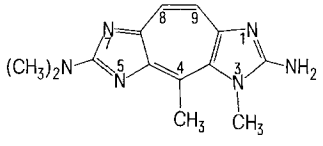
New Zoanthoxanthins from the Mediterranean Zoanthid *Parazoanthus axinellae*[1]

Parazoanthus axinellae is a yellow colonial anthozoan without skeleton belonging to the order Zoanthidea, a group of marine coelenterates related to anemones and stony corals. From ethanolic extracts of this species we recently isolated zoanthoxanthin, a yellow metabolite exhibiting visible-fluorescence in daylight, which by chemical and X-ray analysis was shown to have structure (I), characterized by the hitherto unknown heteroaromatic system 1,3,5,7-tetrazacyclopent[*f*]azulene[2,3]. By further scrutiny of related metabolites in the same



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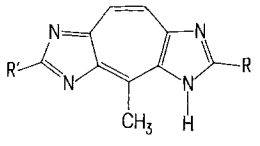

II R=R'=NH_2
III R=NH_2, R'=$N(CH_3)_2$
IV R=Cl, R'=$N(CH_3)_2$
V R=OH, R'=$N(CH_3)_2$
VI R=NH_2, R'=$NHCH_3$

organism, we have now obtained 4 additional pigments, 2 of which, named parazoanthoxanthin A and D, proved to have structure (II) and (III). The remaining, parazoanthoxanthin B and C, were found to correspond to a N-methyl- and to a N,N'-dimethyl-parazoanthoxanthin A, respectively, but on account of the very small quantities available they could not be characterized unequivocally.

The isolation of these new pigments was performed by dipping whole colonies of *P. axinellae*[4] (1 kg wet weight) in ethanol containing 1% of conc. HCl. The resulting yellow extract, purified by liquid-liquid extraction with ether, was passed through a 2.2×25 cm column of Dowex 50 W-X2 (100–200 mesh, H^+ form). After washing the bed with 0.1 *N* HCl and water, a fraction containing mainly[5] the pigments under investigation was eluted with 1 *N* NH_4OH and was extracted with *n*-butanol. The residue obtained after evaporating the organic layer was then fractionated by preparative TLC on silica (F_{254}, Merck) with $CHCl_3$-MeOH-25% NH_4OH (80:20:2, v/v) to give, besides some zoanthoxanthin (Rf 0.68), 120 mg of parazoanthoxanthin D (Rf 0.49), 3 mg of A (Rf 0.22), and trace amounts of B (Rf 0.32) and C (Rf 0.43).

The major co-pigment, thin yellow needles from water, m.p. 303–304° (dec), had a molecular formula of $C_{12}H_{14}N_6$[6] and exhibited an intense blue fluorescence with an emission maximum at 455 nm on excitation at 380 nm (methanol[7]). Parazoanthoxanthin D showed absorption maxima in neutral and acidic media (Table) consistent with the presence in the molecule of a tetrazacyclopentazulene chromophore. Apart from the molecular ion peak at *m/e* 242 (100%), the mass spectrum of the pigment showed significant fragment ions at *m/e* 227 (78%), 213 (38%), and 172 (17%), arising from a $-N=\overset{|}{C}-NMe_2$ grouping. In addition, the IR-spectrum (in K Br) of the pigment was very similar to that of zoanthoxanthin, including an intense broadened band at 3380 cm^{-1}, suggesting the presence of a primary amino function.

Diazotization of parazoanthoxanthin D (50 mg) with $NaNO_2$ (300 mg) in 3 *N* HCl (5 ml) for 18 h at 4 °C gave, along with some unchanged (III), 2 products which were extracted from the reaction mixture with *n*-butanol. Purification of the extract by preparative TLC on silica gel (eluent: $CHCl_3$-MeOH, 90:10) afforded the desaminochloro derivative (IV) (4 mg, Rf 0.63), $C_{12}H_{12}N_5Cl$[8], λ_{max} (MeOH) 405, 304, 292 (infl.), 262 nm, and the corresponding hydroxy-derivative (V) (10 mg, Rf 0.19), $C_{12}H_{13}N_5O$[8], existing in solution predominantly in the tautomeric ureido form $\nu CO(CHCl_3)$ 1708 cm^{-1}; yellow prisms (from MeOH) decomposing at 270° with λ_{max} (MeOH-H^+) 393, 292, 253 nm (log ε 4.31, 4.70, 4.44) and λ_{max} (MeOH-H^-) 418, 307, 293 nm (log ε 4.42, 4.66, 4.65).

Conclusive information indicating structure (III) for parazoanthoxanthin D followed from its PMR-spectrum (CF_3COOH) showing a pair of doublets centred at δ 8.82 and 8.91 (J = 11 Hz) for the 2 aromatic protons at C_8 and C_9 and 2 sharp singlets at δ 3.65 (6H) and 3.29 (3H), assigned respectively to the $-NMe_2$ grouping at C_6 and to a Me group at C_4, by comparison with the corresponding signals of zoanthoxanthin[2].

Thus, the only remaining uncertainty in the structure assigned to parazoanthoxanthin D is the position of the imino hydrogen, due to the possible existence of tautomeric forms in which this proton is attached to one of the other nitrogens of the ringsystem.

The identification of parazoanthoxanthin A, m.p. > 310° (from ethanol), followed easily from the molecular formula $C_{10}H_{10}N_6$[8], and from the UV-spectrum (Table) which closely parallels that of its congener (III). The fully *N*-demethylated structure (II) for the pigment was further substantiated by its PMR-spectrum (CF_3COOH) showing in the methyl region a sole singlet at δ 3.30, attributable to the Me group at C_4.

The remaining two trace metabolites, parazoanthoxanthin B and C, had molecular formula $C_{11}H_{12}N_6$[8] and

Absorption spectra (λ_{max} nm) of zoanthoxanthins

Pigment	MeOH	MeOH-H^+
Zoanthoxanthin	427, 293	392, 293, 259
Parazoanthoxanthin D	415, 306	394, 300, 255
Parazoanthoxanthin C	412, 302	392, 297
Parazoanthoxanthin B	404, 294	384, 287
Parazoanthoxanthin A	404, 295	381, 284

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[4] Various colonies of animals, collected in different period of the year in the Bay of Naples, were analyzed and in all cases they were found to contain the same zoanthoxanthin pattern.

[5] During the elution of the column with 1 *N* ammonia solution, zoanthoxanthin (I) is also desorbed but, owing to its insolubility in water, it precipitates mostly on the resin as fine yellow needles.

[6] Found: C, 58.95; H, 6.12; N, 34.21. $C_{12}H_{14}N_6$ requires: C, 59.49; H, 5.82; N, 34.69%.

[7] Fluorescence measurements were made with an Aminco-Bowman spectrofluorometer. IR- spectra are recorded with a Perkin-Elmer 457 instrument, UV-spectra with an Optica CF4R spectrophotometer, and PMR-spectra with a Varian HA-100 spectrometer; chemical shifts are expressed in ppm from TMS. Mass spectra and exact mass measurements were obtained by direct insertion technique with an A.E.I. MS-902 double-focus spectrometer (70 eV and 50 μA) at the lowest temperature which gave a definite spectrum (170–190°).

[8] Determined by high resolution mass spectral analysis.

$C_{12}H_{14}N_6$[8], respectively, and displayed absorption maxima (Table) consistent with a tetrazacyclopentazulene chromophore. Unfortunately, due to the very small quantities available, they were not further investigated.

To correlate the structures of the new pigments isolated from *P. axinellae* with that of zoanthoxanthin, a sample of the latter was subjected to *N*-demethylation with boiling 40% hydrobromic acid. Fractionation of the butanolic extract of the reaction mixture on silica gel gave, as major product, 3-norzoanthoxanthin, identical with natural parazoanthoxanthin D, along with the dinor-derivative (VI) and fully *N*-demethylated zoanthoxanthin[9]. While the latter was found to correspond in all respects (TLC, UV and MS) to parazoanthoxanthin A, (VI) had chromatographic and spectral properties different from those of the isomeric parazoanthoxanthin B which, consequently, may be either 1- or 3- methylparazoanthoxanthin A.

The simple structural relationship existing among the pigments isolated from *P. axinellae* suggests that the various related fluorescent pigments occurring in zoanthids differ only in the number and position of methyl group linked to the diaminotetrazacyclopentazulene chromophore. The basic skeleton of this new group of marine nitrogen metabolites, for which we propose the generic name zoanthoxanthins, probably arises biogenetically from two C_5N_3 units derived from arginine.

Riassunto. Ulteriori studi degli estratti etanolici di *Parazoanthus axinellae* hanno condotto all'isolamento di altri quattro pigmenti fluorescenti, denominati parazoantoxantina A, B, C e D, che differiscono dalla zoantoxantina (I) unicamente per il numero di gruppi metilici legati al cromoforo diamminotetrazaciclopentazulenico.

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11 February 1974.

[9] Notably, the hydrolysis of zoanthoxanthin afforded also small amounts of the 2-desaminohydroxyderivative of (I), (II) and (III).

Two New Anthraquinones from the Seeds of *Cassia occidentalis* Linn

An anthraquinone glycoside from *Cassia occidentalis* seeds has been isolated[1]. We now report here the presence of 2 new anthraquinones in the seeds of this plant.

Materials and methods. The chloroform extract of the de-fatted seeds (7.5 kg) was fractionated with petroleum ether (b.p. 40–60°) and benzene. The benzene fraction (1.8 g) was chromatographed on silica gel column and eluted with petroleum ether, benzene, ethyl acetate and also with their mixtures. Petroleum ether eluate yielded physcion (510 mg) as has also been reported by KING[2]. Petroleum ether: benzene (1:1) eluate yielded a yellow compound (106 mg). The compound was designated as 'compound A'. The benzene: ethyl acetate (1:1) eluate was concentrated and separated by preparative thin layer chromatography using benzene: ethyl acetate (1:1) as developer. Two bands, yellow and dark red, were obtained. Yellow band yielded emodin (120 mg) as is also quoted by CHOPRA[3]. Dark red band was extracted with hot chloroform. It yielded a dark red compound (120 mg). The compound was designated as 'compound B'.

Results and discussion. Compound A, m.p. 307–09°, $C_{15}H_{10}O_4$, was soluble in benzene, chloroform, pyridine, ethanol and glacial acetic acid; sparingly soluble in petroleum ether, acetone, ethyl acetate and methanol. Colour reactions were characteristic for an anthraquinone compound. An orange colour with 0.5% methanolic magnesium acetate[4] was obtained. Insolubility of the compound in 5% aqueous sodium carbonate indicate[5] the absence of free hydroxyl group in β-position.

Acetylation gave a diacetyl derivative: from methanol yellow green needles, m.p. 145–47°. The compound gave no characteristic colour reaction with cerric ammonium nitrate indicating that both the hydroxyl groups are phenolic in nature. The compound did not contain any methoxyl group as determined by the semi-micro method of BELCHER[6]. On zinc dust distillation, compound A gave 2-methyl anthracene.

The UV-spectrum of the compound showed λ_{max} at 432 nm indicating the presence of two α-hydroxyl groups[7,8]. The two α-hydroxyl groups might be expected to be at positions 1,4, 1,5 or 1,8. However, the possibility of 1,4 hydroxyls have been excluded due to the absence of fluorescence in glacial acetic acid[9], likewise 1,4 as also 1,5 possibilities were excluded[10] due to the presence of 2 peaks at 1675 and 1620 cm^{-1} in the IR-spectra. Thus, the only possibility left is 1,8 position for the 2 hydroxyl groups. The specific colour reaction with 0.5% methanolic magnesium acetate is in conformity with this possibility.

On the basis of all these observations, compound A can be represented either as 1,8-dihydroxy-2-methyl-anthraquinone or as 1,8-dihydroxy-3-methyl-anthraquinone. The latter is known as chrysophanol. Therefore, compound A possesses the structure of 1, 8-dihydroxy-2-methyl-anthraquinone.

Compound B, m.p. 285–87°, $C_{16}H_{12}O_6$, was soluble in benzene, acetone, chloroform, carbon tetrachloride, pyridine, dioxan and glacial acetic acid; sparingly soluble in petroleum ether, ethyl acetate, methanol and ethanol. Colour reactions were characteristic for an anthraquinone compound. Purple colour with 0.5% methanolic magnesium acetate[4] was obtained. It was insoluble in 5% aqueous sodium carbonate, thereby showing[5] the absence

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