treatment of the dried mycelium with acid, a procedure which avoids Kögl's cumbersome method of refluxing pyridine *in vacuo*.[2]

Treatment of phomazarin (4) with silver oxide and methyl iodide in chloroform, either under reflux (2 h), or overnight (room temperature) gave a quantitative yield of tri-*O*-methylphomazarin methyl ester (8). Shorter reaction times at room temperature gave mixtures of the partial methylation products (5), (6), and (7) which were readily isolated by preparative-layer chromatograhhy. The i.r. spectrum of phomazarin methyl ester

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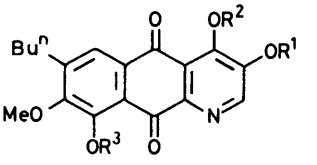
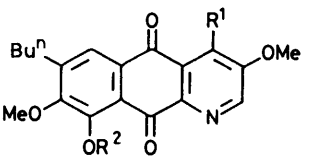
(5) showed no carbonyl absorption above 1 685 cm^{-1} (Table 1), whereas its mono-*O*-methyl derivative (6) showed an ester carbonyl at 1 748 cm^{-1}, indicating the presence of a hydroxy adjacent to the carboxy group in phomazarin (4). Mild hydrolysis of the di-*O*-methylphomazarin methyl ester (7) cleaves the ester and the labile pyridinoid methoxy, re-esterification then affording the mono-*O*-methylphomazarin methyl ester (6). This establishes the relationship of (7) to (6), and in particular the presence of the free 8-hydroxy group in both compounds. Treatment of (8) to give ' dimethylphomazarin hydrate ' as described by Kögl, and recrystallisation from non-aqueous solvents gave material, m.p. 123 °C, analysing for $C_{21}H_{21}NO_8$. Thus Kögl's material, m.p. 116 °C from aqueous methanol, is probably a hydrate of di-*O*-methylphomazarin (9) arising by hydrolysis of the ester and labile pyridinoid methoxy groups. Consequently, his evidence for the structure of the heterocyclic ring of phomazarin is invalid. Esterification of (9) with methanolic hydrochloric acid gave the methyl ester (10) as expected. Similarly, mild hydrolysis of di-*O*-methylphomazarin methyl ester (7), followed by re-esterification, gave the mono-*O*-methylphomazarin methyl ester (6), also obtained as a minor product from direct methylation of phomazarin.

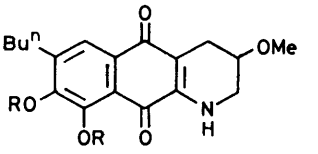
Hydrolysis and decarboxylation of tri-*O*-methylphomazarin methyl ester (8) with hot sulphuric acid gave directly di-*O*-methyldecarboxyphomazarin (13). palladium–charcoal resulted in the uptake of 2 or 4 mol of hydrogen, depending on the reaction time, to give di-*O*-methyldeoxydecarboxyphomazarin (17), along



(11) $R^1 = R^2 = R^3 = H$
(12) $R^1 = Me$, $R^2 = R^3 = H$
(13) $R^1 = R^3 = Me$, $R^2 = H$
(14) $R^1 = R^2 = R^3 = Me$



(15) $R^1 = Cl, R^2 = H$
(16) $R^1 = Cl, R^2 = Me$
(17) $R^1 = H, R^2 = Me$



(18) R = Me
(19) R = H

with the corresponding 1,2,3,4-tetrahydro-derivative (18), after aerial re-oxidation of the quinonoid system. This process was accompanied by considerable decomposition unless triethylamine was added to the

TABLE 1

I.r. data (cm^{-1}) for phomazarin derivatives ($CHCl_3$ solutions)

Compound		ν(CO) bonded	ν(CO) non-bonded	ν(CO) ester or acid	ν(NH)	ν(OH)
(4)		1 633		1 694		
(5)		1 637		1 685		
(6)		1 637		1 748		
(7)		1 641	1 670	1 737		
(8)			1 670	1 735		
(9)		1 640	1 675	1 735		
(10)		1 640	1 675	1 735		
(11)		1 630	1 665			
(12)		1 632				
(13)		1 640	1 675			
(14)			1 675			
(15)		1 640	1 670			
(16)			1 675			
(17)			1 673			
(18)	1 568	1 614 [a]	1 667		3 405	
(19)	1 598	1 636 [b]			3 410	3 525
(34)		1 640	1 670			3 520

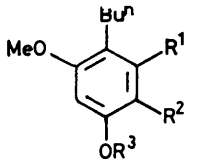
[a] ν(CO) vinylogous amide. [b] Includes vinylogous amide.

In this reaction a methyl ether function has again been hydrolysed, indicating that it must be *peri* to a quinone carbonyl, or *ortho* or *para* to an aromatic nitrogen, or both. That it is located in an active position in a pyridine ring was demonstrated by the replacement of the derived hydroxy by chlorine to give the chloro-compound (16) on treatment of (13) with phosphorus oxychloride. The chlorine atom was itself readily displaced by either hydroxide to give back (13), or by methoxide to give tri-*O*-methyldecarboxyphomazarin (14). Hydrogenation of the chloro-compound (16) over hydrogenolysis mixture. Compound (17) failed to react with *ortho*-phenylenediamine, even under forcing conditions, confirming the presence of an anthraquinone rather than a phenanthraquinone structure in phomazarin.

In contrast to the i.r. spectrum of (17) in which both quinonoid carbonyls absorb at 1 673 cm^{-1}, the spectrum of the tetrahydro-compound (18) showed separate carbonyl bands at 1 667 and 1 614 cm^{-1}. This lowering of a quinone carbonyl absorption frequency can only be due to the presence of a vinylogous amide system,[7] and

so locates the nitrogen function adjacent to the quinonoid ring. In confirmation of the vinylogous amide system, the tetrahydro-compound (18) is non-basic. That the remaining methoxy group in the heteroaromatic ring of (17) is *meta* to the ring-nitrogen was suggested by its resistance to acid hydrolysis and the failure of (17) to rearrange to an *N*-methylpyridone on heating with methyl iodide in a sealed tube. Thus the original hydroxy and carboxy substituents which have been removed during the formation of (17) must occupy the 2- and 4-positions of phomazarin itself. Their relative disposition is indicated by ^{1}H n.m.r. spectroscopy. The ^{1}H n.m.r. spectrum of tri-*O*-methyldecarboxyphomazarin (14) contains *inter alia* an aromatic proton singlet at τ 1.33 (Table 2), corresponding in chemical shift to a proton adjacent to a heteroaromatic nitrogen.[8] In ^{15}N-enriched (13), see below, this proton is coupled to the ring nitrogen, $^2J(^{15}N-^1H)$ 10 Hz. In [^{15}N]pyridine[9] and [^{15}N]quinoline,[10] the 2-proton shows coupling of 10.9 and 11.8 Hz, respectively, to the ring ^{15}N atom. In the ^{1}H n.m.r. spectrum of the deoxydecarboxy-compound (17), the proton at τ 1.33 is *meta*-coupled (J 3 Hz) to the new proton signal at τ 2.12. Thus phomazarin is a 2-carboxy-3,4-dihydroxy-1-aza-anthraquinone. In agreement, the tetrahydro-compound (18) shows resonances at τ 6.2 (C*H*OMe), 6.56 (CHOCH_3 and NHCH_2), and 7.3 (C=CCH_2) (Table 2).

The substitution pattern of the benzenoid ring in Kögl's formulation (2) followed from his conclusion that the phthalic acid isolated from degradation of phomazarin had structure (20). This structure was based on decarboxylation to a benzoic acid (22) and a phenol (24), which were compared with synthetic samples. However, the initial product of decarboxylation should have been the isomeric acid (23) due to preferential loss of the pseudo-β-ketoacid. Moreover, the phthalic acid (20) could not be crystallised but was converted to a crystalline methyl ether (21), m.p. 174 °C. The isolation of this methoxy-phthalic acid is unexpected as experience with such acids, substituted adjacent to both carboxys with groups other than hydroxy, is that the anhydride



(20) $R^1 = R^2 = CO_2H$, $R^3 = H$
(21) $R^1 = R^2 = CO_2H$, $R^3 = Me$
(22) $R^1 = R^3 = H$, $R^2 = CO_2H$
(23) $R^1 = CO_2H$, $R^2 = R^3 = H$
(24) $R^1 = R^2 = R^3 = H$

is formed spontaneously[11,12] in contrast to Kögl's reported isolation of (21), which only formed the anhydride, m.p. 170 °C, on vacuum sublimation.

TABLE 2

Hydrogen-1 chemical shifts (τ) and multiplicities (J/Hz) in the 100 MHz n.m.r. spectra [a] of phomazarin and derivatives

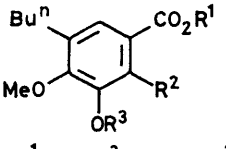
Compound	2-H [b]	5-H [b]	3-OMe [b,c]	4-OMe [b,c]	4-OH [b,d]	7-OMe [b,c]	8-OMe [b,c]	8-OH [b,d]	11-OMe [b,c]	12-CH$_2$ [e]	13CH$_2$, 14CH$_2$ [f]	15-Me [g]	Others
(4) [h]		1.95				5.75				7.06	8.4	8.95	−2.48 (3-OH) [d]
(5)		2.25			−3.12	5.86		−2.91	5.88	7.23	8.4	9.03	
(6)		2.28	6.02		−3.15	5.85		−2.82	5.92	7.23	8.4	9.03	
(7)		2.40	6.00	5.98		5.88		−2.60	5.92	7.25	8.4	9.04	
(8)		2.12	6.05	6.00		5.88	6.00		5.96	7.25	8.4	9.03	
(10)		2.02	6.04		−3.23	5.84	5.96		5.92	7.22	8.4	9.00	
(11)	1.44	1.97				5.80				7.13	8.4	8.98	
(12)	1.51	2.31	5.92		−3.05	5.92		−2.89		7.26	8.4	9.03	
(13)	1.54	2.06	6.04		−2.92	5.95	5.98			7.24	8.4	9.01	
(14)	1.33	2.08	6.05	6.01		5.80	5.80			7.24	8.4	9.05	
(15)	1.31	2.32	5.82			5.93		−2.70		7.24	8.4	9.02	
(16)	1.32	2.06	5.86			5.98	6.02			7.23	8.4	9.01	
(17)	1.33 [i]	2.06	6.05			5.90	5.90			7.30	8.4	9.03	2.12 (4-H) [i]
(18)		2.11	6.56			6.08	6.08			7.30	8.4	9.08	4.11,[d] 6.2, 6.6, 7.3
(19)		2.56	6.59					−2.57		7.23	8.4	9.05	4.05,[d] 4.5,[d] 6.3, 6.6, 7.3
(35)	1.34	2.30	5.83					−2.62		7.24	8.4	9.03	3.62 (7-OH) [d]

[a] For $CDCl_3$ solutions except where stated otherwise. [b] Singlet. [c] Assignments may be interchanged. [d] Exchangeable with D_2O. [e] Triplet (J 7—8 Hz). [f] Broad multiplet. [g] Triplet (J 7 Hz). [h] for CF_3CO_2H solution. [i] Doublet (J 3 Hz).

This conflict has been resolved by an unambiguous synthesis of the phthalic acids (20) and (21) *via* Diels–Alder reaction of dimethyl acetylenedicarboxylate with 1,5-di-n-butyl-2,4-dimethoxycyclohexa-1,3-diene, which was prepared by base-catalysed conjugation of the corresponding 1,4-diene obtained by Birch reduction of 1,5-di-n-butyl-2,4-dimethoxybenzene. The di-n-butyl functionality was required since base-catalysed conjugation of 1-n-butyl-2,4-dimethoxycyclohexa-1,4-diene led to the 1,3-diene with an allylic rather than vinylic butyl group. This diene then formed a Diels–Alder adduct which underwent an Alder–Rickert reaction on pyrolysis with the loss of hex-1-ene to give simply dimethyl 3,5-dimethoxyphthalate. The di-n-butyl functionality overcame this difficulty. The adduct from this diene also lost hex-1-ene on pyrolysis but a butyl group remained on the resultant phthalate ester, saponification of which gave a mixture of acid (21) and the corresponding anhydride, from which no free acid could be isolated. On complete conversion, in refluxing acetic anhydride, the anhydride melted at 134 °C,

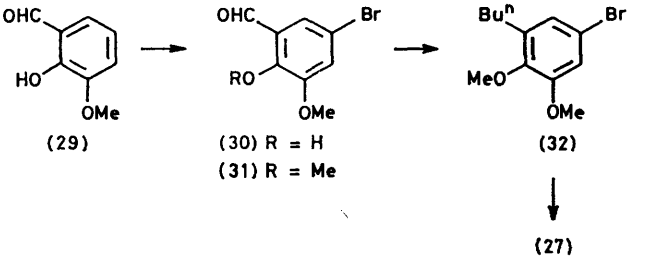
clearly differing from Kögl's anhydride and so phomazarin cannot have the benzenoid substitution pattern depicted in structure (2). Selective demethylation [13] of the anhydride followed by hydrolysis gave the hydroxy-acid (20) which was crystalline, m.p. 156 °C, as expected.

The correct 6-n-butyl-8-hydroxy-7-methoxyazaanthraquinone structure was suggested by the ^{1}H n.m.r. and i.r. spectra of phomazarin derivatives. In contrast to 1,3-dimethoxyanthraquinones [14] where the 2-proton resonates at *ca.* τ 3.5, in the ^{1}H n.m.r. spectrum of tri-*O*-methylphomazarin methyl ester (8), the benzenoid proton resonates at a low value of τ 2.12, a value clearly inconsistent with structure (2). However, in 2-methyl-3,4,5,7-tetramethoxy- and 3-hexyl-2,4,5,7-tetramethoxyanthraquinones [15,16] the 1-protons *peri* to a carbonyl resonate at τ 2.13 and τ 2.47, respectively, indicating that the aromatic proton in phomazarin occupies a *peri* position. Further, irradiation of the benzylic methylene triplet at τ 7.25 in (8) caused a nuclear Overhauser enhancement of 26% in the intensity of the aromatic proton signal, showing that the butyl group is *ortho* to the aromatic proton.[17] Thus the methoxy and hydroxy substituents must occupy the remaining two benzenoid positions. That the hydroxy occupies the remaining *peri*-position was proved by heating the di-*O*-methylphomazarin methyl ester (7) with hot sulphuric acid to give mono-*O*-methyldecarboxyphomazarin (12), which with $POCl_3$ gave the chloro-compound (15). The corresponding compound (16) obtained from tri-*O*-methylphomazarin methyl ester (8), see above, has only one carbonyl absorption band at $\nu_{max.}$ 1 675 cm^{-1}, despite the presence of two quinone carbonyls. In contrast compound (15) has absorptions at 1 640 and 1 670 cm^{-1} indicating one chelated and one free carbonyl. The former is supported in the ^{1}H n.m.r. spectrum of (15) by the presence of a low-field exchangeable proton at τ −2.7, which can only be due to a *peri*-hydroxy on the benzenoid ring since the heterocyclic *peri*-hydroxy has been replaced. Confirmation of this substitution pattern has been obtained by repetition of Kögl's chromic acid degradation of phomazarin triacetate. The initially obtained acetoxy-n-butylmethoxyphthalic acid (25) was converted by hydrolysis and removal of



(25) R^1 = H, R^2 = CO_2H, R^3 = COMe
(26) R^1 = R^2 = R^3 = H
(27) R^1 = R^2 = H, R^3 = Me
(28) R^1 = R^3 = Me, R^2 = H

the carboxy *ortho* to the hydroxy to give the benzoic acid (26), which was methylated to give methyl 5-n-butylveratrate (28), identical with a synthetic sample prepared as shown in the Scheme. The bromo-aromatic (32) was obtained in good yield *via* bromination of *ortho*-vanillin (29) to (30), methylation to give (31), and



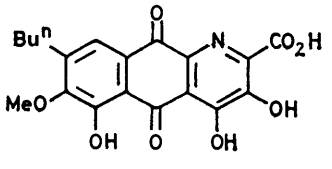
SCHEME

elaboration of the aldehyde to an n-butyl group by standard procedures. Attempts to carboxylate the Grignard reagent from (32) resulted in low yields due to solubility problems. However, the lithium derivative, formed from (32) with n-butyl-lithium, reacted smoothly with carbon dioxide to produce the acid (27) in good yield. The acid was readily methylated with ethereal diazomethane to the ester (28).

The remaining ambiguities in the structure of phomazarin were (*a*) the tautomeric form of the heterocyclic ring and (*b*) the relative orientation of the unsymmetrical benzenoid and heterocyclic rings. 2- and 4-hydroxypyridines and quinolines normally exist as the oxo-tautomers. However, ^{13}C n.m.r. studies of phomazarin derivatives, discussed in the following paper, suggest that the unexpected hydroxy-tautomer predominates. Many methods have been used to investigate tautomerism in heterocyclic compounds, including basicity measurements, i.r. and u.v. spectroscopy, *X*-ray crystallography, and dipole-moment studies,[18] none of which are readily applicable in this case. Nitrogen chemical shifts have been used to distinguish the tautomeric forms of hydroxy- and amino-pyridines and quinolines, being obtained either by direct measurements [19] or by double irradiation (INDOR) [20] techniques. Nitrogen chemical shifts in normal heteroaromatics range from about 60 to 100 p.p.m., *e.g.* 85 and 90 p.p.m. in 3-hydroxy- and 4-methoxy-pyridines respectively, whereas the amide nitrogen shift is *ca.* 200 p.p.m., *e.g.* 209 and 201 p.p.m. in 2- and 4-pyridone respectively.[21] In di-*O*-methyldecarboxyphomazarin (13) the 2-proton appears to be broadened by coupling to the ^{14}N atom. However an INDOR experiment failed to detect the corresponding ^{14}N resonance frequency, presumably because it is very broad due to a large ^{14}N quadrupole relaxation rate. With ^{15}N, spin $\frac{1}{2}$, this problem is removed, and in addition ^{15}N–^{1}H coupling has been used to measure tautomeric equilibria.[22] Thus ^{15}N-enriched phomazarin was prepared by addition of $Na^{15}NO_3$, as the main nitrogen source, to cultures of *P. terrestris*. The ^{1}H n.m.r. spectrum of the derived [^{15}N]di-*O*-methylphomazarin methyl ester (10), has a low-field proton singlet, τ −3.23, which shows no evidence of ^{15}N–^{1}H coupling (typically 90 Hz) indicating that it is a pyridol-O*H* rather than a pyridone-N*H*, though the possibility that the lack of coupling is due to

exchange processes [22] could not be excluded. However, on further conversion to [^{15}N]di-*O*-methyldecarboxyphomazarin (13), the 2-proton exhibits a two-bond ^{15}N–^{1}H coupling of 10 Hz, consistent with the value observed in aromatic heterocycles.[22] In confirmation, the INDOR method allows the ^{15}N chemical shift of 79 p.p.m. to be determined.[23] This value is clearly consistent only with an aromatic ring, and so established the predominance of the 4-hydroxy-tautomer in phomazarin. Recent studies on the protomeric equilibria of simple hydroxypyridines and related compounds show that, in contrast to observations in solution, the hydroxy tautomer is often the more stable form in the gas phase.[24] In solution the main factors influencing the equilibrium are the polarity of the solvent, the presence of strongly electron-withdrawing substituents α to the nitrogen atom, which tend to shift the equilibrium to favour the pyridinoid form by altering the basicity of the nitrogen atom, and the presence of substituents which stabilise one or other of the tautomers by hydrogen bonding.[25] The predominant influence in stabilising the hydroxy form in phomazarin derivatives would appear to be hydrogen bonding to the quinone carbonyl, as this form predominates even after removal of the electron-withdrawing 2-methoxycarbonyl group.

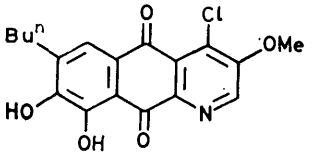
Incorporation studies with [^{14}C]acetate suggest that phomazarin is polyketide in origin, with the carboxy group originating from the methyl carbon of acetate.[26] Of the possible orientations of phomazarin, (4) and (33), only (4) would be consistent with formation of phomazarin *via* the normal processes of polyketide biosynthesis. In the ^{13}C n.m.r. spectrum of tri-*O*-methylphomazarin methyl ester (8), the quinone carbonyls appear at lowest field at 178.9 and 181.3 p.p.m. In the fully ^{1}H-coupled ^{13}C n.m.r. spectrum of (8), the quinone carbonyl resonance at 181.3 p.p.m. appears as a doublet (J 4 Hz) which can only be due to a 3-bond coupling to the 5-proton. In the proton-noise-decoupled spectrum of ^{15}N-enriched (8), the other quinone carbonyl resonance at 178.9 p.p.m. appears as a doublet (J 8 Hz) which must be due to a 2-bond coupling to ^{15}N.[10] A similar coupling is observed on the methoxycarbonyl resonance at 164.4 p.p.m. These couplings are discussed further in the following paper. The occurrence of the ^{1}H and ^{15}N couplings on the alternate quinone carbonyl resonances proves the orientation shown in structure (4) is the correct orientation in phomazarin. Reversing the



(33)

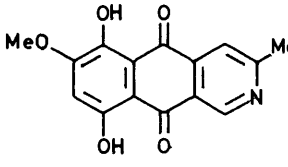
heterocyclic ring, as in (33), would require that both the ^{1}H and ^{15}N couplings appear on the same quinone carbonyl resonance.

The i.r. spectra of phomazarin and its derivatives are summarised in Table 1. Previous attempts to interpret the carbonyl region were hampered by uncertainties due to pyridol–pyridone tautomerism, and the differing hydrogen-bonding possibilities offered by the different possible structures. However, with one exception, all the spectra are readily accounted for. In all compounds with 4-hydroxy and/or 8-hydroxy substituents the *peri*-carbonyl absorption is lowered by chelation, as is the ester carbonyl in phomazarin methyl ester (5). The corresponding hydroxys appear as a very broad band in the 3 500—3 000 cm^{-1} region. In the ^{1}H n.m.r. spectra of these compounds (Table 2) the bonded hydroxys can be seen as sharp singlets at very low field, τ *ca.* −3, and the effect of chelation on the carbonyl resonance in the ^{13}C n.m.r. spectrum is discussed in the following paper. The lowering of the C-10 carbonyl absorption in the tetrahydro-compound (18) is in agreement with the i.r. spectrum of 2-amino-1,4-naphthoquinone which shows $\nu_{max.}$ 1 686 and 1 640 cm^{-1}.[7] On demethylation of (18) to the 7,8-dihydroxy-derivative (19), chelation to the 8-hydroxy lowers the absorption of the other carbonyl to 1 636 cm^{-1}. Both (18) and (19) show strong N–H stretching bands at 3 405 cm^{-1}; and (19) also shows a strong O–H stretch at 3 525 cm^{-1}, and an exchangeable proton singlet at τ 4.50 due to the 7-hydroxy. Both these features due to a 7-hydroxy

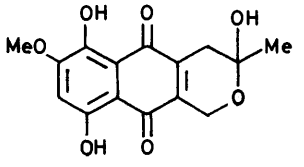


(34)

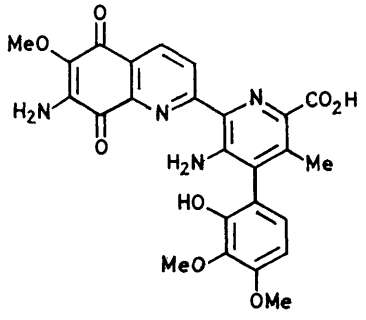
are also apparent in the spectra of the demethyl-derivative (34).



(35)



(36)



(37)

Decarboxyphomazarin (11) unexpectedly shows two bands at 1 665 and 1 630 cm^{-1} and so would appear to

possess a quinone carbonyl not involved in either mesomerism with the nitrogen, or hydrogen-bonding to a *peri*-hydroxy, and this led to the wrong orientation being assigned to phomazarin in a previous study.[5a] However, 3-*O*-methyldecarboxyphomazarin (12), shows no carbonyl absorption above 1 632 cm^{-1}. Decarboxyphomazarin is an extremely insoluble compound, so its anomalous i.r. behaviour may be due to some form of strong inter-molecular association.

Phomazarin is the only naturally occurring 1-azaanthraquinone known. Bostrycoidin (35), is a 2-azaanthraquinone, which co-occurs in *Fusarium solani* with fusarubin (36), and it seems likely that bostrycoidin is formed *in vivo* by condensation of the corresponding aldehyde with ammonia.[27] Streptonigrin (37) is a complex 5-azanaphthoquinone isolated from *Streptomyces flocculus*.[28]

EXPERIMENTAL

Melting points were taken with a Kofler hot-stage microscope. Unless otherwise stated i.r. spectra were measured for solutions in chloroform, u.v. spectra in methanol, and ^1H n.m.r. spectra at 100 MHz in deuteriochloroform with $SiMe_4$ as internal reference. Mass spectra were recorded at 70 eV with an A.E.I. MS9 high-resolution spectrometer. Light petroleum refers to the fraction with boiling point 60—80 °C. Silica gel GF (Merck) was used for preparative layer chromatography (p.l.c.).

Cultivation of P. terrestris *and Isolation of Metabolites.*—The medium, consisting of starch (50 g), sodium nitrate (2 g), potassium dihydrogenphosphate (1 g), magnesium sulphate heptahydrate (0.5 g), calcium chloride (0.5 g), and ferrous sulphate heptahydrate (0.01 g), was made up to 1 l with distilled water, divided amongst 10 250-ml conical flasks and sterilised at 120 °C for 20 min. Each flask was then innoculated with a mycelial suspension of *Pyrenochaeta terrestris* Hansen (CBS 37752) in distilled water and incubated on a rotary shaker for 5 d at 25 °C. This 5-d culture was used to innoculate 42 flasks of the above medium (10 ml of shake culture mycelial suspension per flask) and these were incubated at 25 °C for 1 month on the rotary shaker. The deep purple mycelium was then filtered off and dried. The dried mycelium (90 g) was finely powdered and exhaustively extracted with ether, acetone, and methanol in a Soxhlet apparatus. The residual mycelium (80 g) was washed with light petroleum and air-dried and then acidified with cold 2M aqueous hydrochloric acid, washed with water until neutral, dried, and further extracted with ether, chloroform, and acetone. Chromatography of the chloroform extract (2 g) on acid-washed silica gel produced cynodontin (400 mg) on elution with light petroleum. It crystallised from pyridine with m.p. 260 °C (lit.,[29] m.p. 260 °C); the tetra-acetate had m.p. 223 °C (lit.,[29] m.p. 224—225 °C). Further elution with chloroform produced a red solid consisting of a mixture of two compounds of similar R_F value [t.l.c. using silica gel G containing 3% oxalic acid, eluant benzene–ethyl formate (10 : 3)]. The solid was taken up in chloroform and diluted with methanol to precipitate the less polar of the two compounds (500 mg) which crystallised from methanol with m.p. 202—203 °C with evolution of gas and re-solidification, remelting at 258 °C. ^1H N.m.r. spectroscopy showed this compound to be an adduct of phomazarin and methanol. Addition of water destroyed the adduct and precipitated phomazarin, m.p. 196 °C (lit.,[2] m.p. 196 °C); $\lambda_{max.}$ 231, 277, and 430 nm (log ε 4.54, 4.77, and 3.92 respectively); *m/e* 387 (98%), 343 (100), 341 (50), 328 (14), 314 (36), 300 (44), 296 (34), and 271 (34). The remaining solution, after precipitation of phomazarin, was concentrated to about one-fifth its former volume, and a red solid (contaminated with phomazarin) (80 mg) was obtained on cooling. Three crystallisations from glacial acetic acid produced deep red needles, m.p. 215—216 °C (60 mg). This metabolite, *isophomazarin*, is the subject of a later paper. The remaining mycelium was washed with light petroleum and air-dried. It was then refluxed with 10% dry hydrogen chloride in methanol for 2.5 h, cooled to 0 °C, filtered free of reagent and air-dried. Soxhlet extraction of the mycelium with chloroform gave a red solid (1.5 g). Several crystallisations of this material from methanol yielded pure phomazarin methyl ester (5) (500 mg), m.p. 213 °C (lit.,[1] m.p. 213 °C); $\lambda_{max.}$ 229, 278, and 427 nm (log ε 4.34, 4.60, and 3.92 respectively); *m/e* 401 (100%), 341 (51), 313 (19), and 271 (19).

Production of [^{15}N]*Phomazarin.*—The medium (500 ml) was made up as before, but with replacement of the sodium nitrate with sodium [^{15}N]nitrate (1 g) (95 atom-%), and distributed among 5 flasks, which were innoculated from a pre-grown 5-d culture. After 14 d growth the mycelium was filtered off and phomazarin isolated as before. Mass spectra showed the phomazarin to contain mainly [^{15}N]phomazarin methyl ester, M^+ 402 (100%).

Purdie Methylation of Phomazarin.—(*a*) Silver oxide (750 mg) and methyl iodide (2 ml) were added to a solution of phomazarin (120 mg) in chloroform (20 ml) and the reaction mixture was stirred at room temperature for 24 h. The solid residues were then filtered off, and the solvent removed to give an orange solid. Recrystallisation from methanol gave tri-*O*-methylphomazarin methyl ester (8) as orange needles, m.p. 136—138 °C (lit.,[2] m.p. 131 °C); $\lambda_{max.}$ 270 and 355 nm (log ε 4.69 and 3.80 respectively); *m/e* 443 (100%), 429 (8), 412 (5), 383 (10), 368 (15), 354 (4), and 340 (7). (*b*) Phomazarin (120 mg) was treated as above, but was worked-up after only 5 h. T.l.c. [$CHCl_3$–MeOH (100 : 4)] showed the presence of two spots at R_F *ca.* 0.6 and 0.5. These products were isolated by p.l.c. The upper band gave an orange solid (45 mg), recrystallised from methanol to give tri-*O*-methylphomazarin methyl ester (8), m.p. 136—138 °C. The lower band gave an orange solid (60 mg) which recrystallised from methanol to give *di-O-methylphomazarin methyl ester* (7) as orange rods, m.p. 131—133 °C; $\lambda_{max.}$ 258, 273, and 414 nm (log ε 4.32, 4.40, and 3.94); *m/e* 429 (100%), 414 (10), 400 (14), 398 (6), 302 (7), 369 (9), 368 (10), 354 (15), and 340 (7) (Found: C, 61.71; H, 5.69; N, 3.01. $C_{22}H_{23}NO_8$ requires C, 61.53; H, 5.40; N, 3.26%). (*c*) Phomazarin (100 mg) was treated as above but was worked-up after 2 h. T.l.c. showed the presence of starting material ($R_F = 0$) and two further products (R_F *ca.* 0.1 and 0.3), which were isolated by p.l.c. The more polar compound crystallised from methanol to give phomazarin methyl ester as yellow needles, m.p. 213 °C. The less polar compound crystallised from methanol to give *mono-O-methylphomazarin methyl ester* (6) as orange needles, m.p. 180—182 °C; $\lambda_{max.}$ 236, 265, 293 (sh), and 446 nm (log ε 4.23, 4.28, 3.98, and 3.61 respectively); *m/e* 415 (100%), 400 (6), 355 (40), 354 (34), 326 (12), 313 (9), 312 (10), 303 (9), and 285 (11) (Found: C, 60.77; H, 4.93; N, 3.10. $C_{21}H_{21}NO_8$ requires C, 60.72; H, 5.10; N, 3.37%).

Preparation of 'Dimethyl Phomazarin Hydrate'.—This

compound was prepared as described by Kögl. Careful recrystallisation in the dark from benzene–light petroleum gave the product (9) as yellow needles, m.p. 122—123 °C (with decarboxylation), re-solidifies at 125 °C and re-melts at 180.3 °C; $\lambda_{max.}$ 272 and 370 nm (log ε 4.53 and 3.90 respectively) (Found: C, 60.82; H, 5.16; N, 3.70; OMe, 21.76. $C_{21}H_{21}NO_8$ requires C, 60.72; H, 5.10; N, 3.37; OMe, 21.0%). Esterification of (9) by refluxing in 10% dry hydrogen chloride in methanol for 30 min gave *di-O-methylphomazarin methyl ester* (10), as orange needles, m.p. 138—140 °C; $\lambda_{max.}$ 259, 272, and 404 nm (log ε 4.36, 4.44, and 3.73 respectively) (Found: C, 61.76; H, 5.32; N, 3.20. $C_{22}H_{23}NO_8$ requires C, 61.53; H, 5.40; N, 3.26%); *m/e* 429 (100%), 401 (5), 369 (78), 356 (75), 354 (30), 328 (25), 326 (25), 312 (15), and 298 (30).

Preparation of Mono-O-methylphomazarin Methyl Ester (6).—Di-*O*-methylphomazarin methyl ester (7) (40 mg) was refluxed for 35 min in 1N sodium hydroxide (2 ml) in ethanol (4 ml). Water (30 ml) was added, and the deep purple solution was acidified (concentrated HCl), and the resultant orange precipitate collected by filtration. This material was then refluxed in 2% methanolic HCl (10 ml) for 15 min. On removal of solvent and recrystallisation from methanol, mono-*O*-methylphomazarin methyl ester (6), m.p. 180—181 °C was obtained.

Acid Hydrolysis and Decarboxylation of Tri-O-methylphomazarin Methyl Ester (8) *and Di-O-methylphomazarin Methyl Ester* (7).—A solution of (8) (44 mg), 6N H_2SO_4 (20 ml) and methanol (6 ml) was boiled under reflux for 5.5 h. Carbon dioxide was collected as $BaCO_3$ (yield 15.2 mg, 77% of one carboxy group). The red solution was cooled in ice, neutralised to pH 3.5 with alkali and the resulting fine orange needles filtered off and washed with water, to give di-*O*-methyldecarboxyphomazarin (30 mg), m.p. 184—186 °C (lit.,[3] m.p. 185 °C); $\lambda_{max.}$ 225, 268, and 350 nm (log ε 4.39, 4.55, and 3.86 respectively).

Similar treatment of (7) gave *mono-O-methyldecarboxyphomazarin* (12), as orange needles from chloroform–methanol, m.p. 228—230 °C; $\lambda_{max.}$ 260, 272 (sh), 290 (sh), and 432 nm (log ε 4.42, 4.37, 4.07, and 3.86 respectively); *m/e* 357 (100%), 343 (45), 328 (31), 314 (53), 301 (55), 286 (23), and 272 (17) (Found: C, 63.30; H, 5.18; N, 3.60. $C_{19}H_{19}NO_6$ requires C, 63.81; H, 5.36; N, 3.92%).

Preparation of Chlorodeoxydecarboxyphomazarins (16) *and* (15).—Di-*O*-methylcarboxyphomazarin (13) (29 mg), phosphorus oxychloride (0.75 ml), and dry benzene (0.75 ml) were refluxed for 30 min. This mixture was then evaporated to dryness *in vacuo* and the remaining orange oil taken up in chloroform (20 ml). This solution was washed with water, dried ($MgSO_4$), and evaporated to dryness to afford a yellow solid. Recrystallisation from methanol gave *di-O-methylchlorodeoxydecarboxyphomazarin* (16) (22 mg), m.p. 160—161 °C; $\lambda_{max.}$ 262, 292, and 350 nm (log ε 4.46, 4.38, and 3.82 respectively) (Found: C, 61.80; H, 5.15; N, 3.84. $C_{20}H_{20}ClNO_5$ requires C, 61.71; H, 5.17; N, 3.59%); *m/e* 391 (45%), 389 (100), 374 (50), and 346 (25).

Analogous treatment of mono-*O*-methyldecarboxyphomazarin (12) gave the *mono-O-methylchloro-derivative* (15) as yellow needles, m.p. 197—198 °C (from methanol); $\lambda_{max.}$ 245, 270, 297, and 402 nm (log ε 4.17, 4.31, 4.16, and 3.68 respectively) (Found: C, 60.50; H, 5.05; N, 3.30. $C_{19}H_{18}ClNO_5$ requires C, 60.73; H, 4.83; N, 3.73%); *m/e* 377 (42%), 375 (100), 360 (30), 347 (31), 329 (93), 319 (15), and 305 (14).

Preparation of Tri-O-methyldecarboxyphomazarin (14).—A solution of sodium methoxide in methanol (3.0 ml, 0.4N) was heated at reflux for 2.5 h with di-*O*-methyldeoxydecarboxyphomazarin (16) (19 mg). The solution was evaporated to dryness, taken up in benzene and washed with water. The dried benzene solution was then chromatographed on Florisil, the yellow chloroform eluate evaporated to dryness, and the residue recrystallised to afford tri-*O*-methyldecarboxyphomazarin (16 mg), m.p. 159—160 °C (lit.,[2] m.p. 160 °C); $\lambda_{max.}$ 266 and 355 nm (log ε 4.38 and 3.74 respectively).

Catalytic Hydrogenation of Di-O-methylchlorodeoxydecarboxyphomazarin (16).—Di-*O*-methylchlorodeoxydecarboxyphomazarin (77 mg) was dissolved in warm methanol (10 ml) and added to a pre-hydrogenated palladium–charcoal catalyst (5%, 70 mg) in methanol (25 ml) containing triethylamine (0.3 ml). This mixture was hydrogenated at room temperature until 2 mol of hydrogen were absorbed, the colour changing from yellow to green. The catalyst was then filtered off and air passed through the filtrate for 30 min. After evaporation of the solvent *in vacuo*, the residue was dissolved in benzene and chromatographed on Florisil. A trace of a red product was eluted with 20% chloroform in benzene followed by a yellow product eluted with 20% methanol in chloroform. This was recrystallised from methanol to give *di-O-methyldeoxydecarboxyphomazarin* (17) (43 mg), m.p. 138—139 °C; $\lambda_{max.}$ 256, 295, and 355 nm (log ε 4.31, 4.30, and 3.65 respectively) (Found: C, 67.59; H, 5.71; N, 4.16. $C_{20}H_{21}NO_5$ requires C, 67.59; H, 5.96; N, 3.94%). A repeat hydrogenation allowing 4 mol of hydrogen to be absorbed and working-up as above afforded *di-O-methyltetrahydrodeoxydecarboxyphomazarin* (18), recrystallised from light petroleum (b.p. 30—40 °C), m.p. 92—93 °C; $\lambda_{max.}$ 236, 281, 361, and 480 nm (log ε 4.23, 4.47, 3.65, and 3.45 respectively) (Found: C, 66.98; H, 7.30; N, 4.34; OMe, 25.6. $C_{20}H_{25}NO_5$ requires C, 66.83; H, 7.01; N, 3.90; OMe, 25.9%); *m/e* 359 (100%), 335 (16), 328 (93), 314 (16), and 229 (5).

Demethylation of the Tetrahydro-compound (18).—The tetrahydro-compound (18) (32 mg) was dissolved in dry methylene chloride (10 ml) and boron trichloride (0.5 ml) was added. After stirring for 45 min at room temperature, the reaction mixture was diluted with chloroform (40 ml) and washed with water (2 × 30 ml). Removal of solvent gave a dark gum which was purified by p.l.c. Elution with chloroform–methanol (96 : 4), removal of the band at R_F 0.44, and recrystallisation, gave the demethylated product (19) as purple crystals, m.p. 158—159 °C (from acetone–light petroleum); $\lambda_{max.}$ 277, 338, and 432 nm (log ε 4.32, 3.74, and 3.50 respectively) (Found: M^+, 331.141 9. $C_{18}H_{21}NO_5$ requires M, 331.142 0); *m/e* 331 (91%), 316 (8), 300 (100), 298 (21), and 286 (15).

Demethylation of Di-O-methylchlorodeoxydecarboxyphomazarin.—The chloro-compound (16) (20 mg) was dissolved in methylene chloride and cooled to −10 °C. Boron trichloride in slight excess was added and the mixture was set side for 3 h. Destruction of excess of reagent with water, followed by isolation of the product by chloroform extraction, gave a red solid (15 mg) which crystallised from methanol, to give the demethylated product (34), m.p. 211—212 °C.

Preparation of Decarboxyphomazarin.—Phomazarin was decarboxylated as described by Kögl,[2] by subliming at 220—230 °C under high vacuum. Recrystallisation from methanol gave decarboxyphomazarin (11), m.p. 253—255 °C (lit.,[1] m.p. 254 °C); $\lambda_{max.}$ 229, 270, and 435 nm (log ε 4.34,

4.47, and 3.96 respectively); *m/e* 343 (100%), 328 (13), 314 (30), 310 (11), 300 (38), 296 (29), 272 (26), and 271 (21).

Synthesis of 6-*n*-*Butyl*-3,4-*dimethoxyphthalic Acid* (21).—(*a*) 2,4-*Dimethoxybutyrophenone*. Polyphosphoric acid was prepared by equilibrating a mixture of orthophosphoric acid (250 g), and phosphorus pentaoxide (250 g) at 100 °C for 1 h. Butyric acid (50 g, 0.57 mol) was added and the mixture was equilibrated for a further 15 min. Resorcinol dimethyl ether (50 g, 0.36 mol) was then added and the deep red mixture was reacted for 45 min, when it was quenched with ice and extracted with ether. The organic extract was washed with 2M aqueous sodium hydroxide and water, dried, and evaporated to dryness to leave a red oil. Distillation gave a clear, colourless oil (80 g), b.p. 156—158 °C at 3 mmHg (lit.,[30] 146—147 °C at 2 mmHg); $\nu_{max.}$ (film) 1 660s, 1 590s, and 1 560s cm^{-1}.

(*b*) 1-*n*-*Butyl*-2,4-*dimethoxybenzene*. The phenone (above, 65.5 g, 0.32 mol) was treated with lithium aluminium hydride–aluminium chloride.[31] The compound was obtained as a clear oil on distillation, b.p. 81—82 °C at 0.1 mmHg (44 g); $\nu_{max.}$ (film) 1 620s and 1 590s cm^{-1}; τ 3.12 (br d, *J* 7 Hz, 6-H), 3.71 (br s, 3-H), 3.74 (dd, *J* 7 and 2 Hz, 5-H), 6.25 and 6.30 (s, 2 ArO*Me*), 7.51 (br t, *J* 7 Hz, $ArCH_2C_3H_7$), 8.56 (m, $ArCH_2C_2H_4Me$), and 9.09 (br t, *J* 6 Hz, ArC_3H_6Me) (Found: C, 74.4; H, 9.13. $C_{12}H_{18}O_2$ requires C, 74.19; H, 9.34%).

(*c*) 5-*n*-*Butyl*-2,4-*dimethoxybutyrophenone*. This was prepared by condensation of the butylresorcinol dimethyl ether (above, 3.94 g, 0.22 mol) with butyric acid (50 g, 0.57 mol) in polyphosphoric acid (500 g) as previously described. Reaction time was 2.5 h at 100 °C. The phenone was a pale yellow liquid, b.p. 139 °C at 0.05 mmHg (60 g), which solidified on cooling, forming yellow prisms, m.p. 34—35 °C; $\nu_{max.}$ (film) 1 670s, 1 615s, and 1 585s cm^{-1}; τ 2.52 (s, 6-H), 3.70 (s, 3-H), 6.14 and 6.16 (s, 2 ArO*Me*), 7.22 (t, *J* 6 Hz, $ArCOCH_2Et$), 7.50 (br t, *J* 8 Hz, $ArCH_2C_3H_7$), 8.42 (sextet, *J* 6 Hz, $ArCOCH_2CH_2Me$), 8.53 (m, $ArCH_2C_2H_4Me$), and 9.05 (br t, *J* 6 Hz, $ArCOC_2H_4Me$ and ArC_3H_6Me) (Found: C, 73.37; H, 8.74. $C_{16}H_{24}O_3$ requires C, 72.69; H, 9.15%).

(*d*) 1,5-*Di*-*n*-*butyl*-2,4-*dimethoxybenzene*. The phenone (above, 42.5 g, 0.16 mol) was reduced with lithium aluminium hydride–aluminium chloride reagent as previously described. Distillation of the crude dialkyl aromatic gave a clear oil (36 g), b.p. 102 °C at 0.05 mmHg; $\nu_{max.}$ (film) 1 615s and 1 590s cm^{-1}; τ 3.28 (s, 6-H), 3.74 (s, 3-H), 6.26 (s, 2 ArO*Me*), 7.53 (br t, *J* 7 Hz, 2 $ArCH_2C_3H_7$), 8.56 (m, 2 $ArCH_2C_2H_4Me$), and 9.08 (br t, *J* 6 Hz, 2 ArC_3H_6Me).

(*e*) 1,5-*Di*-*n*-*butyl*-2,4-*dimethoxycyclohexa*-1,3-*diene*. The dimethoxydibutylbenzene (above, 12 g) was dissolved in a mixture of tetrahydrofuran (40 ml) and t-butyl alcohol (30 ml). Ammonia (150 ml) was distilled off sodium and ferric nitrate into the reaction mixture. Lithium (2.8 g) was slowly added and the reaction was allowed to proceed for 3 h. Excess of lithium was destroyed by the addition of ethanol, and the reaction mixture was then diluted with water and extracted with light petroleum. The extracts were washed with water, dried (sodium sulphate), and evaporated to dryness to leave a pale yellow oil (11.5 g). This oil, without further purification, was added to a solution of potassium t-butoxide in dry DMSO (7 g, 40 ml) and maintained under a dry nitrogen atmosphere at 25 °C for 3 h. The reaction was then quenched with water and extracted with light petroleum. The extracts were washed with water and dried (sodium sulphate). Evaporation left a yellow oil which distilled as a clear, colourless liquid (10.7 g), b.p. 70—75 °C at 0.03 mmHg. This was shown to consist of the required conjugated diene (90%) by ^1H n.m.r. and u.v. spectroscopy; $\lambda_{max.}$ 276 nm (calc. 275);[32] τ (CCl_4) 5.26 (s; olefinic proton).

(*f*) 6-*n*-*Butyl*-3,5-*dimethoxyphthalic anhydride*. The conjugated diene mixture (above, 10.7 g, 0.04 mol) was treated with dimethyl acetylenedicarboxylate (8 g, 0.06 mol) at room temperature. The reaction temperature rose to 120 °C during 15 min with the formation of a red solution. The mixture was set aside overnight and then heated to 190 °C for 45 min and vacuum-distilled to remove excess of reagent. Crude dimethyl 6-n-butyl-3,5-dimethoxyphthalate distilled as a yellow viscous oil, b.p. 160—165 °C at 0.1 mmHg. Treatment of this material with ethanolic potassium hydroxide (6 g in 60 ml) at reflux for 1 h followed by acidification gave a mixture of 6-n-butyl-3,5-dimethoxyphthalic acid (21) and the anhydride. The mixture was dissolved in acetic anhydride (20 ml) and maintained at 100 °C for 1 h. Removal of volatile material by vacuum distillation left a tacky residue which solidified on treating with ether. The solid crystallised from glacial acetic acid as plates, m.p. 134 °C, and was identified as the required anhydride (1.2 g); $\nu_{max.}$ 1 830s, 1 770s, 1 630m, and 1 595m cm^{-1}; τ 3.34 (s, 4-H), 5.94 and 6.01 (s, ArO*Me*), 7.00 (br t, *J* 7 Hz, $ArCH_2C_3H_7$), 8.65 (m, $ArCH_2C_2H_4Me$), and 9.05 (br t, *J* 6 Hz, ArC_3H_6Me); $\lambda_{max.}$ 226, 247, and 346 nm (ε 25 000, 25 200, and 7 900 respectively) (Found: C, 63.62; H, 6.30. $C_{14}H_{16}O_5$ requires C, 63.62; H, 6.10%).

(*g*) 6-*n*-*Butyl*-3-*hydroxy*-5-*methoxyphthalic anhydride*. The dimethoxy anhydride (above, 620 mg, 3 mmol) was dissolved in anhydrous dichloromethane (20 ml) and cooled to −10 °C. Boron trichloride was added in slight excess and the mixture was left to warm to room temperature overnight. Destruction of the excess of reagent with water, followed by extraction of the organic material with ethyl acetate in the usual manner, yielded a pale yellow solid which on washing with ether–light petroleum gave the white hydroxy-anhydride (530 mg). It crystallised from acetone–ether as plates, m.p. 160—162 °C; $\nu_{max.}$ 3 200s, 1 830s, 1 745s, 1 645m, and 1 620s cm^{-1}; τ 3.32 (s, 4-H), 6.06 (s, ArO*Me*), 7.02 (br t, *J* 7 Hz, $ArCH_2C_3H_7$), 8.55 (m, $ArCH_2C_2H_4Me$), and 9.05 (br t, *J* 6 Hz, ArC_3H_6Me); $\lambda_{max.}$ 227, 246, and 346 nm (ε 25 400, 12 500, and 3 200 respectively).

(*h*) 6-*n*-*Butyl*-3-*hydroxy*-5-*methoxyphthalic acid* (20). The hydroxy anhydride (above, 175 mg) was shaken with 2M aqueous sodium hydroxide solution (10 ml) until dissolution was complete. Acidification with dilute sulphuric acid and extraction of the acid with ethyl acetate gave a colourless solid which crystallised from ether–light petroleum as rhomboids (110 mg), m.p. 155—165 °C; $\nu_{max.}$ 3 190s, 1 710s, 1 650s, and 1 600s cm^{-1}; τ (hexadeuterioacetone) 3.48 (s, 4-H), 4.20 (br, $W_{\frac{1}{2}}$ 60 Hz, ArO*H*, and 2 $ArCO_2H$), 6.09 (s, ArO*Me*), 7.48 (br t, *J* 6 Hz, $ArCH_2C_3H_7$), 8.56 (m, $ArCH_2C_2H_4Me$), and 9.08 (br t, *J* 6 Hz, ArC_3H_6Me); $\lambda_{max.}$ 258 and 307 nm (ε 6 000 and 4 000 respectively) (Found: C, 58.31; H, 6.05. $C_{13}H_{16}O_6$ requires C, 58.20; H, 6.01).

5-*Bromo*-ortho-*vanillin* (30).—*ortho*-Vanillin was brominated by the method of Brink in 95% yield. The compound crystallised from dilute ethanol, m.p. 128 °C (lit.,[33] m.p. 129 °C); τ 0.16 (s, ArC*H*O), 2.72 and 2.86 (d, *J* 2 Hz, ArH_2), and 6.10 (s, ArO*Me*).

5-*Bromo*-ortho-*veratraldehyde* (31).—5,Bromo-*ortho*-vanillin (2.31 g, 10 mmol) was dissolved in DMF (20 ml) containing sodium hydroxide (400 mg, 10 mmol) and the mixture

was stirred at 70 °C for 30 min. Methyl iodide (3 equiv.) was added and the mixture was set aside for 12 h and then poured into water. The mixture was extracted with ether, washed with 2M aqueous sodium hydroxide and water, dried, and evaporated to dryness to lave a white solid (1.5 g) which crystallised from dilute ethanol, m.p. 84 °C; $\nu_{max.}$ 1 675s and 1 580s cm^{-1}; τ −0.35 (s, ArC*H*O), 2.44 and 2.74 (d, *J* 2 Hz, ArH_2), and 6.00 and 6.08 (s, 2 ArO*Me*).

1-(5-*Bromo*-2,3-*dimethoxyphenyl*)*butan*-1-*ol*.— 5-Bromo-*ortho*-veratraldehyde (800 mg) was reacted with the Grignard reagent (in ether) formed from magnesium (2 equiv.) and n-propyl bromide (2 equiv.) in the usual manner to give the alcohol esentially quantitatively; a 14-h reaction time was required; $\nu_{max.}$ (film) 3 400s and 1 575s cm^{-1}; τ 2.95 and 3.12 (d, *J* 2 Hz, ArH_2), 5.10 [br t, *J* 6 Hz, ArC*H*(OH)Pr^n], 6.20 (s, 2 ArO*Me*), 7.64 [br s, ArCH(O*H*)Pr], 8.50 [m, ArCH(OH)C_2H_4Me], and 9.08 [br t, *J* 8 Hz, ArCH(OH)-C_2H_4*Me*]. The alcohol was used in the next step without further purification.

1-*n*-*Butyl*-5-*bromo*-2,3-*dimethoxybenzene* (32).—The alcohol was quantitatively dehydrated to the styrene by refluxing in benzene solution containing toluenesulphonic acid hydrate (0.2 equiv.). Water was removed by a Dean–Stark trap over a reaction time of 6 h; $\nu_{max.}$ (film) 1 645w, 1 580s, 1 560s, and 970s cm^{-1}; τ 2.85 and 3.18 (d, *J* 2 Hz, ArH_2), 3.40 and 3.80 (centres of AB quartet, J_{AB} 16 Hz, with high-field portion further split into t, *J* 6 Hz, ArC*H*=C*H*Et), 6.20 and 6.28 (s, 2 ArO*Me*), 7.78 (quintet, *J* 6 Hz, ArCH=CHCH_2Me), and 8.94 (t, *J* 6 Hz, ArCH=CHCH$_2$*Me*). The styrene was hydrogenated at 15 °C and 1 atm in ethanol using platinum oxide catalyst (0.05 equiv.) to yield the alkylbenzene quantitatively; $\nu_{max.}$ (film) 1 585s and 1 570s cm^{-1}; τ 3.14 (coalesced AB quartet, ArH_2), 6.20 and 6.24 (s, 2 ArO*Me*), 7.44 (br t, *J* 7 Hz, ArCH_2Pr), 8.56 (m, $ArCH_2C_2H_4$Me), and 9.10 (br t, *J* 7 Hz, ArC_3H_6*Me*).

Methyl 5-*n*-*Butylveratrate* (28).—The bromo-aromatic (31) (1.09 g, 4 mmol) was dissolved in dry ether and cooled to 0 °C. n-Butyl-lithium (2.25M in hexane, 4.2 mmol) was added and the mixture was stirred at 0 °C for 45 min. Excess of solid carbon dioxide was added and the mixture was set aside until it had returned to room temperature. Dilute hydrochloric acid was added and the substituted benzoic acid was extracted with ether. The extract was separated into neutral and acid fractions and thus yielded the crude substituted benzoic acid (27) as a red gum (660 mg). The gum was sublimed at 120 °C at 0.2 mmHg as a white solid, m.p. 77 °C; τ 2.39 and 2.49 (d, *J* 2 Hz, ArH_2), 6.08 and 6.11 (6, 2 ArO*Me*), 7.52 (br t, *J* 6 Hz, ArC$H_2C_3H_7$), 8.50 (m, $ArCH_2C_2H_4$Me), and 9.04 (br t, *J* 6 Hz, ArC_3H_6Me) (Found: C, 65.13; H, 7.54. $C_{13}H_{18}O_4$ requires C, 65.53; H, 7.61%). The acid was methylated quantitatively with diazomethane to give the required ester (28); $\nu_{max.}$ (film) 1 715s and 1 585s cm^{-1}; $\tau(CCl_4)$ 2.62 and 2.67 (d, *J* 1 Hz, ArH_2), 6.14 and 6.18 (s, 2 ArO*Me* and CO_2*Me*), 7.41 (br t, *J* 7 Hz, ArC$H_2C_3H_7$), 8.52 (m, $ArCH_2$-C_2H_4Me), and 9.05 (br t, *J* 6 Hz, ArC_3H_6*Me*).

Methyl 5-*n*-*Butylveratrate from Phomazarin*.—Phomazarin (250 mg) was converted to the triacetate (150 mg) as described by Kögl. The acetate was a yellow powder of indefinite m.p. Oxidation of this material by chromic acid as described gave an orange-red gum (20 mg) after hydrolysis of the acetoxy group. This gum was dissolved in hot water (10 ml), filtered, and the resulting clear solution was acidified to pH 2 with concentrated sulphuric acid (1 drop). The acidified solution was refluxed for 2.5 h and then concentrated to 2 ml *in vacuo*. This solution was extracted with ether and the ether extracts were washed with saturated brine solution, dried, and evaporated to leave an orange gum (10 mg). Purdie methylation of this material followed by purification *via* preparative g.l.c. (5% CW20M at 180 °C) gave a clear oil (2 mg), the mass spectrum and u.v. spectrum of which were identical with those of synthetic methyl 5-n-butylveratrate (28).

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