The Structure of Arenicochromine, a Trihydroxymethoxybenzpyrenequinone from *Arenicola marina*

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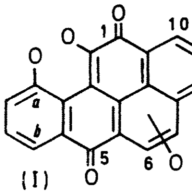
Summary Arenicochromine, a pigment obtained from the lugworm, *Arenicola marina*, is shown to be a trihydroxymethoxybenzpyrenequinone; a possible derivation from 3,4-benzpyrene is suggested.

SOME years ago Dutch workers[1] isolated from the skin of the lugworm, *Arenicola marina*, a pigment, arenicochrome, which was obtained as a dark violet tripotassium salt. On acid hydrolysis it liberated sulphuric acid (2 mols) and a dark purple compound, arenicochromine, for which they suggested the molecular formula, $C_{40}H_{32}O_{15}$ or $C_{42}H_{34}O_{16}$. The u.v.–visible, redox, and indicator properties suggested that arenicochromine was a hydroxyquinone, but no structure was put forward.

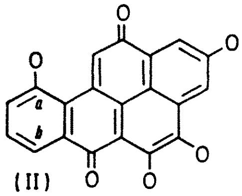
We have now confirmed most of the earlier work but find that the molecular formula of arenicochromine is $C_{21}H_{12}O_6$ (Found: *M*, 360·0621; required 360·0634); it shows ν_{max} (KBr) 3530sh, 3420, 3220, 1635sh, and 1612 cm^{-1}. Treatment with acetic anhydride in cold pyridine gave an orange triacetate, m.p. 210—211°, $C_{27}H_{18}O_9$ (mass spectrum) which, on reductive acetylation, afforded a yellow leucopenta-acetate, m.p. 232—233°, $C_{31}H_{24}O_{11}$ (mass spectrum). The n.m.r. and mass spectra of these derivatives show that they possess one methoxy-group and, respectively, three and five acetoxy-groups. Zinc-dust distillation of arenicochromine yielded 3,4-benzpyrene (u.v. spectrum showing fine structure identical with that of authentic material), and the u.v. spectrum of the leucopenta-acetate is similar to that of 1,5-diacetoxy-3,4-benzpyrene, bathochromically shifted [diacetate, λ_{max} (EtOH) 254, 264, 274, 285, 298, 338sh, 354, 372, 392, and 407 nm (log ϵ 4·64, 4·71, 4·51, 4·69, 4·78, 3·91, 4·21, 4·47, 4·51, and 4·00), leucopenta-acetate, λ_{max} 253, 275, 288, 299, 311, 397, and 418 nm (log ϵ 4·62, 4·55, 4·51, 4·64, 3·64, 4·20, and 4·04)]. We conclude that the parent compound is a 3,4-benzpyrenequinone and the u.v.–visible spectrum of the 1,5-isomer resembles that of arenicochromine triacetate [λ_{max} (EtOH) 231, 241sh, 269, 282sh, 303sh, 345, 357, and 410 nm (log ϵ 4·59, 4·48, 4·35, 4·31, 4·15, 3·96, 4·00, and 4·12)]; 3,4-benzpyrenequinone, λ_{max} 226, 235sh, 278sh, 288, 299, 348, 367, and 429 nm (log ϵ 4·51, 4·47, 4·34, 4·38, 4·30, 4·00, 4·02, and 3·79)]. Thus the pigment is probably a trihydroxymethoxy-3,4-benzpyrene-1,5-quinone.

The n.m.r. spectrum (100 MHz, $CDCl_3$) of the triacetate shows, in addition to signals from the acetate (9H) and methoxy (3H) groups, the presence of six ring protons of which one is isolated (τ 2·28, 1H, s), two are *meta*-coupled (τ 2·00 and 3·08, each 1H, d, J 2·0 Hz) and three form an AMX system (τ 1·42, 1·86, and 2·21, 3H). The couplings indicated were confirmed by spin-decoupling experiments, and from this information the substitution patterns (I) and (II) emerge (in each case one oxygen function could be either at *a* or *b*). The location of the methoxy-group was ascertained by irradiation of the methoxy-protons at τ 6·00 when a nuclear Overhauser effect was observed, the signal from the higher-field *meta*-coupled proton at τ 3·08 showing a marked enhancement (47% increase in height, 17% increase in area). Accordingly, this proton must be at C-8 and hence the methoxy-group must lie at C-7. As the singlet signal was unaffected, C-6 must be occupied by a hydroxy-group (see II), the consequent *o*-hydroxymethoxy-substitution accounting for the significant $M - 18$ peak (28%) in the mass spectrum of the parent pigment, and the *peri*-hydroxyquinone system for the chelated carbonyl absorption at 1612 cm^{-1}. This band is broad, with several shoulders (principally 1635 cm^{-1}) and must overlap the C-1 carbonyl peak. Accordingly, arenicochromine is of type (II) and we prefer structure (III; R = H) as methylation with diazomethane gives, as principal product, a dimethyl ether (III; R = Me) possessing one *peri*-hydroxy-group (τ −3·57). Moreover, the *meta*-coupled protons of the AMX system are both deshielded, implying that both the methoxy and the 5-carbonyl group directly affect the AMX system. Consequently, the methoxy-group must be at *a* rather than *b* in (III). Arenicochromine triacetate may be a derivative either of (III; R = H) or a tautomeric form.

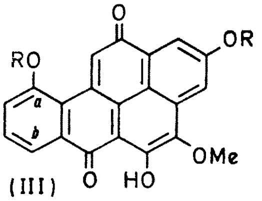
The positions of the *O*-sulphate groups in arenicochrome have yet to be established. Arenicochrome itself is an artefact; the native greenish-yellow pigment occurs in a reduced form which undergoes oxidation during chromatographic purification.



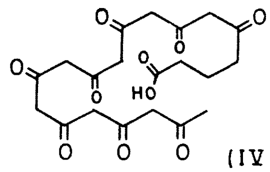
(I)



(II)



(III)



(IV)

The origin of this polycyclic system is intriguing; we offer two hypotheses. One is that the pigment is biosynthesised *de novo* from acetate by way of (IV) (after considerable modification), and the other assumes that 3,4-benzpyrene is the precursor. Trace amounts of this hydrocarbon have been reported[2] in marine sands and muds, and in various marine organisms including lugworms. Mass spectrometry of a sample of the muddy sand inhabited by "our" lugworms in the Ythan estuary, Aberdeenshire, failed to detect the presence of polycyclic hydrocarbons but showed the characteristic spectrum of sulphur from *m/e* 256 (S_8) down to *m/e* 32 (S_2) accompanied by the appropriate isotopic satellite peaks. Extraction of 6 kg sand yielded 340 mg of sulphur (presumably of bacterial origin[3]), and a fluorescent fraction with the same R_F value as 3,4-benzpyrene although the u.v. spectrum (λ_{max} 221, 258, 267, and 288 nm) did not disclose a polycyclic component. However, the mass spectrum of this fraction revealed the presence of a tetracyclic hydrocarbon ($C_{18}H_{12}$) and 3,4-benzpyrene (M 252·0926. Calc. for $C_{20}H_{12}$, M 252·0939) which was confirmed by the fluorescence spectrum. From the latter the 3,4-benzpyrene content was estimated as 0·12 μg/kg dry sand. This, then, may be the origin of arenicochrome [Mallet and Priou[2] found 125 μg/kg *Arenicola marina* (dry wt.) from St. Malo bay] but the origin of the 3,4-benzpyrene is still uncertain.

The wide occurrence of this hydrocarbon, in trace amounts, is usually attributed to industrial pollution and the discharge of oil from ships. However, as it has been reported in soil[4] far removed from industrial areas and in marine sediments[5] remote from shipping lanes, a bacterial[6] or algal[7] origin has been suggested.

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[1] P. van Duijn, E. Havinga, and G. O. E. Lignac, *Experientia*, 1951, **15**, 376; P. van Duijn, *Rec. Trav. chim.*, 1952, **71**, 585, 595.

[2] L. Mallet and Le Theule, *Compt. rend.*, 1961, **252**, 565; J. Bourcart, C. Lalou, and L. Mallet, *ibid.*, 640; L. Mallet and M.-L. Priou, ibid., 1967, **264**, *D*, 969.

[3] "Bergey's Manual of Determinative Bacteriology," eds. R. S. Breed, E. G. D. Murray and N. R. Smith, 7th edn., Williams and Wilkins, Baltimore, 1957.

[4] M. Blumer, *Science*, 1961, **134**, 474; J. Borneff and R. Fischer, *Arch. Hyg. Bakteriol.*, 1962, **146**, 430; J. Borneff and H. Kunte, *ibid.*, 1963, **147**, 401.

[5] L. Mallet, L. V. Perdriau, and J. Perdriau, *Compt. rend.*, 1963, **256**, 3487.

[6] L. Mallet, L. Zanghi, and J. Brisou, *Compt. rend.*, 1967, **264**, *D*, 1534.

[7] J. Borneff, F. Selenka, H. Kunte, and A. Maximos, *Environmental Res.*, 1968, **2**, 22.