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THE ZOOCHROME OF THE SPONGE *VERONGIA AEROPHOBA* ("URANIDINE")

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Abstract.- The yellow zoochrome of the sponge Verongia aerophoba, which blackens when exposed to the air, has been isolated and its structure established as 3,5,8-trihydroxy-4-quinolone. The pigment is easily oxidized to an unstable blue quinone which quickly gives rise to an unsoluble black material.

The sponge *Verongia aerophoba* (Schmidt) draws its species name (*aerophoba* = air fearing) from the fact that its dense sulphur yellow colour becomes black when exposed to the air. Some one hundred years ago Krukenberg[1] described the presence in this sponge of a yellow pigment that blackens in the body after death and also in aqueous solution, especially at high pH values, and coined for it the name "uranidine". "Uranidine(s)" characterizes many Verongidae[2]: the yellow colour is exhibited by the intact sponge, but when the sponge is damaged, a navy blue colour develops immediately which quickly becomes red-purple or black. This sequence is striking and rapid. Investigations directed to the chemical characterization of the zoochrome of *V. aerophoba* were unsuccessful. Christomanos[3] showed that the black colour is not due to the formation of a melanin-type pigment, while Čmelik[4] claimed the isolation of a chromoprotein with indicator properties from the same organism. We report here the isolation and the structural elucidation of the zoochrome of *V. aerophoba* as well as an explanation, on chemical grounds, of the observed colour changes.

The sponge (800 g, dry weight after extraction) was extracted with acetone. The extract was evaporated under reduced pressure and the resulting aqueous suspension was extracted sequentially with diethyl ether and *n*-butyl alcohol. During this manipulation the formation of a copious black precipitate was observed. The butanolic extract was evaporated under reduced pressure to afford 64 g of an oily material, part of which (3 g) was chromatographed on Sephadex LH-20 with methanol. The pigment (*1*: 110 mg) was eluted soon after the previously described bromo-compounds[5]. *1* is very unstable but it can be stored in dilute methanolic solution for several months at -20°C. Neutral or acidic solutions of *1* are yellow [λ_{max} (H_2O-pH 7.0), 365 (ε = 2,900), 319 (ε = 2,600), 239 (ε = 15,000); λ_{max} (HCl-pH 1.0), 368 (ε = 2,900), 321 (ε = 2,600), 240 (ε = 15,000)]; addition of alkali gives first a reversible shift [λ_{max} (0.1 M phosphate buffer--pH 8.0), 553 (ε = 1,100), 364 (ε = 2,700), 304 (ε = 3,400), 239 (ε = 13,200)] and then a blue solution [λ_{max} (NaOH - pH 13), 641 (ε = 3,100), 604 (ε = 3,000), 315 (ε = 2,900), 236 (ε = 14,700)] which turn to orange by acidification [λ_{max} (solution at pH 13 acidified with HCl) 438, 252, 226]. The blue alkaline solutions of *1* give rise in few minutes to a black precipitate which is insoluble in the common organic solvents.

1 has a molecular formula $C_9H_7NO_4$ (high resolution E.I. mass spectrometry) which, in conjunction with the NMR data (see tables), suggested a quinolone structure. The oxygenated functions were placed as depicted in *1* on the following evidence. *1* on acetylation with acetic anhydride-acetic acid yields an acetyl derivative (*2*; m/z 319; $M^+-CH_2=CO$) which was shown to be a tetraacetate by ^1H--NMR (table 1); in particular in the spectrum of *2* the singlet at δ 8.80 was assigned to the C-2 proton of a quinoline derivative for its chemical shift.

On the other hand, in the ^1H-NMR spectrum of *1* (table 1) the C-2 proton resonates as a doublet at δ 7.58 coupled with one of the four exchangeable protons (N<u>H</u>; δ 10.27), suggesting that *1* has a 4-quinolone structure bearing an OH substituent in the position 3. The other two mutually coupled protons must be therefore situated on the benzenoid ring in a *ortho* position (J = 8.1 Hz); they could be present, in principle, either in the 5-6, 6-7 or 7-8 positions. The 5-6 position was discarded since for the C-5 proton a considerably larger chemical shift value was expected in the ^1H-NMR spectrum of *1* due to the consistent *peri* effect of the C-4 carbonyl group[6]. Moreover, comparison of the chemical shifts of the *ortho* coupled benzenoid protons in the ^1H-NMR spectrum of *1* with those of the model compounds *3*[7] and *4*[9] (table 1), taking into account known[10] substituent effects, clearly suggests the 3,5,8-trihydroxy-4-quinolone structure for *1*. Comparison of the ^{13}C-NMR spectra of *1* and *3* (table 2) confirmed the assigned structure.

1 is structurally related to the highly fluorescent compound *5* previously isolated from *V. cavernicola*[11,5], from which it could derive from a biogenetic point of view.

When sodium hydroxide was added to a solution of *1* under a nitrogen atmosphere the blue colour was not observed; on the other hand addition of ascorbic acid, a mild reducing agent, to the alkaline blue solution of *1* restores the original UV spectrum. Therefore the blue colour which is developed at high pH values should be regarded as an air oxidation of *1* to a quinone for which structure *6* could be supposed. To observe the ^1H-NMR spectrum of *6*, a CD_3OD solution of *1* was treated with two drops of 40% NaOD directly in the NMR tube and the resulting blue

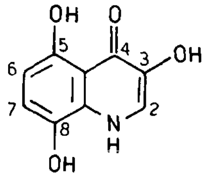
Table 1 - ^1H-NMR data for compounds *1*, *2*, *3*, *4* and *6*; recorded in DMSO-d_6 solution ($CDCl_3$ for *2*; CD_3OD-NaOD, acidified with DCl, for *6*) at 500 MHz.

Protons	Chemical shifts (δ) *1*	*2*	*3*	*4*	*6*
H-2	7.58(d;J=5.8 Hz)	8.80(s)	7.57(t;J=7.3 Hz)	7.92(d;J=7.2 Hz)	7.87(s)
H-3			5.90(d;J=7.3 Hz)	6.09(d;J=7.2 Hz)	
H-5				7.58(overlapped with H-8)	
H-6	6.26(d;J=8.1 Hz)	7.23(d;J=8.0 Hz)	6.63(d;J=8.7 Hz)		7.01(d;J=10.4 Hz)
H-7	6.82(d;J=8.1 Hz)	7.43(d;J=8.0 Hz)	7.11(d;J=8.7 Hz)	7.33(dd;J=2.9 and 8.9 Hz)	7.08(d;J=10.4 Hz)
H-8				7.58(overlapped with H-5)	
$-O\underline{H}$	8.56(s) 8.69(bs) 9.84(bs)				
$C\underline{H}_3-\overset{\overset{O}{\|}}{C}-O-$		2.35(s) 2.38(s) 2.41(s) 2.48(s)			
$C\underline{H}_3-O-$			3.72(s) 3.91(s)	3.87(s)	
$-N\underline{H}$	10.27(d;J=5.8 Hz)		3.33(d;J=7.3 Hz)	3.56(bs)	

Table 2 - ^{13}C-NMR data for compounds *1* and *3*; recorded in DMSO-d_6 solution at 125.76 MHz.

Carbons	Chemical shifts (δ) *1*	*3*
C-2	123.9 (d)	136.9 (d)
C-3	137.1* (s)	111.7 (d)
C-4	173.1 (s)	175.9 (s)
C-4a	112.7 (s)	117.0 (s)
C-5	151.5 (s)	152.7 (s)
C-6	104.0 (d)	103.6 (d)
C-7	114.4 (d)	111.3 (d)
C-8	139.2* (s)	141.8 (s)
C-8a	128.8 (s)	132.8 (s)
$\underline{C}H_3$-O		56.1 (q) 56.2 (q)

* Signals may be interchanged



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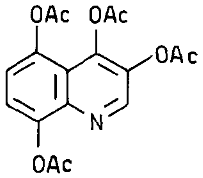

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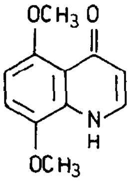

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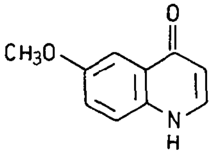

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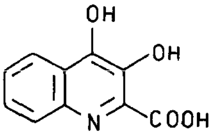

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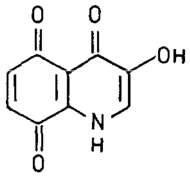

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solution was acidified with conc. DCl to record the spectrum under acidic conditions , in which *6* is a little more stable. The spectrum (table 1) is in accordance with the depicted structure *6*; this quinone is unstable and readily polymerizes, giving rise to the black precipitate. The same sequence is observed on the sponge and, similarly, should be ascribed to raising of the pH in the damaged cells and subsequent air oxidation.

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