Phomazarin. Part 3.[1] The Structure of Isophomazarin

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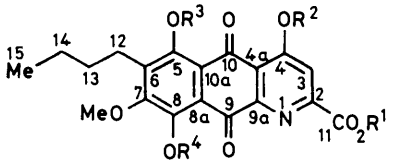
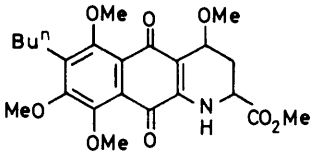
Isophomazarin, a minor pigment isolated from the mycelium of *Pyrenochaeta terrestris* has been assigned the structure 6-n-butyl-4,5,8-trihydroxy-7-methoxy-1-aza-anthraquinone-2-carboxylic acid from biogenetic considerations and a comparison of its chemical behaviour, and of spectral data of its derivatives with those of phomazarin.

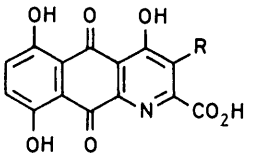
FROM chromatography of the mycelial extracts of *Pyrenochaeta terrestris*, we have isolated, in low yield, a



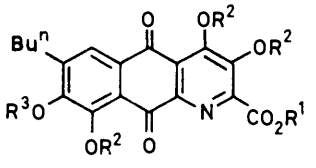
(1) $R^1 = R^2 = R^3 = R^4 = H$
(2) $R^1 = Me, R^2 = R^3 = R^4 = H$
(3) $R^1 = R^2 = R^3 = Me, R^4 = H$
(4) $R^1 = R^2 = R^4 = Me, R^3 = H$
(5) $R^1 = R^3 = R^4 = Me, R^2 = H$
(6) $R^1 = R^2 = R^3 = R^4 = Me$



(7)



(8a) R = H
(8b) R = OMe



(9) $R^1 = R^2 = H, R^3 = Me$
(10) $R^1 = R^3 = Me, R^2 = H$
(11) $R^1 = R^2 = R^3 = Me$
(12) $R^1 = R^2 = R^3 = H$

red pigment, $C_{19}H_{17}NO_8$, m.p. 215—216 °C, isomeric with phomazarin (9), whose structure determination and biosynthesis have been discussed in previous papers.[1,2] We now report studies leading to structure (1) for this new metabolite, isophomazarin.

RESULTS AND DISCUSSION

The similarity of their physical properties and spectral data suggested a close structural relationship between phomazarin and isophomazarin. The ^1H n.m.r. spectrum of iso-phomazarin (Table 1) showed the presence of an uncoupled aromatic proton, methoxy, and n-butyl groups as in phomazarin.[2] On treatment with methanolic HCl, isophomazarin formed a methyl ester (2), m.p. 201—202 °C, whose i.r. spectrum (Table 2) indicated the presence of an unchelated ester carbonyl, ν(CO) at 1 730 cm^{-1}, in contrast to phomazarin methyl ester (10) in which the ester is chelated and has ν(CO) at 1 685 cm^{-1}, and chelated quinone carbonyls at ν(CO) 1 615 cm^{-1}. In addition to the butyl, methoxy, and aromatic proton resonances, the ^1H n.m.r. spectrum of (2) showed three low-field exchangeable protons indicating the presence of three strongly hydrogen-bonded phenolic groups in isophomazarin. If isophomazarin has the same 1-aza-anthraquinone structure as phomazarin then, as none of these phenolic hydroxys can be adjacent to the ester function, they must occupy the three remaining *peri*-positions to the quinone carbonyls, the fourth being occupied by the heteroaromatic nitrogen, see below. Thus isophomazarin must have the partial structure (8).

The presence of three phenolic hydroxys was confirmed by Purdie methylation of isophomazarin to give both di-*O*-methylisophomazarin methyl ester (3), m.p. 197—201 °C and tri-*O*-methylisophomazarin methyl ester (6), m.p. 171—175 °C. The latter compound was clearly different from tri-*O*-methylphomazarin methyl ester (11), m.p. 136—138 °C, and so isophomazarin is not a simple methylation isomer of phomazarin, in accord with partial structure (8). The i.r. spectrum of the di-*O*-methyl compound (3) shows the presence of both chelated and non-chelated quinone carbonyls with ν(CO) at 1 625 and 1 680 cm^{-1}, respectively, whereas in the tri-*O*-methyl derivative both quinone carbonyls absorb showing ν(CO) at 1 680 cm^{-1}. The di-*O*-methylisophomazarin methyl ester is assigned structure (3) by analogy with phomazarin, in which the last position to be methylated is the 8-OH.[1] However, structure (4) is also a possibility for this compound and cannot be definitely excluded on the available evidence.

In a series of nuclear Overhauser experiments with tri-*O*-methylisophomazarin (6) it was found that irradiation of the benzylic methylene protons at τ 7.21 caused no enhancement of the aromatic proton intensity and so they were not adjacent to each other as in phomazarin. However, irradiation of the methoxy resonance at τ 5.89 did result in an enhancement of *ca.* 15% in the intensity of the aromatic proton resonance, indicating the presence of a methoxy *ortho* to the aromatic proton. This behaviour, considered with the chemical shift of

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the aromatic proton, is in accord with partial structure (8a) only and means that the remaining butyl and methoxy substituents must be on carbons 6 and 7. The chemical shift of the aromatic proton is much too low for it to be on C-6 or C-7.

TABLE 1

Hydrogen-1 n.m.r. spectra of isophomazarin and related compounds [a]

Compound	3-H	12-CH_2 [b]	13-CH_2, 14-CH_2 [c]	15-Me [b]	OH [d,e]	OMe [d]	Other
(1) [f]	1.72 [d]	7.06	8.4	8.97		5.86	
(2)	2.12 [d]	7.22	8.4	9.02	−3.68, −2.68, −2.49	5.82, 5.93	
(3)	2.01 [d]	7.20	8.4	9.00	−3.40	5.79, 5.90, 5.97, 6.02	
(5)	2.10 [d]	7.20	8.4	9.00	−2.90	5.84, 5.91, 5.95, 6.00	
(6)	2.08 [d]	7.21	8.4	9.00		5.84, 5.92, 5.89, 6.02, 602	
(7)	8.4 [c]	7.24	8.4	9.01		6.02, 6.10, 6.14, 6.30, 6.64	3.80 (N*H*),[e] 5.24 (4-*H*),[c] 5.78 (2-*H*) [b]

[a] For $CDCl_3$ solutions except where stated otherwise. [b] Triplet (J 7 Hz). [c] Multiplet. [d] Singlet. [e] Exchangeable with D_2O. [f] For CF_3CO_2H solution.

TABLE 2

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

	Compound				
	(2)	(3)	(5)	(6)	(7)
ν(CO) bonded	1 615	1 625	1 620		1 625 *
ν(CO) unbonded		1 680	1 675	1 680	1 670
ν(CO) ester	1 730	1 725	1 730	1 730	1 740
ν(NH)					3 390

* Vinylogous amide.

The presence of the heterocyclic ring in isophomazarin was confirmed by the ease of reduction of (6) over palladium–charcoal to give, after aerial re-oxidation of the quinone system, the tetrahydro-derivative (7), whose i.r. spectrum shows absorptions at 1 740, 1 670, and 1 625 cm^{-1}. The latter absorption can only be due to a quinone carbonyl whose absorption frequency has been lowered by vinylogous amide tautomerism, and so locates the nitrogen adjacent to the quinonoid ring as in phomazarin. The ^1H n.m.r. spectrum of (7) is consistent with the proposed heterocyclic ring structure, and showed that one of the methyl ester functions has moved upfield to τ 6.64, a shift typical of aliphatic methoxys, and the aromatic proton resonance in (6) has been replaced by a multiplet at *ca.* τ 8.4. In addition the methyl ester resonance moves upfield to τ 6.30. The presence of a 4-hydroxy substituent in isophomazarin is also confirmed by treatment of tri-*O*-methylisophomazarin methyl ester (6) with hot sulphuric acid, to give an acidic product which, on esterification with methanol–HCl, gave di-*O*-methylisophomazarin methyl ester (5), m.p. 190—191 °C. The ready hydrolysis of the 4-*O*-methyl ether function is in accord with it being *para* to the heteroaromatic nitrogen, and that it must be *peri* to a quinone carbonyl is evidenced by the lowering of a carbonyl absorption frequency to 1 620 cm^{-1} in the infrared spectrum of (5), and by the presence of a low-field exchangeable proton at τ −2.90 in the ^1H n.m.r. spectrum of (5). It is noteworthy that no decarboxylation occurred in this reaction as similar treatment of di- and tri-*O*-methylphomazarin methyl esters resulted in the formation of the corresponding decarboxy-derivatives. However on prolonged treatment of phomazarin methyl ester itself (10), with hot sulphuric acid, no decarboxylation occurred, the sole product being the 7-demethyl derivative (12). Thus it would appear that under acidic conditions, the participation of an *ortho*-methoxy function is necessary to effect decarboxylation.

The ^{13}C n.m.r. spectra of isophomazarin methyl ester (2) and the tri-*O*-methyl derivative (6) (Table 3) are in full agreement with the above structure. In (2) the quinone carbonyl resonances appear at 188.3 and 183.6 p.p.m. and can be assigned to C-10 and C-9 respectively. In the fully ^1H-coupled ^{13}C spectrum, both these resonances appear as sharp singlets as there is no possibility of coupling to the aromatic proton, as is observed for phomazarin derivatives. The resonance at 134.0 p.p.m. appears as a partially resolved multiplet in the coupled spectrum due to coupling to the benzylic methylene protons, and so can be assigned to C-6. In phomazarin methyl ester (11), C-6 resonates at 144.9 p.p.m. (Table 3) and so in (2), C-6 is moved upfield by 10.9 p.p.m. due to

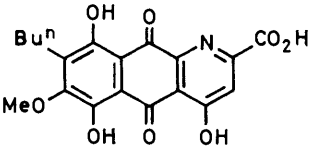
TABLE 3

Carbon-13 n.m.r. spectra [a] of isophomazarin derivatives (2) and (6), and the corresponding phomazarin derivatives (10) and (11)

	Compound			
Carbon	(2)	(6)	(10)	(11)
C-2	153.2 [b]	153.0	132.2	149.7
C-3	118.0	111.4	152.5	151.4
C-4	169.0	167.4	158.3	160.6
C-4a	117.5	129.3	117.3	130.3
C-5	164.3	164.8	122.2	124.8
C-6	134.0	134.0	144.9	144.9
C-7	154.9	153.6	153.6	153.6
C-8	156.6	159.9	156.6	157.9
C-8a	111.3	120.3 [b]	115.8	125.0
C-9	183.6	178.9	184.8	178.9
C-9a	150.1 [b]	148.6 [c]	140.4	146.7
C-10	188.3	186.6	187.6	181.3
C-10a	107.0	122.3 [b]	126.1	125.1
C-11	160.0	159.9	169.5	164.4
C-12	23.8	23.8	30.2	30.4
C-13	31.0	31.2	31.7	32.2
C-14	22.9	23.0	22.6	22.7
C-15	13.9	13.9	13.9	13.9
MeO	61.6	61.4	60.5	61.3
MeO	53.4	61.4	53.5	52.3
MeO		58.4		62.5
MeO		57.3		62.5
MeO		53.5		61.6

[a] In p.p.m. relative to $SiMe_4$, $CDCl_3$ solutions. [b,c] Assignments may be interchanged.

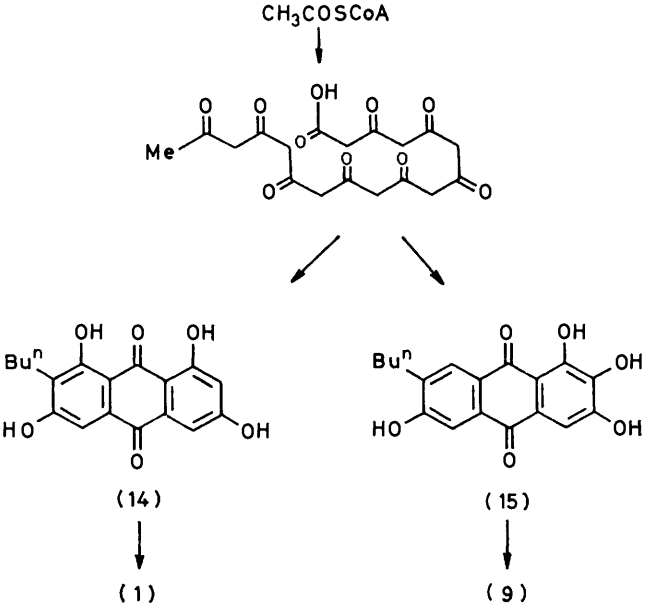
the *ortho*-shielding effect of the 5-hydroxy. Similarly, see below, C-10a is moved upfield from 126.1 p.p.m. in (10) to 107.0 p.p.m. in (2). The resonance at 164.3 p.p.m. in (2) is assigned to C-5 as in the fully ^{1}H-coupled spectrum it appears as a doublet of triplets [$^2J(^{13}C–O–^1H)$ 6 Hz and $^3J(^{13}C–C–C–^1H)$ 4 Hz]. On addition of D_2O, the doublet coupling to the chelated hydroxy proton was removed, the remaining coupling being to the benzylic methylene protons. In phomazarin methyl ester, the protonated aromatic carbon appears as a doublet of triplets due to coupling to the H-5 and H-12 protons respectively: however, in isophomazarin methyl ester, C-3 gives a doublet [$^1J(^{13}C–^1H)$ 171 Hz] of broad signals which sharpen considerably on addition of D_2O, consistent with C-3 being adjacent to the chelated 4-hydroxy. On the basis of expected chemical shifts and couplings in the fully ^{1}H coupled spectrum, the resonances at 117.5, 111.3, and 107.0 p.p.m. are assigned to C-4a, C-8a, and C-10a, respectively. In the coupled spectrum the resonance at 117.5 p.p.m. appears as a broad signal, which sharpened to a doublet [$^3J(^{13}C–C–^1H)$ 4 Hz] on addition of D_2O and so must be assigned to C-4a, the remaining coupling being to H-3. Similarly the resonances at 111.3 and 107.0 p.p.m. appeared in the coupled spectrum as a broad singlet and a doublet [$^2J(^{13}C–O–^1H)$ 4 Hz] respectively, both collapsing to sharp singlets on addition of D_2O and resultant exchange of the adjacent chelated hydroxy protons. The highest field resonance (107.0 p.p.m.) was assigned to C-10a as it should appear at higher field than C-8a due to the extra shielding it experiences from the *para* 7-methoxy group. Of the remaining non-aliphatic resonances in (2), all showed coupling to either methoxy or hydroxy except the resonances at 150.1 and 153.2 p.p.m. and so these were assigned to C-9a and C-2. Both C-2 and C-4 appeared at much lower field than in phomazarin methyl ester (10), as the shielding effect of the 3-hydroxy in phomazarin methyl ester has been removed. The remaining resonances were readily assigned by comparison with phomazarin derivatives. The differences in chemical shifts in the trimethyl derivative (6), particularly those of C-4a, C-8a, and C-10a, were consistent with the previously observed effect of removal of chelation on methylation of the phenolic hydroxys. No significant change is observed in the C-4 resonance on methylation, consistent with isophomazarin having a pyridol rather than a pyridone ring structure, in agreement with previous observations with phomazarin.[2]



(13)

It must be noted that structure (13) with the opposite orientation of the heterocyclic ring cannot be positively excluded on the above data but it is unlikely in view of the co-occurrence of phomazarin and isophomazarin. Moreover structure (1) is fully consistent with a polyketide derivation whereas (13) is not. The shortage of material available precluded any further experiments to clarify this point.

The co-occurrence of phomazarin and isophomazarin is biosynthetically interesting as isophomazarin retains the oxygen from the terminal carboxy of the precursor polyketide chain which is lost in phomazarin itself but lacks the 'extra' 3-hydroxy function. Thus neither phomazarin nor isophomazarin is derived from the other but most likely represent different initial products, *e.g.* (14) and (15) (Scheme), after condensation of the pre-



SCHEME Parallel biosynthetic pathways to phomazarin and isophomazarin

cursor nonaketide which then both undergo an exactly parallel sequence of secondary modifications, including (not necessarily in this order) C-4 hydroxylation, *O*-methylation, oxidative ring-cleavage, transamination, and heterocyclic ring closure to give the final metabolites.

EXPERIMENTAL

General details have been described previously.[2]

Isophomazarin (1).—Chromatography of the chloroform extract of the acidified mycelia of *P. terrestris* gave a red gum, which after several recrystallisations from acetic acid produced pure *isophomazarin* (1) as red needles (60 mg), m.p. 215—216 °C (Found: C, 59.2; H, 4.5; N, 3.9. $C_{19}H_{17}NO_8$ requires C, 58.9; H, 4.4; N, 3.6%).

Isophomazarin Methyl Ester (2).—Isophomazarin (20 mg) was dissolved in methanol (2 ml) containing one drop of concentrated hydrochloric acid and the solution was refluxed for 3 h. A fluffy red precipitate formed which could be recrystallised from methanol as red needles (20 mg), m.p. 201—202 °C; $\lambda_{max.}$ 248 and 263 nm (log ε 4.53 and 4.60 respectively) (Found: C, 59.6; H, 4.9; N, 3.7. $C_{20}H_{19}NO_8$ requires C, 59.9; H, 4.7; N, 3.5%).

Purdie Methylation of Isophomazarin Methyl Ester.—The

methyl ester (24 mg) was dissolved in chloroform (15 ml) and silver oxide (200 mg) and methyl iodide (1 ml) were added and the mixture stirred at room temperature for 15 h. After filtering off solid residues and removal of solvent an orange gum was obtained. This was purified by preparative t.l.c. on a 20 × 20 Kieselgel GF plate (0.5 mm thick), eluent chloroform–methanol (96 : 4); removal of the yellow band (R_F 0.53) and recrystallisation from methanol gave *di*-O-*methylisophomazarin methyl ester* (3) as orange crystals (10 mg), m.p. 197—201 °C; $\lambda_{max.}$ 256, 276(sh), and 450 nm (log ε 4.29, 4.10, and 3.63 respectively) (Found: C, 61.49; H, 5.41; N, 3.30. $C_{22}H_{23}NO_8$ requires C, 61.53; H, 5.40; N, 3.26%); *m/e* 429 (100%), 414 (13), 400 (10), 398 (8), 386 (48), 373 (14), and 357 (9). Isophomazarin (30 mg) was treated as above, but the reaction was worked-up after 24 h. Recrystallisation of the resultant orange-red gum from methanol gave *tri*-O-*methylisophomazarin methyl ester* (6) as orange needles (12 mg), m.p. 171—175 °C; $\lambda_{max.}$ 260 and 452 nm (log ε 4.31 and 3.54 respectively) (Found: M^+, 443.158 0. $C_{23}H_{25}NO_8$ requires M, 443.158 0); *m/e* 443 (100%), 429 (57), 427 (40), 414 (15), 400 (15), 386 (21), and 368 (17).

Di-O-*methylisophomazarin* (5).—Tri-*O*-methylisophomazarin was dissolved in methanol (1 ml), 6N sulphuric acid (4 ml) was added, and the mixture refluxed for 18 h. The reaction mixture was worked-up to give a red gum which was refluxed in 5% methanolic HCl for 2 h. Removal of solvent gave a red solid which was recrystallised from methanol to give *di*-O-*methylisophomazarin methyl ester* (5) as orange needles (2 mg), m.p. 190—191 °C; $\lambda_{max.}$ 250, 265, and 440 nm (log ε 4.32 and 3.60 respectively) (Found: M^+, 429.143 4. $C_{22}H_{23}NO_8$ requires M, 429.142 4); *m/e* 429 (100%), 414 (4), 400 (11), 386 (44), 373 (15), and 357 (19).

Catalytic Hydrogenation of Tri-O-*methylisophomazarin Methyl Ester* (6).—Compound (6) (10 mg) was dissolved in methanol (10 ml). Triethylamine (0.2 ml), and 5% palladium–charcoal catalyst (20 mg) were added and the mixture was hydrogenated at room temperature. The catalyst was then filtered off and air passed through the filtrate for 10 min. After removal of solvent, the orange residue was purified by t.l.c. on a 20 × 20 silica GF_{254} plate, eluent chloroform–methanol (96 : 4). The yellow band, R_F 0.6, was removed and eluted to give *tri*-O-*methyltetrahydroisophomazarin methyl ester* (7) as an orange gum (4 mg); $\lambda_{max.}$ 272, 313, and 374 nm (log ε 4.34, 3.97, and 3.66 respectively) (Found: M^+, 447.189 1. $C_{23}H_{29}NO_8$ requires M, 447.189 3); *m/e* 447 (14), 416 (45), 356 (100), 342 (21), and 328 (16).

Acid Hydrolysis of Phomazarin Methyl Ester (10).—A solution of phomazarin methyl ester (20 mg), 6N H_2SO_4 (10 ml), and methanol (4 ml) was refluxed for 24 h. The resulting solution was cooled, extracted with chloroform, and the red residue obtained on removal of solvent was recrystallised from methanol to give 7-*demethylphomazarin* (12) as red needles (10 mg), m.p. 192—196 °C; *m/e* 373 (100%), 329 (40), 300 (67), 287 (50), and 286 (47); τ(CF_3CO_2H) 1.92 (1 H), 7.08 (2 H, t, *J* 7 Hz), 8.4 (4 H, m), and 9.00 (3 H, t, *J* 7 Hz).

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[1] Part 2; A. J. Birch and T. J. Simpson, preceding paper.
[2] A. J. Birch, D. N. Butler, R. Effenberger, R. W. Rickards, and T. J. Simpson, *J.C.S. Perkin I*, 1979, 807.