Chemistry of Quinones. Part V.[1] Structure of Cladofulvin, a Bianthraquinone from *Cladosporium Fulvum* Cooke

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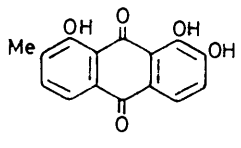
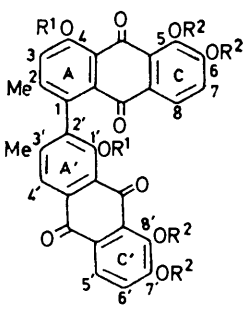
Chemical and spectroscopic evidence indicates that the natural quinone cladofulvin is the unsymmetrical 5,7'-didehydro-dimer of 1,2,8-trihydroxy-6-methylanthraquinone (nataloe-emodin), 1',4,5,6,7',8'-hexahydroxy-2,3'-dimethyl-1,2'-bianthracene-9,9',10,10'-tetrone (II; $R^1 = R^2 = H$).

AGOSTI *et al.* isolated a pigment, cladofulvin, from the phytopathogenic fungus *Cladosporium fulvum* Cooke [syn. *Fulvia fulvum* (Cooke) Ciferri] and on the basis of classical degradations and spectroscopic (i.r. and u.v.–visible) data suggested it had structure (I).[2] This structure was questioned by Chari *et al.*[3] and in Part IV we showed that cladofulvin was different from a synthetic sample of the quinone (I).[1] We have now re-isolated cladofulvin from cultures of *C. fulvum* and on the basis of the additional evidence presented below conclude that cladofulvin is the bianthraquinone (II; $R^1 = R^2 = H$). Cladofulvin appears to be the first example of a natural αβ'-bianthraquinone.



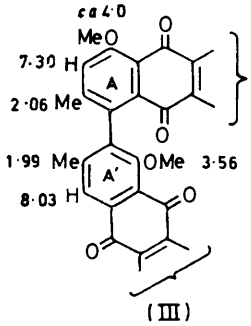
(I)



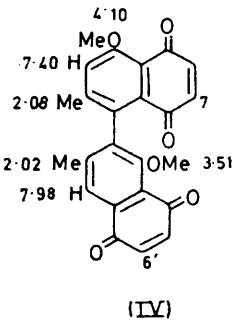
(II)

The pigment isolated had essentially the same m.p., i.r. spectrum (all the 19 bands previously reported), and u.v.–visible spectrum as those reported by Agosti *et al.*,[2] but we have shown by mass spectrometry and elemental analysis that the pigment has the molecular formula $C_{30}H_{18}O_{10}$, and is thus a bianthraquinone. Free rotation about the C–C bond joining the quinone nuclei is evidently not possible as cladofulvin is optically active ($[\alpha]_D^{20}$ −743°). The pigment reacted with diazomethane to give a tetramethyl ether with the same m.p. and i.r. carbonyl frequencies as those reported [2] for the similarly prepared 'cladofulvin dimethyl ether.' However, the properties of the hexamethyl ether which we obtained by exhaustive methylation of the pigment differ from those reported [2] for 'cladofulvin trimethyl ether' (found; m.p. 151·5—152·5°, *M* 622; reported;[2] m.p. 212—214°, *M* 312). Agosti *et al.* did not report a yield for the methylation reaction and it is possible that the product they isolated was derived from an impurity in the pigment: *C. fulvum* is known to produce other anthraquinones.[2] Unfortunately none of the samples of Agosti *et al.* were available for comparison.

The ^1H n.m.r. spectrum of the hexamethyl ether contained signals due to six *O*-methyl groups, two slightly different *C*-methyl groups, and six aromatic protons. In the ^1H n.m.r. spectra of other bianthraquinones the signals due to protons in groups *ortho* to the bond joining the quinone nuclei resonate at higher field than usual,[4,5] because the nuclei are approximately at right angles to each other (to minimise steric interactions between the *ortho*-substituents) and this results in each *ortho*-substituent being in the shielding cone of the neighbouring aromatic ring. In the present case a methoxy-group and two β-methyl groups resonated at unusually high field. Other positions in the rings which contain these substituents are, in view of the results below, occupied by a methoxy-group and two protons which appear as singlets at chemical shifts which indicate that one is an α- and one a β-proton. These spectral data indicate that rings A and A' of cladofulvin hexamethyl ether [see structure (III)] resemble the benzenoid



(III)



(IV)

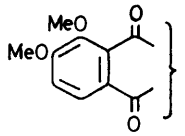
Chemical shifts (δ): the assignments of the *C*-methyl groups are possibly reversible

rings of isodiospyrin dimethyl ether (IV) [6] and in agreement with this the shift values for the two compounds are very similar.

The four remaining aromatic protons gave two AB systems (each with *J* 8 Hz), indicating two pairs of *ortho*-protons. Decoupling experiments showed that the proton at δ 8·10 was coupled to that at 7·22 and that at 7·72 was coupled to that at 7·10. These chemical shifts are consistent with each AB system being due to

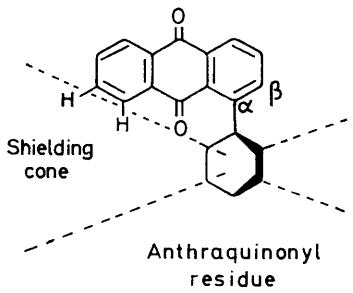
[1] Part IV, D. G. Davies, P. Hodge, and P. Yates, preceding paper.
[2] G. Agosti, J. H. Birkinshaw, and P. Chaplen, *Biochem. J.*, 1962, **85**, 528.
[3] V. M. Chari, S. Neelakantan, T. R. Seshadri, *Tetrahedron Letters*, 1967, 999.
[4] Y. Ogihara, N. Kobayashi, and S. Shibata, *Tetrahedron Letters*, 1968, 1881.
[5] N. L. Dutta, A. C. Ghosh, P. A. Nair, and K. Venkataraman, *Tetrahedron Letters*, 1964, 3023.
[6] A. L. Fallas and R. H. Thomson, *J. Chem. Soc.* (*C*), 1968, 2279.

one α- and one β-proton and hence rings c and c′ must have the partial formula (V). One α-proton resonated at somewhat higher field than the other, and the α-protons in some α,α′-bianthraquinonyls resonate at



(V)

higher field than those in the corresponding monomeric quinones.[4] These differences arise because each anthraquinonyl residue shields the α-protons in the other half of the molecule [4] [see (VI)]. In contrast, only protons which are very close to an anthraquinonyl residue in a β-position will be within its shielding cone. This suggests that in the present case the α-proton resonating at higher field is at C-8 in ring c [formula (II)] and hence



(VI)

this quinone nucleus has α-methoxy-groups in a 1,8-relationship.*

It is clear from the above discussion and the results of Agosti *et al.*[2] that one anthraquinone nucleus in cladofulvin has α-hydroxy-groups in a 1,8-relationship and the only structural point remaining is whether the α-hydroxy-groups in the second nucleus are in a 1,5- or 1,8-relationship. The behaviour of cladofulvin with diazomethane indicates that the latter is the case. As noted above the pigment reacts with this reagent to give a tetramethyl ether. As the longest wavelength maximum in the u.v.–visible spectrum [7] of the ether is only at 410 nm, each nucleus must contain only one α-hydroxy-group and thus when cladofulvin reacted with diazomethane the β-hydroxy-group and only *one* of the two α-hydroxy-groups in each nucleus were methylated. Usually only β-hydroxy-groups are readily methylated with diazomethane but it is sometimes possible to methylate *one* of the two α-hydroxy-groups in a 1,8-dihydroxyanthraquinone.[8] This suggests, therefore, that both quinone nuclei have α-hydroxy-groups in a 1,8-relationship, and the tetramethyl ether probably has structure (II; $R^1 = H$, $R^2 = Me$). None of the methoxy-signals in the ^1H n.m.r. spectrum appeared at unusually high field so the α-hydroxy-group at position 1′ was not methylated. By analogy, it is probable that the α-hydroxy-group at position 4 was also not methylated. Some support for structure (II; $R^1 = H$, $R^2 = Me$) comes from the fact that oxidation of 'cladofulvin dimethyl ether' gives 3,4-dimethoxyphthalic anhydride in low yield.[2]

The structure proposed for cladofulvin (II; $R^1 = R^2 = H$) is consistent with its i.r. and u.v.–visible spectra. Cladofulvin is formally a didehydro-dimer of 1,2,8-trihydroxy-6-methylanthraquinone (nataloemodin) and, as expected, the properties of cladofulvin and its derivatives have strong similarities with those of nataloe-emodin and its derivatives. For example, both hydroxy-quinones give a violet colouration with methanolic magnesium acetate [9] and the u.v.–visible spectra of cladofulvin and nataloe-emodin are very similar (see Experimental section). Attempts to cleave cladofulvin reductively to give nataloe-emodin by using alkaline dithionite [10] were unsuccessful. The results obtained by cleaving cladofulvin hexamethyl ether (II; $R^1 = R^2 = Me$) with the potassium t-butoxide–water reagent [11] are consistent with the proposed structure. Cleavage (34% yield) gave 2,3- and 3,4-dimethoxybenzoic acids in comparable yields, as expected [11] for a structure containing two 1,2,8-trimethoxyanthraquinone units, and 3,4-dimethoxyphthalic acid.

EXPERIMENTAL

M.p.s were determined with a Kofler hot-stage apparatus. Organic solutions were dried with magnesium sulphate. ^1H N.m.r. spectra were recorded at 60 or 100 MHz for *ca.* 10% solutions in deuteriochloroform. Mass spectra were obtained with a Varian-MAT CH7 single focusing instrument. Preparative t.l.c. (p.l.c.) was carried out with plates coated with silica gel (Merck Kieselgel HF_{254}) and chloroform–hexane as eluant.

Growth of Cladosporium fulvum *Cooke.*—Twenty-six penicillin flasks each containing Czapek–Dox [12] liquid medium (500 ml) supplemented with 0·15% of Marmite and containing glass wool to the surface of the liquid were innoculated with *C. fulvum* Cooke, strain number 54,978 from the Commonwealth Mycological Institute, Kew. After incubation at 25° for 2 months the aqueous medium was covered with a thick mat of grey fungus with a purple and brown reverse.

Isolation of Cladofulvin.—The mycelia were separated from the glass wool, dried, macerated, and extracted (Soxhlet) first with light petroleum (b.p. 40—60°) for 3 days then with ether for 3 days. Evaporation of the ethereal extract afforded a viscous, orange oil. This was washed

* The one quinonoid proton which is shifted to relatively high field in the spectrum of quinone (IV) [6] is probably in the shielding cone of an α-quinonyl substituent and is, therefore, probably H-7.

[7] R. H. Thomson, 'Naturally Occurring Quinones,' Butterworths, London, 1971, pp. 57—64.

[8] Ref. 7, p. 43.

[9] S. Shibata, M. Takito, and O. Tanaka, *J. Amer. Chem. Soc.*, 1950, **72**, 2789.

[10] S. Shibata, T. Murakami, I. Kitagawa, and T. Kishi, *Pharm. Bull.* (*Tokyo*), 1956, **4**, 111; B. Howard and H. Raistrick, *Biochem. J.*, 1954, **56**, 56.

[11] D. G. Davies, P. Hodge, and P. Yates, *J.C.S. Perkin I*, 1973, 2299.

[12] 'The Oxoid Manual,' 3rd edn., Oxoid Ltd., London, 1967, p. 111.

repeatedly with hot light petroleum (b.p. 40—60°) and the residue was recrystallised from benzene to give *cladofulvin* (*1′,4,5,6,7′,8′-hexahydroxy-2,3′-dimethyl-1,2′-bianthracene-9,9′,10,10′-tetrone*) (II; $R^1 = R^2 = H$) as an orange solid (470 mg). Paper chromatography of the pigment on Whatman No. 1 paper using toluene as eluant (descending) gave one yellow spot, R_F 0·40, which gave a violet colouration with methanolic magnesium acetate solution.[9] Cladofulvin had m.p. 310—315° (decomp.) [lit.,[2] 310° (decomp.)], $[\alpha]_D^{20}$ −743° (*c* 0·1154 mg ml^{-1} $CHCl_3$), $\nu_{max.}$ (Nujol) 3360, 3200, 1660, 1617, 1582, 1272, 1196, 1176, 1150, 1113, 1066, 1043, 1019, 891, 872, 850, 825, 817, 772, 758, 749, 720, and 716 cm^{-1}, $\lambda_{max.}$ (EtOH) 235, 262infl, 270, 296infl, and 447 nm (log ε 4·56, 4·64, 4·65, 4·30, and 3·91), $\lambda_{max.}$ (EtOH + NaOH) 269, 342, 425, and 558 nm (log ε 4·68, 4·27, 4·02, and 4·20), *m/e* 538 (M^+, 100%) (Found: C, 66·8; H, 3·8. $C_{30}H_{18}O_{10}$ requires C, 66·9; H, 3·4%).

Methylation of Cladofulvin.—Cladofulvin (200 mg) in acetone (10 ml) was treated with excess of diazomethane in ether for 1 h. Excess of reagent was then destroyed with acetic acid and the mixture evaporated to dryness. P.l.c. of the residue afforded one major product (108 mg), and two minor products (6 mg each). A portion of the major product was recrystallised (×3) from ethanol to afford *cladofulvin tetramethyl ether* (*1′,4-dihydroxy-5,6,7′,8′-tetramethoxy-2,3′-dimethyl-1,2′-bianthracene-9,9′,10,10′-tetrone*) (II; $R^1 = H$, $R^2 = Me$) as yellow needles, m.p. 169—172° (lit.,[2] 169—171°), $\nu_{max.}$ (Nujol) 1659 and 1625 cm^{-1}, $\lambda_{max.}$ (EtOH) 229, 275, 296infl, and 410 nm (rel. absorbance 1·00, 0·90, 0·49, and 0·28), *m/e* 594 (M^+, 100%), δ (60 MHz) 2·05br (s, 2 CMe), 3·97, 3·99, and 4·01 (rel. intensities, 2 : 1 : 1; 4 OMe), 7·19 (d, *J* 8 Hz, Ar*H*), 7·29 (d, *J* 8 Hz Ar*H*), 7·30 (s, Ar*H*), 7·79 (s, Ar*H*), 7·89 (d, *J* 8 Hz, Ar*H*), 8·16 (d, *J* 8 Hz, Ar*H*), 13·1 (s, α-OH), and 13·3 (s, α-O*H*) (Found: C, 60·5; H, 4·2. $C_{34}H_{26}O_{10}$ requires C, 60·6; H, 4·4%).

The combined products from the above reaction (110 mg) were treated with sodium hydride (40 mg) and methyl iodide (4 ml) in 1,2-dimethoxyethane (40 ml) at reflux temperature for 2 days. Water (10 ml) was then added and the mixture evaporated to dryness. P.l.c. of the chloroform-soluble part of the residue gave *cladofulvin hexamethyl ether* (*1′,4,5,6,7′,8′-hexamethoxy-2,3′-dimethyl-1,2′-bianthracene-9,9′,10,10′-tetrone*) (II; $R^1 = R^2 = Me$) (101 mg, 44% overall yield) as yellow needles, m.p. 151·5—152·5° (from ethanol), $[\alpha]_D^{20}$ −231° (*c* 2·22 mg ml^{-1} $CHCl_3$), $\nu_{max.}$ (CCl_4) 1675 cm^{-1}, $\lambda_{max.}$ (EtOH) 225, 277, and 372 nm (log ε 4·71, 4·66, and 4·16), *m/e* 622 (M^+, 100%) (Found: C, 69·7; H, 5·1. $C_{36}H_{30}O_{10}$ requires C, 69·5; H, 4·8%), for ^{1}H n.m.r. spectrum (60 and 100 MHz) see Discussion section.

Spectral Data of Nataloe-emodin (*1,2,8-Trihydroxy-6-methylanthraquinone*).—This quinone, prepared by the method of Haynes, Henderson, and Tyler,[13] had $\lambda_{max.}$ (EtOH) 232, 259, 292, and 437 nm (log ε 4·34, 4·38, 4·06, and 3·91), $\lambda_{max.}$ (EtOH + NaOH) 260, 298sh, 342, 424, and 548 nm (log ε 4·43, 3·87, 3·90, 3·65, and 3·94).

Spectral Data of Nataloe-emodin Trimethyl Ether (*1,2,8-Trimethoxy-6-methylanthraquinone*).—This quinone, prepared by methylation of nataloe-emodin, had m.p. 184—185° (lit.,[14] 164—165°), $\nu_{max.}$ (Nujol) 1680 cm^{-1}, $\lambda_{max.}$ (EtOH) 226, 270, 287infl, and 375 nm (log ε 4·45, 4·30, 4·09, and 3·86), δ (60 MHz) 2·47 (s, CMe), 3·98, 4·01, and 4·06 (3 s, 3 OMe), 7·08 (s, H-7), 7·18 (d, *J* 8 Hz, H-3), 7·67 (s, H-5), and 8·03 (d, *J* 8 Hz, H-4).

Cleavage of Cladofulvin Hexamethyl Ether.—The quinone (9·5 mg) was cleaved using the procedure described in Part III,[11] the reaction mixture being heated under reflux for 4 h. The acidic products which were not biphenyl derivatives were 2,3-dimethoxybenzoic acid, 3,4-dimethoxybenzoic acid, and 3,4-dimethoxyphthalic acid (molar ratio, 6 : 4 : 13). The cleavage yield [11] was 34%.

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[13] L. J. Haynes, J. I. Henderson, and J. M. Tyler, *J. Chem. Soc.*, 1960, 4879.
[14] J. L. Simonsen, *J. Chem. Soc.*, 1927, 721.