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ZOANTHOXANTHINS OF A NEW STRUCTURAL TYPE FROM *EPIZOANTHUS ARENACEUS* (ZOANTHARIA)

L. Cariello, S. Crescenzi, G. Prota and L. Zanetti
Stazione Zoologica di Napoli and Istituto di Chimica Organica dell'Università,
Via Mezzocannone 16, Napoli, Italy

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Abstract—Four new fluorescent nitrogenous pigments belonging to the group of zoanthoxanthins have been isolated from ethanolic extracts of the Mediterranean zoanthid *Epizoanthus arenaceus*. Two of them, named epizoanthoxanthin A and B, have been identified as **2** and **3** respectively, while the others, pseudozoanthoxanthin and 3-norpseudozoanthoxanthin, are formulated by chemical and spectroscopic evidence as the structures **4** and **5**, characterized by the novel isomeric 1,3,7,9-tetrazacyclopent[*e*]azulene skeleton.

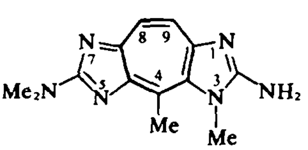
Previously we reported the occurrence in *Parazoanthus axinellae* of zoanthoxanthin (**1**)[1] and six closely related copigments[2,3], differing in the number and position of the methyl groups attached to the nitrogens of the 2,6-diamino-1,3,5,7-tetrazacyclopent[*f*]azulene chromophore.

In pursuing our research on zoanthoxanthins we have examined the nitrogenous pigments in *Epizoanthus arenaceus*, another zoanthid commonly found in the Bay of Naples. Although closely related to *P. axinellae*, this species was found to contain a quite different pattern of four new fluorescent pigments, two of which, designated epizoanthoxanthin A (**2**) and B (**3**), were found to have the same skeleton of the previously described zoanthoxanthins, while the remaining two are characterized by an isomeric tetrazacyclopentazulene system, and accordingly were named pseudozoanthoxanthin (**4**) and 3-norpseudozoanthoxanthin (**5**).

The isolation of these new metabolites was performed by fractionation of the ethanolic extracts of *E. arenaceus* on a Dowex 50 W column, followed by purification of the zoanthoxanthin fractions by TLC on silica and/or crystallization. The yields and some properties of the four pigments so obtained are reported in Table 1.

Epizoanthoxanthins. The identification of these minor pigments was aided by the results of a previous study[3] on the methylation of zoanthoxanthin (**1**) and parazoanthoxanthin D (**6**), leading to various N-methyl derivatives which were fully characterized by spectral methods. Indeed, one of the pigments isolated from *E. arenaceus*, epizoanthoxanthin A, $C_{13}H_{16}N_6$, m.p. 191–192°, showed chromatographic, electrophoretic and spectral (UV, MS, PMR) properties identical with those of the derivative **2**, obtained from parazoanthoxanthin D (**6**) by selective methylation of the amino group at C-2[3].

The other congener, epizoanthoxanthin B, $C_{14}H_{18}N_6$, of which only few milligrams were available, showed a typical zoanthoxanthin chromophore, and its PMR spectrum (Table 2) was similar to that of **2**, except for an additional 3H singlet at $\delta 4\cdot24$, attributable to a Me group on a cyclic nitrogen. As the chromatographic and spectral properties of epizoanthoxanthin B differed from those of the two known[3] zoanthoxanthins **7** and **8**, it followed that the pigment could be either **9** or **3**. However, the latter formulation appeared more compatible with the chemical shift of the C–Me group, resonating at $\delta 3\cdot27$ as expected for a zoanthoxanthin unsubstituted at position N-3 and



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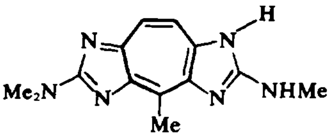

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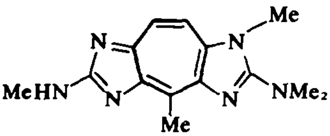

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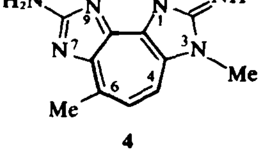

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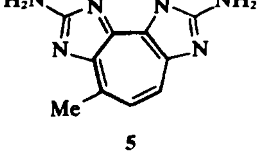

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Table 1. Yields and properties of zoanthoxanthins from *E. arenaceus*

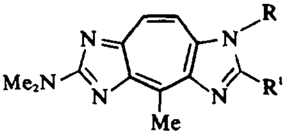
Pigment	Yield, mg/kg (wet weight)	R_f values[a]	Electrophoretic migration[b] pH 1·8	pH 5·4	λ_{max} nm (log ϵ) in MeOH, and in MeOH-H$^+$
Epizoanthoxanthin A (2)	15	0·59	0·77	0·24	419, 310, 295 infl (4·37, 4·65, 4·51); 396, 303 (4·31, 4·69).
Epizoanthoxanthin B (3)	6	0·79	0·85	0·48	412, 310 (4·13, 4·70); 396, 308 (4·17, 4·65).
Pseudozoanthoxanthin (4)	300	0·86	1·00	1·00	421, 367, 307, 281 (3·93, 4·07, 4·67, 4·48); 399, 348, 335, 290 (4·33, 3·78, 3·77, 4·88).
3-Norpseudozoanthoxanthin (5)	10	0·56	0·87	0·37	400, 360, 296, 253 (3·80, 3·74, 4·52, 3·84); 398, 332 br, 287 (4·02, 3·61, 4·60).

[a] In $CHCl_3$-MeOH-25% NH_4OH (90:10:1, v/v).
[b] On Whatman No. 1 paper relative to pseudozoanthoxanthin; pH 1·8, formic-acetic acid buffer; pH 5·4, pyridine-acetic acid buffer.

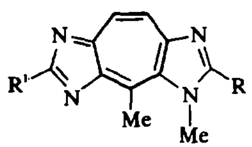
Table 2. PMR data (δ) in CF_3COOH of zoanthoxanthins and their derivatives

Compound	Aromatic protons	Me-N (cyclic)	-NHMe	-NMe$_2$	C-Me
2	8·91[a]		3·46	3·62	3·26
3	~8·80[b]	4·24	3·49	3·70	3·27
5	8·15 and 8·29[c]	4·56			3·01
4	8·21[d]	4·05 and 4·61			3·03
12	8·73[a]	4·13 and 4·68			3·24
13	8·61[a]	4·11 and 4·45			3·09
18	8·07[d]	4·02 and 4·62		3·56	3·00
11	8·33 and 8·40[c]	3·86 and 4·27			3·04
15	8·37[a]	3·88 and 4·40			3·14
14	8·19 and 8·43[c]	3·86 and 4·25			3·02
19	7·92 and 8·04[c]	3·80 and 4·36		3·51	2·93

[a] Broadened signal; [b] broadened AB quartet; [c] doublets, J ≈ 11 Hz; [d] singlet.



6: R = H; R' = NH$_2$
7: R = Me; R' = NHMe
10: R = Me; R' = NMe$_2$



8: R = NHMe; R' = NMe$_2$
9: R = NMe$_2$; R' = NHMe

N-5*. Proof that 3 was the correct structure of epizoanthoxanthin B, was obtained by selective methylation of the pigment at NHMe group with MeI and sodamide in liquid NH_3, which gave the desired homolog, identical in all respect with the previously described[3] zoanthoxanthin derivative **10**.

Pseudozoanthoxanthins. The principal nitrogenous metabolite of *E. arenaceus*, pseudozoanthoxanthin **4**, crystallized from methanol as yellow prisms which in solution exhibited an intense blue fluorescence with an emission maximum at 446 nm on excitation at 340 nm. Elemental analyses suggested for the pigment the molecular formula $C_{12}H_{14}N_6$ which was confirmed by exact measurements of the molecular ion peak at 242 *m/e* (base peak). Other diagnostic ions in the mass spectrum were at 227, 214, 200 and 159 *m/e*, the latter arising by loss of a $C_3H_5N_3$ grouping. The UV spectrum (Table 1) of pseudozoanthoxanthin was consistent with a tetrazacyclopentazulene chromophore, but it displayed some characteristic features with respect to those of zoanthoxanthins. Typical was also the PMR spectrum (Table 2) showing, in addition to two Me group on cyclic nitrogens, two aromatic protons and a C-Me group resonating at relatively higher field, as compared with the corresponding signals in zoanthoxanthins. On the whole these signals accounted for 11 of the 14 protons of the molecular formula of pseudozoanthoxanthin, the remaining were probably located on N atoms, as suggested by a broadened band at δ7·92 removed on addition of D_2O.

When refluxed in 6N HCl pseudozoanthoxanthin gave the oxo analogue **11**, $C_{12}H_{13}N_5O$, whose IR spectrum displayed a carbonyl band at 1724 cm^{-1}. On the other hand, diazotisation of the pigment with sodium nitrite in 3N HCl, afforded two reaction products which were separated by TLC on silica and identified as the desaminochloroderivative **12**, $C_{12}H_{12}N_5Cl$, and the corresponding desaminohydroxyderivative **13**, $C_{12}H_{13}N_5O$. In contrast with its isomer **11**, the compound **13** showed lack of absorption in the IR carbonyl region and on treatment

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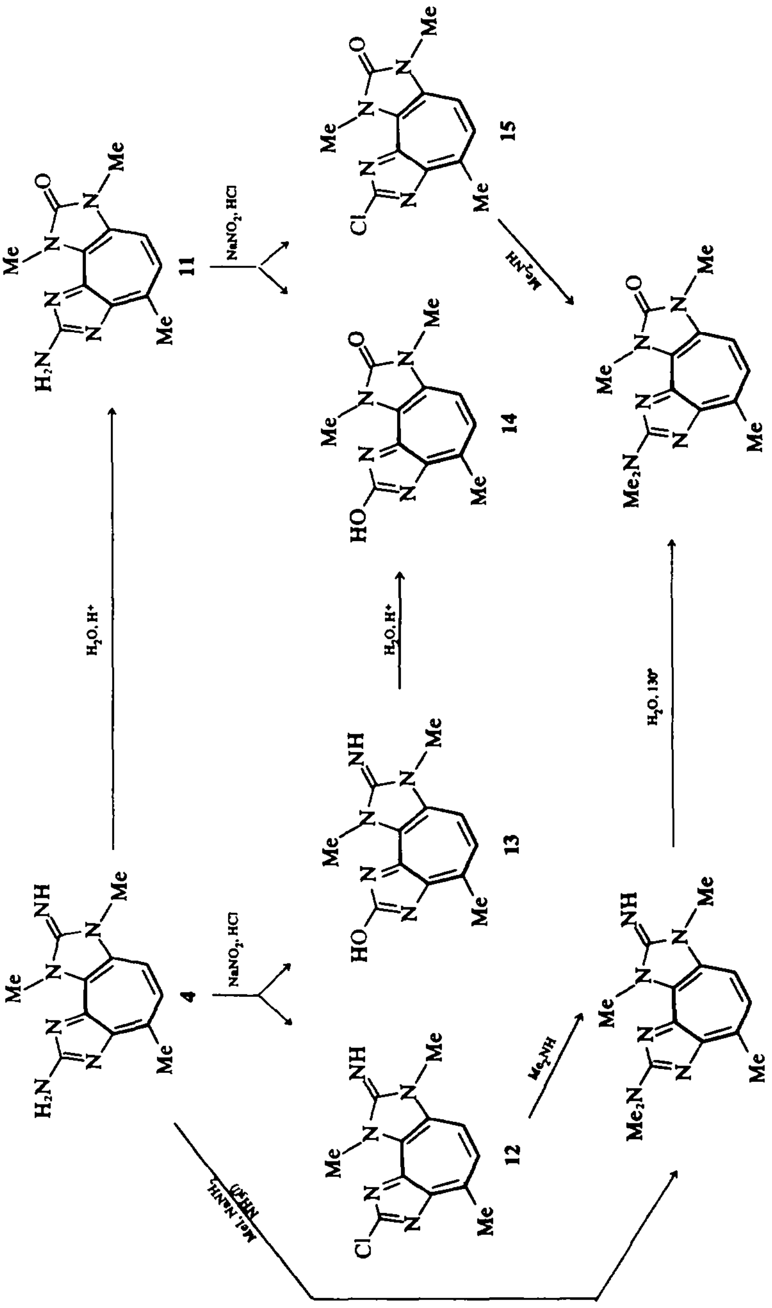
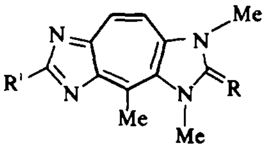
*In zoanthoxanthin (**1**) and its analogues, the resonance of the C-Me group is shifted to low field at δ3·41 ± 0·01 by the effect of the neighbouring N-Me group[3].


4
11
12
13
14
15
18
19
H₂O, H⁺
NaNO₂, HCl
NaNO₂, HCl
H₂O, H⁺
Me₂NH
Me₂NH
H₂O, 130°
MeI, NaNH₂ NH₃(l)

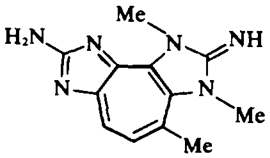
with boiling 6N HCl it underwent, as expected, the hydrolysis of the imino function to give **14**, $C_{12}H_{12}N_4O_2$, $\nu_{C=O}$ 1728 cm^{-1}. This latter, together with the chlorocompound **15**, $C_{12}H_{11}N_4OCl$, $\nu_{C=O}$ 1725 cm^{-1}, was also obtained by diazotising the derivative **11** under conditions similar to those used for pseudozoanthoxanthin.

In addition to information on the nature of the nitrogen functions, these reactions suggested that the two N–Me groups were located on the same imidazole ring, since a different structural situation would lead to pairs of isomers for each of the foregoing products. In agreement with this view, examination of the PMR spectra (Table 2) of the pseudozoanthoxanthin derivatives revealed that the resonances of N–Me groups were little influenced by the substitution of the primary amino group with OH or Cl, whereas they were both significantly shifted diamagnetically when the imino function was replaced by oxygen. Notably, in the PMR spectrum of the derivative **14**, the two aromatic protons gave a well defined AB quartet (J ≅ 11 Hz), thus indicating that they were next to each other.

On the basis of the foregoing data, three alternative structures (**16**, **4** and **17**) for pseudozoanthoxanthin appeared to be possible, although the first, having the usual zoanthoxanthin skeleton, seemed rather unlikely in the light of the characteristic spectroscopic features of the pigment.



16: R = NH; R' = NH_2
20: R = NH; R' = NMe_2
21: R = O; R' = NMe_2



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To rule out definitively structure **16**, pseudozoanthoxanthin was converted into the dimethylderivative **18** either by methylation of the NH_2 group with MeI and sodamide in liquid NH_3 or by way of the chlorocompound **12** (see Scheme). Subsequent hydrolysis of **18** with water at 130° gave the oxo analogue **19**, $C_{14}H_{17}N_5O$, $\nu_{C=O}$ 1718 cm^{-1}, which could be also obtained from **15** by treatment with Me_2NH. As expected, **18** and **19** showed chromatographic and spectral properties different from those of the corresponding known isomeric derivatives **20**[3] and **21**[1].

Hence, pseudozoanthoxanthin may be either **4** or **17**, which differ only for the position of the C–Me group on the 1,3,7,9-tetrazacyclopent[*e*]azulene chromophore. Both structures account for the unusual methyl δ-value 4·61 attributable to the Me group at N-1 spatially close to the other imidazole ring, as well as for the relatively higher chemical shifts of both the C–Me group and the aromatic protons with respect to those of zoanthoxanthins. However, irradiation at the frequency of the 3-Me signal in the PMR spectrum of the chloroform soluble derivative **19**, produced no appreciable modification of the resonance from the C–Me protons,* while the same experiment with the isomeric analogue **21** resulted in an increase in the intensity (by *ca.* 16%) of the C–Me signal at δ3·27. On the basis of these NOE experiments we propose structure **4** for pseudozoanthoxanthin.

The characterization of the other closely related pigment as 3-norpseudozoanthoxanthin (**5**), followed easily from the molecular formula $C_{11}H_{12}N_6$ and from the PMR spectrum which differed from that of **4** essentially for the lack of the N-3 methyl signal at δ4·05 (Table 2).

Conclusive evidence indicating structure **5** for the pigment was obtained by treatment of pseudozoanthoxanthin (**4**) with boiling conc. HBr. Fractionation of the butanolic extracts of the reaction mixture on silica gel gave as major product the oxo analogue **11**, along with the desired 3-norderivative showing chromatographic, electrophoretic and spectral (UV, MS, PMR) properties identical with those of the natural pigment.

EXPERIMENTAL

M.ps were determined with a Kofler-hotstage apparatus and are uncorrected. IR spectra were recorded with a Perkin-Elmer Infracord 137 E, UV spectra with an Optica CF4R spectrophotometer, and PMR spectra with a Varian HA-100 instrument; chemical shifts are expressed in ppm from TMS. The fluorescence measurements were measured in MeOH on an Aminco-Bowman spectrophotofluorometer. Mass spectra and exact mass measurements were obtained by direct insertion technique with an AEI MS-902 spectrometer (70 eV); besides the molecular ion peak the most significant ions in the mass spectrum are given with their relative intensities. The chromatographic column of Dowex 50 W (100–200 mesh; 2% cross linkage; H^+ form) was prepared according to standard procedure. Analytical and preparative TLC were carried out on precoated silica gel F_{254} plates (E. Merck) and all solvents used for development and for elution were redistilled; proportions given for mixed solvents are by volume. The chromatograms were examined by UV irradiation at 366 nm.

Isolation of the pigments. A batch of living specimens of *Epizoanthus arenaceus* (1 kg, wet weight), collected in the Bay of Naples, were homogenated in a Waring blendor with EtOH (800 ml). The homogenate was centrifuged and the residue re-extracted a few times until the EtOH was almost colourless. The combined extracts, acidified with conc HCl, were concentrated *in vacuo* to a small volume, diluted with water and extracted with ether to remove lipochromes and other extraneous materials. The aqueous layer was then passed through a column of Dowex 50 W (2·5 × 25 cm) on which zoanthoxanthins were strongly adsorbed. After washing with 0·5 N HCl and water, the column was eluted with 1 N NH_4OH and the fractions containing zoanthoxanthins were collected and combined in two pools on the basis of TLC examination. A third fraction, containing mainly pseudozoanthoxanthin (**4**), was obtained by suspending the bed of the column in 2 N NaOH and by extracting the slurry with butan-1-ol (1 liter in 4 portions).

The first pooled fractions were extracted with butan-1-ol, and the residue obtained by evaporating the organic phase was purified by TLC on silica with $CHCl_3$-MeOH-25% NH_4OH (80:20:2) to give mainly **5**, yellow prisms (from MeOH) which darkens from 230° onwards without melting; very slightly soluble in lower alcohols, dioxan and DMSO; *m/e* 228 (100%), 213 (12%), 200

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*Reverse irradiation gave the same result.

(18%), 186 (11%), 159 (18%), and 158 (17%). (Found: M^+, 228·1112. $C_{11}H_{12}N_6$ requires: M, 228·1123).

Similar work up of the second pooled fractions gave, besides an additional amount of **5**, epizoanthoxanthin A (**2**), orange yellow needles (from H_2O), m.p. 191–192°, identical in all respects with one of the methylated products of **6**, and epizoanthoxanthin B(**3**), as an amorphous yellow powder, *m/e* 270 (100%), 255 (20%), 242 (19%), 214 (6%), and 185 (8%). (Found: M^+, 270·1577. $C_{14}H_{18}N_6$ requires: M, 270·1593).

The third fraction containing pseudozoanthoxanthin was evaporated to dryness and the residue purified by repeated crystallizations from MeOH to give analytically pure sample of **4**, as yellow prisms, m.p. > 310°, insoluble in water and in ordinary organic solvents. (Found: C, 59·41; H, 5·93; N, 34·61%; M^+, 242·1294. Calc. for $C_{12}H_{14}N_6$: C, 59·49; H, 5·82; N, 34·69%; M, 242·1280).

Methylation of epizoanthoxanthin B (**3**). An excess of MeI was added portionwise to a soln of the pigment (5 mg) in liquid NH_3 (1 ml), containing $NaNH_2$ prepared *in situ* by addition of 10 mg of Na. The mixture was stirred at −50° for 30 min. Removal of the volatile constituents left a residue which was purified by preparative TLC with $CHCl_3$-MeOH (80:20) to give a sole methylated product (3 mg) identified as **10** by chromatographic, electrophoretic, UV and MS comparison with an authentic sample.

Hydrolysis of pseudozoanthoxanthin (**4**). In a typical experiment* a soln of **4** (50 mg) in 6 N HCl (3 ml) was refluxed for 24 hr. The hydrolysate was evaporated to dryness *in vacuo* and the residue taken up with water, basified with 2 N NaOH and extracted with butan-1-ol. Fractionation of the organic layer by preparative TLC with $CHCl_3$-MeOH (85:15) gave, along with unchanged **4**, 20 mg of **11** (R_f 0·70), small yellow needles (from H_2O), subliming at 298–300°; λ_{max}(MeOH) 408, 364, 291, and 251sh nm (log ϵ 3·72, 3·98, 4·72, and 3·94); λ_{max}(MeOH/H^+) 403, 337, 294, and 248 nm (log ϵ 4·15, 3·78, 4·91, and 4·08); *m/e* 243 (100%), 228 (19%), 214 (65%), 200 (25%), 188 (17%), 187 (21%), and 159 (42%). (Found: M^-, 243·1124. $C_{12}H_{13}N_5O$ requires: M, 243·1120).

Diazotisation of pseudozoanthoxanthin (**4**). To a cold soln of **4** (50 mg) in 3 N HCl (5 ml), NaCl (100 mg) and $NaNO_2$ (200 mg) was added and the mixture was kept 21 hr at 4° with occasional stirring. After addition of urea, the mixture, adjusted at pH 6 with 2 N NaOH, was extracted firstly with $CHCl_3$ (60 ml in 3 portions) and then with butan-1-ol (40 ml in 2 portions).

The residue obtained after evaporating the chloroform extract was purified by preparative TLC with $CHCl_3$–MeOH (85:15) to give **12** (R_f 0·74; 24 mg), as yellow orange needles (from C_6H_6), m.p. 253–255° (dec); λ_{max}(MeOH) 434, 337, 294, and 241sh nm (log ϵ 4·11, 3·82, 4·52, and 4·12); λ_{max} (MeOH/H^+) 391, 338, 329, 280, and 245 nm (log ϵ 4·23, 3·76, 3·74, 4·74, and 4·22); *m/e* 261 (100%), 246 (20%), 233 (5%), 219 (8%), and 218 (8%). (Found: M^+, 261·0793. $C_{12}H_{12}N_5\ {}^{35}Cl$ requires: M, 261·0781).

Fractionation of the butanolic extract by preparative TLC using $CHCl_3$–MeOH–25% NH_4OH (80:20:1) as the eluent gave, along with an additional amount of **12** (R_f 0·97; 4 mg), the hydroxy derivative **13** (R_f 0·58; 9 mg), yellow needles (from aqueous MeOH), m.p. > 310°; λ_{max} (MeOH) 426, 400, 356 br, 298, 285, and 243 nm (log ϵ 3·48, 3·99, 3·68, 4·39, 4·54, and 4·05); λ_{max} (MeOH/H^+) 379, 352, and 294 nm (log ϵ 3·75, 3·88, and 4·82); λ_{max} (MeOH/OH^-) 418, 371, and 306 nm (log ϵ 3·83, 4·00, and 4·79); *m/e* 243 (100%), 228 (24%), 215 (16%), 214 (14%), and 200 (15%). (Found: M^+, 243·1124. $C_{12}H_{13}N_5O$ requires: M, 243·1120).

Diazotisation of **11**. A soln of **11** (50 mg) in 3 N HCl was diazotised as described above. The mixture was extracted with $CHCl_3$ (90 ml in 4 portions), and the extract was fractionated by preparative TLC with $CHCl_3$–MeOH (85:15) to give mainly two yellow products. The major band (R_f 0·88) gave 11 mg of **15**, fine needles (from MeOH) subliming from 215° and melting at 270–271°; λ_{max} (MeOH) 412, 341, 328, 281, and 245 nm (log ϵ 4·04, 3·74, 3·73, 4·77, and 4·05); λ_{max} (MeOH/H^+) 393, 381 sh, 345, 332, 287, 272, and 245 nm (log ϵ 4·31, 4·11, 3·78, 3·77, 4·63, 4·60, and 4·30; *m/e* 262 (100%), 247 (13%), 233 (19%), and 178 (10%). (Found: M^+, 262·0635. $C_{12}H_{11}N_4O^{35}Cl$ requires: M, 262·0621).

The minor band (R_f 0·60) afforded 8 mg of **14**, prisms (from EtOH), m.p. > 310°; λ_{max} (MeOH) 406, 338, 293, and 245 nm (log ϵ 4·11, 3·50, 4·86, and 4·01); λ_{max} (MeOH/H^+) 382, 341, and 294 nm (log ϵ 3·71, 3·95, and 5·03); λ_{max} (MeOH/OH) 405, 366, 307, and 286 nm (log ϵ 3·54, 4·12, 4·36, and 4·72); *m/e* 244 (100%), 229 (9%), 217 (31%), 216 (23%), 215 (54%), 201 (13%), 189 (25%), and 160 (39%). (Found: M^+, 244·0949. $C_{12}H_{12}N_4O_2$ requires: M, 244·0960).

Compound **14** was also obtained in 60% yield from **13** by treatment with 6 N HCl and work up as described above for the hydrolysis of the parent pigment **4**.

N,N-*dimethyl pseudozoanthoxanthin* (**18**)

(a) *By methylation of pseudozoanthoxanthin* (**4**). To a soln of **4** (70 mg) in liquid NH_3 (5 ml) containing sodamide (50 mg), 0·5 ml of MeI were added dropwise over a period of *ca* 30 min. After 1 hr at −50°, the mixture was evaporated to dryness and the residue extracted with $CHCl_3$. Preparative TLC of the extract on silica with $CHCl_3$–MeOH–25% NH_4OH (90:10:1) afforded 38 mg of **18**, yellow needles (from MeOH), m.p. > 310°, λ_{max} (MeOH) 411sh, 389, 314, 286, and 251 nm (log ϵ 4·16, 4·20, 4·34, 4·41, and 4·30); λ_{max} (MeOH/H^+) 414, 301, and 252 nm (log ϵ 4·32, 4·73, and 4·32); *m/e* 270 (77%), 255 (100%), and 241 (68%). (Found: M^+, 270·1565. $C_{14}H_{18}N_6$ requires: M, 270·1593.

(b) *By amination of* **12**. To a soln of **12** (20 mg) in MeOH (2 ml) excess of $Me_2NH.HCl$ (0·81 g), an equivalent amount of AcONa (0·82 g) and 0·2 ml of AcOH were added sequentially, and the mixture was kept 2 hr at 100° in the sealed tube. After addition of dil NaOH aq and removal of the Me_2NH *in vacuo*, the mixture was extracted with $CHCl_3$ and the extract were purified by preparative TLC with $CHCl_3$–MeOH–25% NH_4OH (90:10:1) to give 12 mg of **18**, identified by chromatographic, UV and MS comparison with an authentic sample.

Hydrolysis of N,N-*dimethyl pseudozoanthoxanthin* (**18**). A suspension of **18** (10 mg) in water (3 ml) was heated in a sealed tube at 130° for 2 hr. The mixture, obtained by repeating the above procedure 5 times, was extracted with $CHCl_3$. TLC of the organic layer on silica with $CHCl_3$–MeOH (95:5) gave, besides some unchanged **18** (R_f 0·10), 28 mg of the oxo compound **19** (R_f 0·72), crystallizing from MeOH as fine yellow needles, m.p. 195–197°. A 7% soln of **19** in $CDCl_3$ was used for the NOE experiments; δ 2·83 (3H, s, C–Me), 3·37 (6H, s, NMe_2), 3·48 (3H, s, Me at N-3), 4·12 (3H, s, Me at N-1), 6·99 and 7·43 (1H each, d, $J \simeq 10·5$ Hz, aromatic protons). λ_{max}(MeOH) 417, 383, 316 infl, 295, and 253 nm (log ϵ 4·09, 4·19, 4·27, 4·60, and 4·21); λ_{max} (MeOH/H^+) 416, 305, and 252 nm (log ϵ 4·33, 4·89, and 4·30); *m/e* 271 (66%), 256 (100%), 242 (67%) and 201 (22%). (Found: M^+, 271·1425. $C_{14}H_{17}N_5O$ requires: M, 271·1433).

Compound **19** was also obtained in excellent yield (72%) from **15** by treatment with $Me_2NH.HCl$ as described above.

Demethylation of pseudozoanthoxanthin (**4**). A soln of **4** (60 mg) in conc HBr (5 ml) was refluxed for 3 hr. The mixture was concentrated *in vacuo*, diluted with 0·5 N NaOH and extracted with butan-1-ol. Fractionation of the extract by preparative TLC with $CHCl_3$–MeOH–25% NH_4OH (80:20:2) gave two products. The major band (R_f 0·90; 21 mg) was identified as **11** by MS and PMR comparison with an authentic sample, while the minor band (R_f 0·55; 9 mg) showed chromatographic, electrophoretic and

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*Because of the unfavourable solubility properties of the pigment and its derivatives, the reactions were usually carried out on a small scale (10–50 mg).

spectral (UV, MS and PMR) properties identical with those of natural **5**.

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REFERENCES

[1] L. Cariello, S. Crescenzi, G. Prota, F. Giordano and L. Mazzarella, *J. Chem. Soc. Chem. Comm.* 99 (1973); L. Cariello, S. Crescenzi, G. Prota, S. Capasso, F. Giordano and L. Mazzarella, *Tetrahedron* **30**, 3281 (1974)

[2] L. Cariello, S. Crescenzi, G. Prota and L. Zanetti, *Experientia* **30**, 849 (1974)

[3] L. Cariello, S. Crescenzi, G. Prota and L. Zanetti, *Tetrahedron* **30**, 3611 (1974)