The Structure of Hallachrome: 7-Hydroxy-8-methoxy-6-methyl-1,2-anthraquinone

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The epidermal pigment of the marine worm *Halla parthenopeia*, originally considered to be an indolequinone derivative and later an aminoquinonesulphonic acid, has been isolated in crystalline form and identified as 7-hydroxy-8-methoxy-6-methyl-1,2-anthraquinone (III) from its chemical and spectral properties. The arrangement of the substituents is proposed on the basis of nuclear Overhauser effect experiments on hallachrome itself and the identification of a previously unknown 9,10-anthraquinone derivative (1,2,7-trihydroxy-8-methoxy-6-methyl-9,10-anthraquinone), obtained from hallachrome leucotriacetate by oxidation with chromic acid.

HALLACHROME is a red pigment occurring in the epidermal–epithelial cells of the sea-worm *Halla parthenopeia* (Delle Chiaje [1]), an errant polychaete of the Eunicidae family which is found chiefly in the Bay of Naples. In 1931 Mazza and Stolfi [2] announced its identity with 2-carboxy-2,3-dihydroindole-5,6-quinone (dopachrome), an important intermediate in the biosynthesis of eumelanins.[3] The indolequinone structure was then accepted until further studies [4-6] showed that the spectral and solubility properties of the natural pigment were significantly different from those of dopachrome. In addition, Nicolaus and Caglioti [7,8] reported experimental evidence suggesting the absence of nitrogen in hallachrome. However, no alternative structure for the pigment was proposed, but later Bielig and Möllinger [9] suggested that hallachrome was an aminoquinonesulphonic acid with the empirical formula $C_{21}H_{24-25}NO_9S$.

We have recently examined hallachrome anew, noting particularly that the properties of the pigment described by Bielig and Möllinger were significantly different from those previously reported for hallachrome (see Table).

TABLE 1

Comparison of solubility and spectral properties reported for hallachrome

	Ref. 5	Ref. 9
Molecular formula		$C_{21}H_{24-25}NO_9S$
Water solubility	Low	High
Chloroform solubility	High	Low
U.v. absorptions	500, 310, 255 nm (EtOH)	540, 310, 260 nm (MeOH)
Effect of alkali	Reversible change to green (650—625, 365, 260 nm)	Reversible change to green (735, 285 nm)
Effect of acid	Change to red-violet (550, 310, 255 nm)	No change

We have already [10] briefly described a new isolation procedure which allowed us to obtain the natural pigment in crystalline form for the first time. The most significant feature of our procedure is the extraction of the pigment, which is performed by dipping living specimens of *Halla parthenopeia* into chloroform at room temperature. Under these mild conditions an almost pure solution of hallachrome is obtained. Consequently, the extracted pigment is stable and can be further purified simply by chromatographing the red chloroform extracts on a polyamide column, with chloroform–benzene (70 : 30 v/v) as eluant. The u.v. spectrum of the crystalline pigment [dark-red prisms, m.p. 224—226° (decomp.)], $\lambda_{max.}$ (MeOH) 500, 312, and 250 nm (log ε 3·75, 4·52 and 4·51); $\lambda_{max.}$ (MeOH–OH^-) 650br, 377, 316infl, and 271 nm, is similar to that described for hallachrome by Bu'Lock and his colleagues.[5] Moreover, elemental analyses and mass spectrometry showed that hallachrome contains neither nitrogen nor sulphur, and established the molecular formula as $C_{16}H_{12}O_4$, including one methoxy-group. On the basis of these data it appears that the pigment isolated by Bielig and Möllinger from glacial acetic extract of *Halla parthenopeia* is an artefact, which probably arises by interaction of hallachrome with an unknown nitrogen- and sulphur-containing substance present in the crude extract.

Aside from the molecular ion peak at *m/e* 268 (58·8%), the mass spectrum of hallachrome showed a prominent $(M + 2)^+$ peak (52·9%) typical of *ortho*-quinones [11] and other diagnostic fragment ions at $M - CO$ (73·5%), $M - (CO + CH_3)$ (base peak) and $M - (2CO + CH_3)$ (35·2%). As expected, on hydrogenation over palladium–charcoal at room temperature the pigment absorbed 1 mol. equiv. of hydrogen, with formation of a pale yellow product; this was readily reoxidized in contact with air to give a red compound identical (u.v.–visible spectrum; t.l.c.) with the original pigment. Moreover, hallachrome reacted with *o*-phenylenediamine to give a quinoxaline derivative, and when heated with acetic anhydride it afforded a crystalline acetate, m.p. 194—196°, $C_{18}H_{14}O_5$. On reductive acetylation, hallachrome, as well as its acetate, gave a leucotriacetate, m.p. 148—

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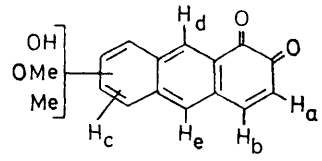
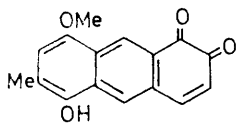
[7] R. A. Nicolaus and L. Caglioti, *Ricerca Sci.*, 1957, **27**, 113.
[8] R. A. Nicolaus, *Rass. Med. Sper.*, 1960, suppl. 2, p. 10 (Idelson, Napoli).
[9] H. J. Bielig and H. Möllinger, *Z. physiol. Chem.*, 1960, **321**, 276.
[10] G. Prota, M. D'Agostino, and G. Misuraca, *Experientia*, 1970, **26**, 15.
[11] R. H. Thomson, 'Naturally Occurring Quinones,' 2nd edn., Academic Press, London, 1971, p. 83.

149°, $C_{22}H_{20}O_7$, which exhibited an anthracene-type absorption spectrum, $\lambda_{max.}$ (MeOH) 388, 368, 350, 334, 252, and 244sh nm (log ε 3·71, 3·79, 3·67, 3·45, 4·97, and 4·53).

Oxidation of hallachrome with alkaline permanganate afforded, as main product, benzene-1,2,4,5-tetracarboxylic acid (pyromellitic acid), identified by comparison with an authentic sample. It thus seemed that the pigment was a 1,2-anthraquinone derivative unsubstituted at positions 9 and 10. This conclusion was confirmed by the n.m.r. spectrum, which suggested the partial structure (I): δ 2·32 (CMe), 3·88 (ArOMe), *ca.* 9·9 (ArOH), 6·31 and 7·68 (ABq, *J* 10 Hz, C-4 and C-3 protons), and 8·42 and 7·80 (each s, C-9 and C-10 protons, assigned by comparison with signals from some synthetic 1,2-anthraquinones [12,13]). A further broadened singlet at δ 7·50 must arise from the aromatic proton (H_c) of the trisubstituted benzene ring, which is probably located next to the methyl group since the signal of the latter also appears as a broadened singlet. In agreement with this view, irradiation at the frequency of the methyl protons sharpened the signal at δ 7·50, without affecting the other aromatic singlets. Moreover, irradiation at the frequency of the methoxy-resonance increased the intensity (by *ca.* 13%) of the signal from the *meso*-proton H_d without appreciable modification of the other aromatic signals.



(I)



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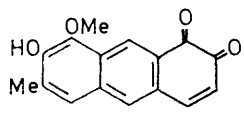
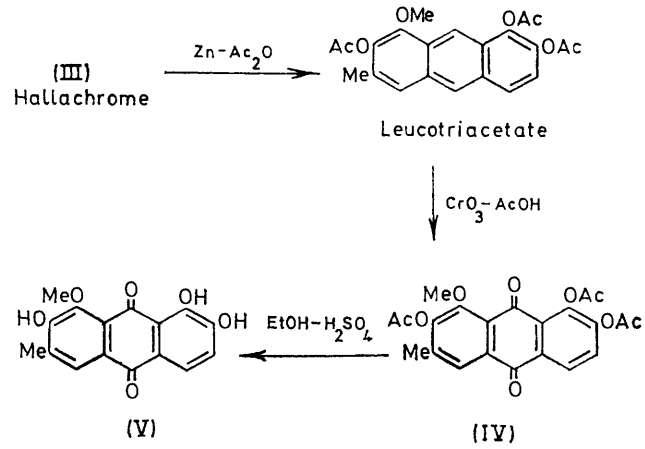


(III)

From these observations we deduced that in hallachrome the methoxy-group is at C-8; and the methyl group could be either at C-7 or at C-6, in both cases next to the proton attached to the trisubstituted benzene ring. Consequently, two alternative structures (II) and (III) appeared to be possible. The latter was correct was deduced from the spectroscopic characteristics of the 9,10-anthraquinone derivative (V), obtained from hallachrome as outlined in the Scheme. Reductive acetylation of hallachrome gave the corresponding leucotriacetate, which was then oxidized with chromium trioxide in acetic acid at 70—75°. The reaction afforded as main product (52%) a pale yellow compound (IV), m.p. 206—208°, $\lambda_{max.}$ (MeOH) 346 and 262 nm (log ε 3·77 and 4·57), ν_{CO} ($CHCl_3$) 1783 and 1686 cm^{-1}, consistent with the presence of a 9,10-anthraquinone chromophore. Deacetylation of compound (IV) with ethanolic sulphuric acid gave an orange compound (V), m.p. 256—259° (decomp.), which was identified as 1,2,7-trihydroxy-8-methoxy-6-methyl-9,10-anthraquinone on the following grounds. The pigment displayed absorption maxima (EtOH) at 436, 372, 285, and 231 nm (log ε 3·84, 3·74,



SCHEME Conversion of hallachrome into the 9,10-anthraquinone derivative (V)

4·54, and 4·37) and was shown by mass spectrometry to have a mol. wt. of 300 (base peak) and a mol. formula of $C_{16}H_{12}O_6$. The mass spectrum also exhibited significant peaks at $M - 17$ (27·9%), $M - 18$ (99·8%), and $M - 29$ (13·2%), consistent with the presence of a *peri*-methoxy-group, and the i.r. spectrum (Nujol) showed a broad OH band at 3268 cm^{-1} and two carbonyl absorptions at 1653m and 1629s cm^{-1}, indicating that only one of the quinone carbonyl groups was chelated. The n.m.r. spectrum [$(CD_3)_2SO$] exhibited signals at δ 2·26 and 3·89 (each 3H, s, β-Me and OMe), 7·12 and 7·53 (each 1H, d, *J* 8·3 Hz, *ortho*-coupled aromatic C-3 and C-4 protons), and 7·77 p.p.m. (1H, s, α-proton adjacent to Me). This assignment was further substantiated by the n.m.r. spectrum ($CDCl_3$) of the triacetate (IV), which exhibited two doublets (*J* 8·6 Hz) at δ 7·55 and 8·22 and a singlet at δ 7·96, in complete agreement with the substitution pattern in (IV). It follows that the 9,10-anthraquinone pigment has structure (V) and hence that hallachrome must have structure (III). Hallachrome is the first representative of a new type of natural quinone, characterized by a 1,2-anthraquinone chromophore unsubstituted at positions 9 and 10. A preliminary investigation on the distribution of hallachrome in polychaetae has revealed that it occurs also in *Lumbriconereis impatiens* (Claparède), an errant worm of the Eunicidae family commonly used as bait by Neapolitan fishermen.

EXPERIMENTAL

M.p.s were determined for samples in capillaries. I.r. spectra were recorded with a Perkin-Elmer Infracord 137 E instrument, u.v. spectra with a Perkin-Elmer 402 spectrophotometer, and n.m.r. spectra with a Perkin-Elmer R-12 or a Varian HA 100 instrument (standard internal

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[13] P. Boldt and K. P. Paul, *Chem. Ber.*, 1966, **99**, 2337.

tetramethylsilane). Mass spectra and exact mass measurements were obtained by the direct insertion technique with an A.E.I. MS-902 double-focus spectrometer (probe temperature 180—200°; electron-beam energy 70 eV). Column chromatography was run on NM-polyamide-SC6 (Macherey, Nagel, and Co., D-S16 Duren). Analytical and preparative t.l.c. was carried out on Merck F_{254} silica gel in the specified solvent systems. Solvents for development and for elution were redistilled; proportions given are by volume. Samples were dried (P_2O_5) *in vacuo* overnight at 60° before analysis.

Isolation of Hallachrome (III).—In a typical run, ten living specimens of *Halla parthenopeia*, between 50 and 80 cm in length, were dipped into chloroform at room temperature. After 30 min the orange-red extract was decanted and the worm was re-extracted a few times until the chloroform assumed a pale pink colour. The combined extracts, were dried (Na_2SO_4), concentrated *in vacuo* to *ca.* 20 ml, and chromatographed in two portions on a polyamide column (4 × 50 cm) [chloroform–benzene (80 : 20) as eluant]. A single red band was eluted; concentration of the solution to a small volume and storage overnight at 4°, gave crystalline *hallachrome* (130 mg) as dark-red prisms, homogeneous on t.l.c. [$CHCl_3$–MeOH (97 : 3)], m.p. 224—226° (decomp.), slightly soluble in ethanol, insoluble in water and in aqueous sodium hydrogen carbonate (Found: C, 71·75; H, 4·6; OCH_3, 12·0%; M^+, 268·0741. $C_{16}H_{12}O_4$ requires C, 71·65; H, 4·45; OCH_3, 11·55%; M^+, 268·0735), $\nu_{max.}$ (Nujol) 3300br, 1695w, 1652s, 1610m, and 1580 cm^{-1}.

In alkaline media (0·1N-NaOH) the colour of the pigment turns reversibly from red to green.

Treatment of hallachrome (III) (10 mg) in ethanol (10 ml) with *o*-phenylenediamine (10 mg) in acetic acid (2 ml); and warming the mixture for 15 min on a water bath gave the *quinoxaline derivative*, which was deposited as a crystalline red-orange precipitate on cooling; m.p. >310° (Found: M^+, 340·1237. $C_{22}H_{16}N_2O_2$ requires M, 340·1211), $\lambda_{max.}$ (MeOH) 478, 358infl, 338, 326infl, and 276 nm (log ε 3·56, 3·94, 4·13, 3·97, and 4·36).

A solution of hallachrome (III) (50 mg) in acetic anhydride (5 ml) was heated for 1 h on a steam-bath. Volatile constituents were removed *in vacuo*, and the residue was chromatographed on a 2 × 20 cm polyamide column [eluant chloroform–benzene (30 : 70)] to give *hallachrome acetate* (36 mg), orange-red needles, m.p. 194—196° (from benzene–light petroleum) (Found: C, 69·2; H, 4·6%; M^+, 310. $C_{18}H_{14}O_5$ requires C, 69·65; H, 4·5%, M, 310), *m/e* 312 (8·5%), 310 (35), 270 (12·5), 268 (30), 255 (10), 240 (100), 225 (57·5), 197 (12·5), and 43 (17·5), $\lambda_{max.}$ (MeOH) 466, 304, 296, and 246 nm (log ε 3·90, 4·59, 4·54, and 4·51), $\nu_{max.}$ ($CHCl_3$) 1764 and 1667 cm^{-1}, δ[$(CD_3)_2SO$] 2·30 (3H, s, ArMe), 244 (3H, s, ArOAc), 3·96 (3H, s, ArOMe), 6·46 (1H, d, *J* 10 Hz, H-3), 7·67br (1H, s, H-5), 7·75 (1H, d, *J* 10 Hz, H-4), 7·96 (1H, s, H-10), and 8·58 p.p.m. (1H, s, H-9).

When an ethanolic solution of hallachrome acetate (15 mg) was treated with *o*-phenylenediamine, a yellow-orange precipitate formed within a few minutes. Preparative t.l.c. of the crude product on silica gel [solvent benzene–methanol (95 : 5)] gave the *quinoxaline derivative*, yellow-orange needles (18 mg), m.p. 269—271° (from ethanol) (Found: M^+, 382·1309. $C_{24}H_{18}N_2O_3$ requires M, 382·1317), $\lambda_{max.}$ ($CHCl_3$) 451, 426, 405sh, 329, 317, 264, and 252 nm (log ε 4·13, 4·09, 3·79, 4·62, 4·53, 4·68, and 4·68).

To a solution of hallachrome (III) (100 mg) in acetic anhydride (10 ml) freshly dried zinc dust (350 mg) was added. The mixture was heated with occasional stirring at 100° for 1·5 h, filtered, and evaporated. The residue was extracted with ether, and the extract washed with water and evaporated. Crystallization from ethanol gave *hallachrome leucotriacetate* (95 mg) as pale yellow needles, m.p. 148—149° (Found: C, 66·3; H, 5·15%; M^+, 396. $C_{22}H_{20}O_7$ requires C, 66·65; H, 5·05%; M, 396), *m/e* 396 (28·3%), 354 (45·2), 270 (100), and 255 (35·8), $\nu_{max.}$ (CCl_4) 1799 cm^{-1}, δ($CDCl_3$) 2·33 (6H, s, ArOAc), 2·43 (3H, s, ArMe), 2·50 (3H, s, ArOAc), 4·01 (3H, s, ArOMe), 7·25 (1H, d, *J* 9·5 Hz, H-3), 7·53br (1H, s, H-5), 7·81 (1H, d, *J* 9·5 Hz, H-4), 8·26 (1H, s, H-10), and 8·62 p.p.m. (1H, s, H-9).

Permanganate Oxidation of Hallachrome.—A solution of hallachrome (III) (10 mg) in 2N-potassium carbonate (3 ml) was treated with 3% potassium permanganate at 60° until the colour of the reagent persisted for 5 min. After addition of sodium hydrogen sulphite, the mixture was filtered, and the filtrate passed through a 2 × 5 cm column of Dowex 50 W (100—200 mesh; H^+ form). The cation-free solution was then evaporated *in vacuo* and in the residue benzene-1,2,4,5-tetracarboxylic acid was identified by t.l.c. on silica gel [solvent butan-1-ol–acetic acid–water (60 : 20 : 20)] by comparison with an authentic sample. For additional confirmation the acid was converted by treatment with excess of ethereal diazomethane into the methyl ester, which was purified by preparative t.l.c. on silica gel [benzene–ether (80 : 20) as eluant]. The purified ester (R_F 0·54, located by u.v. light) had u.v. and i.r. spectra identical with those of the synthetic tetramethyl ester prepared similarly from the authentic acid.

1,2,7-*Triacetoxy*-8-*methoxy*-6-*methyl*-9,10-*anthraquinone* (IV).—A solution of chromic anhydride (100 mg) in acetic acid (7·5 ml) containing water (1 ml) was added dropwise to a solution of hallachrome leucotriacetate (50 mg) in acetic acid (5 ml), kept at 75—80°. After 30 min at this temperature, the mixture was cooled, diluted with water, and extracted with ether. The extract was washed with aqueous sodium hydrogen carbonate, dried (Na_2SO_4), and evaporated *in vacuo*. Crystallization of the residue from ethanol gave 1,2,7-*triacetoxy*-8-*methoxy*-6-*methyl*-9,10-*anthraquinone* (IV) as yellow prisms (56 mg), m.p. 206—208° (Found: C, 62·2; H, 4·05%; M^+, 426. $C_{22}H_{18}O_9$ requires C, 61·95; H, 4·2%; M, 426), δ($CDCl_3$) 2·29 (3H, s, ArMe), 2·33 (3H, s, ArOAc), 2·37 (3H, s, ArOAc), 2·42 (3H, s, ArOAc), 3·88 (3H, s, ArOMe), 7·55 (1H, d, *J* 8·6 Hz, H-3), 7·96 (1H, s, H-5), and 8·22 p.p.m. (1H, d, *J* 8·6 Hz, H-4).

1,2,7-*Trihydroxy*-8-*methoxy*-6-*methyl*-9,10-*anthraquinone* (V).—A solution of pure triacetate (IV) (50 mg) in ethanol (20 ml) containing conc. sulphuric acid (0·5 ml) was refluxed for 3 h. Concentration of the solution to a small volume gave 1,2,7-*trihydroxy*-8-*methoxy*-6-*methyl*-9,10-*anthraquinone* (V) as orange needles (31 mg), m.p. 256—258° (decomp.) (Found: C, 64·35; H, 4·2%; M^+, 300. $C_{16}H_{12}O_6$ requires C, 64·0; H, 4·0; M, 300), *m/e* 300 (100%), 283 (27·9), 282 (98·2), 271 (13·2), 257 (29·5), 254 (66·2), and 229 (14·7).

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