Tetrahedron Vol 30, pp 3281 to 3287. Pergamon Press 1974 Printed in Great Britain

ZOANTHOXANTHIN, A NATURAL 1,3,5,7-TETRAZACYCLOPENT[*f*]-AZULENE FROM *PARAZOANTHUS AXINELLAE*[a]

L. CARIELLO, S. CRESCENZI and G. PROTA*
Stazione Zoologica, Napoli and Istituto di Chimica Organica, Università di Napoli

and

S. CAPASSO, F. GIORDANO and L. MAZZARELLA
Instituto Chimico, Università di Napoli, Italy

(*Received in the UK 5 October* 1973; *Accepted for publication* 10 *April* 1974)

Abstract—Zoanthoxanthin, the principal fluorescent pigment of the mediterranean zoanthid *Parazoanthus axinellae*, has been isolated in crystalline form and identified as 2-amino-3,4-dimethyl-6-dimethylamino-3*H*-1,3,5,7-tetrazacyclopent[*f*]azulene **1** from its chemical and spectral properties and by X-ray crystallographic analysis of its 2-chloro-derivative **3**.

Crystals of **3** are monoclinic, space group $P2_1/c$, with $Z = 4$, in a unit cell of dimensions $a = 7{\cdot}282$, $b = 8{\cdot}408$, $c = 25{\cdot}422$ A°, $\beta = 94{\cdot}31°$. The structural analysis, carried out by direct methods and refined to an R value of 0·040, has shown that the molecule is nearly planar, the 7-membered carbocyclic ring assuming a shallow boat conformation while the two imidazole rings are slightly bent in the opposite direction with respect to the folding of the central ring.

In the course of screening work on new nitrogen metabolites from coelenterates of the class Anthozoa, attention was given to a group of basic yellow-coloured substances with powerful fluorescence in ordinary light, occurring in some colonial organisms of the family Zoanthidea (order Zoantharia) which inhabit mediterranean coastal waters, i.e. *Epizoanthus arenaceus* and *Parazoanthus axinellae*.

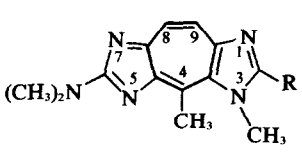
Since the general properties of these metabolites did not correspond with those of any of the known types of animal pigments, a detailed chemical investigation on the more accessible species, i.e. *Parazoanthus axinellae*, was undertaken. In this paper we describe the experiments leading to assignment of structure **1** to principal constituent for which we propose the trivial name zoanthoxanthin. The isolation was performed by dipping the whole animals in ethanol at room temperature and by passing the yellow extract, partially purified by liquid–liquid extraction, through a column of a strongly cationic exchange resin. Under these conditions zoanthoxanthin was strongly adsorbed on the resin, making possible the removal of most of the extraneous substances by washings with dilute acids and water; subsequent treatment with dilute ammonia effected the elution of zoanthoxanthin which, owing to its insolubility in water, precipitated as fine yellow needles on the resin.

Recrystallization from methanol gave analytically pure sample of **1** with m.p. 275–276° (dec) and UV maxima in MeOH at 427 and 293 nm (log ϵ 4·35 and 4·52) while in N HCl the maxima are at 392, 293 and 259 nm (log ϵ 3·94, 4·25 and 3·87).

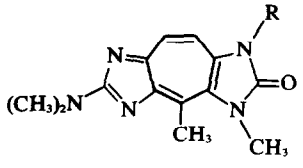
In solution the compound exhibited a caracteristic greenish-blue fluorescence in daylight which was little affected by addition of acids or alkalies.

Elemental analyses and mass spectrometry established molecular formula as $C_{13}H_{16}N_6$, including

[a] A preliminary account of this work has appeared, Ref 1.



1: R = NH_2
3: R = Cl
4: R = Br
5a: R = OH
7: R = OCH_3



5b: R = H
6: R = CH_3

one C-methyl group (Kuhn–Roth analysis). The IR spectrum (Fig 1) offered little information. The NMR spectrum of **1** taken in CF_3COOH showed a broad singlet at 8·80 δ (2H) for two aromatic protons and three sharp Me singlets at δ 4·26 (3H), 3·62 (6H) and 3·42 (3H) assigned respectively to a Me group on cyclic nitrogen, N-Me_2 and C-Me at unusually low field.[2] In DMSO-d_6 the two aromatic protons resonated at δ 7·93 δ, while the Me singlets appeared at δ 3·90, 3·20 and 3·13, respectively. In addition the spectrum exhibited a broadened signal at 8·05 δ for two D_2O-exchangeable protons, suggesting the presence of a primary amino function. Acetylation of zoanthoxanthin gave a monacetylderivative, $C_{15}H_{18}N_6O$ **2**, showing IR bands ($CHCl_3$) at 3320 and 1709 cm^{-1}.

Considering the molecular formula and the nature of the functional groups, it followed that zoanthoxanthin possessed a new tricyclic ring system formed by 4 nitrogens and 9 carbons.

When treated with sodium nitrite and 2N HCl, zoanthoxanthin yielded a crystalline chlorocompound, $C_{13}H_{14}N_5Cl$ (**3**), m.p. 192° (dec), and similarly by diazotisation in hydrobromic acid[3] it gave the bromo analogue, $C_{13}H_{14}N_5Br$ (**4**); in both cases the primary amino group of zoanthoxanthin was replaced by the corresponding halogen atom. In accordance with this view, treatment of **3** with a saturated solution of ammonia in anhydrous dioxane† at 80° resulted in a partial (10% yield) conversion of material into zoanthoxanthin (TLC, MS and UV evidence).

Either halogen containing compound suffer readily hydrolysis with dilute alkalies to give the same hydroxyderivative, $C_{13}H_{15}N_5O$ (**5a**), m.p. 304–306° (dec), which in solution exists predominantly in the tautomeric lactame form (**5b**) [$\nu_{CO}(CHCl_3)$ 1722 cm^{-1}]. As expected, treatment of **4** with diazomethane gave a N-Me derivative, $C_{14}H_{17}N_5O$ (**6**) as major product, along with the methoxy isomer (**7**) (MS and IR evidence).

Further experiments to gain conclusive information on the nature of the chromophore of zoanthoxanthin by degradative studies were unsuccessful owing to the marked stability of the compound to acids, alkalies and various oxidizing agents. Moreover, under very drastic conditions, degradation of zoanthoxanthin usually resulted in extensive cleavage of the molecule with formation of numerous products of low molecular weight, which appeared to be of little or no use in the structural analysis.

Considering the chemical behaviour of zoanthoxanthin and as material was limited, the complete structure was established through X-ray analysis of the chloro-containing derivative (**3**), which gave suitable crystals, monoclinic, space group $P2_1/c$, from aqueous ethanol.

The structure determination, carried out through a straightforward application of direct methods, permitted to locate all the atoms of the molecule, including the hydrogens, and revealed in addition the presence of three water molecules per asymmetric unit. The final atomic parameters and their standard deviations are listed in Tables 1 and 2. From these data it followed that the skeleton of the molecule is formed by a 7-membered carbocyclic ring fused with two imidazole units as depicted in Fig 2.

Perpendicular distances of individual atoms from some least-squares planes through various moieties of the molecule, show that the whole molecule is not strictly planar (see plane I in Table 3). The lack of planarity is essentially due to a slight puckering of the 7-membered ring, which assumes a shallow boat conformation (see planes II, III, and IV in Table 3), with dihedral angles 2·2° (between planes II and III) and 1·9° (between planes III and IV), comparable with those found in other 7-membered rings.[4] As a consequence of this conformation, the two imidazole rings, which in turn are planar in the limit of the accuracy of the structural parameters

†An attempt to convert **3** in zoanthoxanthin with methanolic ammonia under conditions similar to those reported for other heterocyclic halogen derivatives (i.e. 8-chloropurines) resulted mainly in the formation of the methoxy derivative **7**.

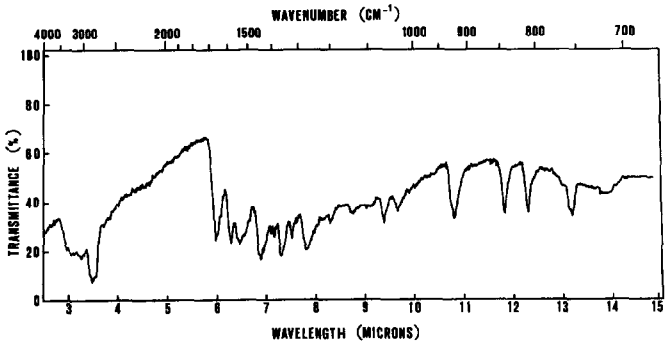


Fig. 1. The IR spectrum (nujol) of zoanthoxanthin (**1**).

Table 1. Final positional ($\times 10^4$) and thermal parameters ($\times 10^2$) of the non-hydrogen atoms with standard deviations in parentheses. The temperature factors are in the form $\exp\{-0{\cdot}25[B_{11}a^{*2}h^2 + B_{22}b^{*2}k^2 + B_{33}c^{*2}l^2 + 2B_{12}a^*b^*hk + 2B_{13}a^*c^*hl + 2B_{23}b^*c^*kl]\}$

	x/a	y/b	z/c	B_{11}	B_{22}	B_{33}	B_{12}	B_{13}	B_{23}
Cl	8457(1)	1379(1)	3237(0)	595(3)	600(3)	285(2)	49(2)	110(2)	113(2)
N(1)	6935(2)	−431(2)	6300(1)	403(6)	386(7)	263(5)	−24(5)	28(5)	−17(5)
N(2)	6365(2)	−2631(2)	5775(1)	390(6)	327(6)	282(6)	−26(5)	39(5)	16(5)
N(3)	7614(2)	−490(2)	4036(1)	394(6)	372(7)	245(5)	22(5)	33(5)	−5(5)
N(4)	8404(2)	2015(2)	4252(1)	400(7)	376(7)	334(6)	−14(6)	34(5)	67(5)
N(5)	5906(2)	−2817(2)	6677(1)	557(8)	469(8)	263(6)	−116(7)	64(6)	22(6)
C(1)	6403(2)	−1983(2)	6261(1)	345(7)	389(8)	276(7)	−22(6)	28(5)	17(6)
C(2)	6885(2)	−1412(2)	5463(1)	290(6)	301(7)	269(6)	7(5)	20(5)	2(5)
C(3)	7009(2)	−1628(2)	4920(1)	318(6)	306(7)	256(6)	2(6)	6(5)	−12(5)
C(4)	7505(2)	−383(2)	4587(1)	290(6)	338(7)	239(6)	27(5)	14(5)	4(5)
C(5)	8142(2)	963(2)	3878(1)	382(7)	441(9)	283(7)	39(7)	43(6)	73(6)
C(6)	8007(2)	1213(2)	4707(1)	308(6)	308(7)	307(7)	2(5)	26(5)	29(5)
C(7)	8191(2)	2034(2)	5186(1)	390(7)	302(7)	351(8)	−30(6)	20(6)	−10(6)
C(8)	7861(2)	1492(2)	5676(1)	412(7)	323(7)	309(7)	−21(6)	7(6)	−44(6)
C(9)	7252(2)	−30(2)	5804(1)	315(6)	331(7)	269(6)	−1(6)	10(5)	−23(5)
C(10)	6193(4)	−2217(3)	7212(1)	741(13)	734(14)	250(7)	−214(11)	33(8)	12(8)
C(11)	5219(3)	−4424(3)	6613(1)	637(11)	498(10)	431(9)	−147(9)	111(8)	58(8)
C(12)	6601(3)	−3281(2)	4718(1)	640(11)	357(8)	343(8)	−84(8)	61(8)	−52(6)
C(13)	7247(4)	−1828(3)	3676(1)	1016(17)	441(10)	297(8)	−66(11)	101(10)	−81(7)
O(1)	10470(2)	4810(2)	4073(1)	604(7)	327(6)	467(7)	25(5)	55(6)	20(5)
O(2)	9935(3)	5382(2)	2959(1)	993(12)	578(9)	383(7)	−132(8)	−102(7)	47(6)
O(3)	8391(3)	3352(2)	2164(1)	979(11)	393(7)	391(7)	19(7)	6(7)	55(5)

Table 2. Positional ($\times 10^3$) and thermal parameters ($\times 10$) of the hydrogen atoms with standard deviations in parentheses

	x/a	y/b	z/c	B(A°²)
H(1)	866(3)	315(2)	516(1)	14(4)
H(2)	808(3)	223(3)	598(1)	25(4)
H(3)	518(3)	−255(3)	741(1)	41(6)
H(4)	619(4)	−100(3)	724(1)	52(6)
H(5)	727(4)	−244(5)	743(1)	89(10)
H(6)	487(4)	−465(4)	624(1)	70(8)
H(7)	413(5)	−459(4)	687(1)	82(10)
H(8)	616(4)	−517(4)	672(1)	64(9)
H(9)	554(4)	−338(3)	443(1)	44(7)
H(10)	771(3)	−397(3)	460(1)	30(5)
H(11)	607(4)	−398(3)	499(1)	53(7)
H(12)	579(4)	−201(4)	367(1)	85(9)
H(13)	793(4)	−270(4)	378(1)	74(9)
H(14)	769(5)	−153(4)	332(1)	88(10)
H(15)	1159(4)	434(3)	412(1)	56(7)
H(16)	966(4)	392(4)	415(1)	58(7)
H(17)	1056(4)	642(3)	292(1)	53(7)
H(18)	1011(4)	513(3)	327(1)	57(7)
H(19)	904(4)	399(4)	237(1)	65(9)
H(20)	781(4)	407(3)	189(1)	51(7)

(planes V and VI in Table 3), are bent in the opposite direction with respect to the folding of the central ring.

A projection of the structure along the *b* axis (Fig 3) shows that the molecules are stacked in piles nearly parallel to the *a** axis, each molecule being related to the next one along the pile by a centre of inversion. Due to the slight puckering of the molecule, the average interplanar separations along the pile are sequentially 3·35 A° and 3·54 A°. The only short intermolecular contacts involve C(10) and N(5) atoms of one molecule and the Cl atom of a molecule at $(1-x, y, 1-z)$. These interactions may well account for the slight bent and rotation of $-N(CH_3)_2$ grouping with respect to the imidazole ring C (see also plane VI in Table 3).

The C–N bond lengths of the imidazole ring A compare well with single C_{sp_2}–N_{sp_2} and double C=N bond lengths, with the exception of the bond C(5)–N(3) = 1·351 A° which has an intermediate value. The BC ring moiety forms a resonating system closely resembling that of azulene. The average C–N distances within ring C conforms to the length generally found in heteroaromatic compounds. However, the transannular C(2)–C(9) bond (1·461 A°) is somewhat shorter than that found in azulene[5,6] and the same applies also for the C(7)–C(8) bond (1·366 A°) and for the C(2)–C(3)–C(4) valency angle (122·3°).

The rather short C(1)–N(5) distance (1·342 A°), coupled with the fact that the Me carbons C(10) and C(11) are very close to the average plane of the molecule, is indicative of a high degree of π-bonding involving the exocyclic N atom and suggests that, in addition to the two main Kekulé structures, forms with formal positive charges placed on the N(5) atom, may also give an important contribution to the structure.

As shown in Fig 3, the water molecules occurring in crystals of **3** form a H-bonding network involving the N(1), N(2), and N(4) nitrogens with all hydrogen atoms well placed along the O–O and O–N centerlines.

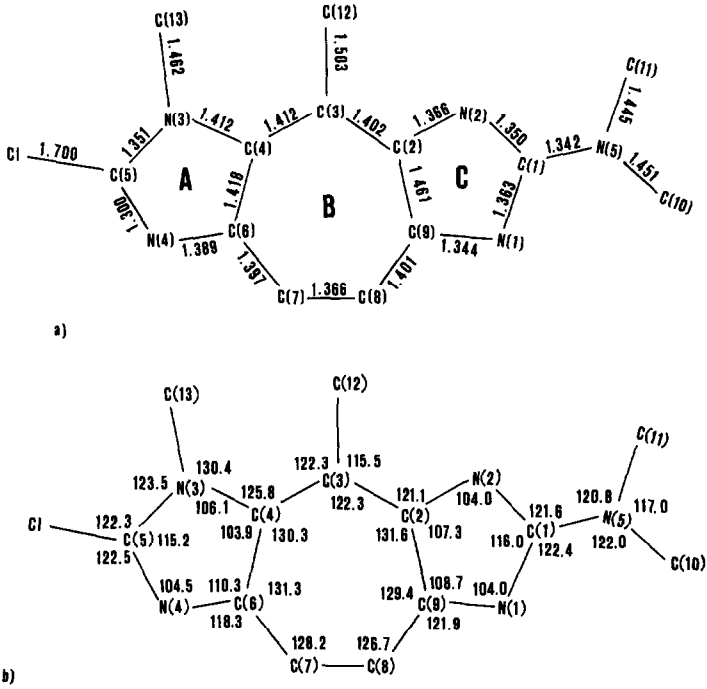


Fig 2. A view of the molecule of the transformed product (3) of zoanthoxanthin with the numbering scheme used in X-ray analysis. (a) Bond lengths (A°). (b) Bond angles (°). The average e.s.d.'s are 0·003 A° and 0·3° respectively.

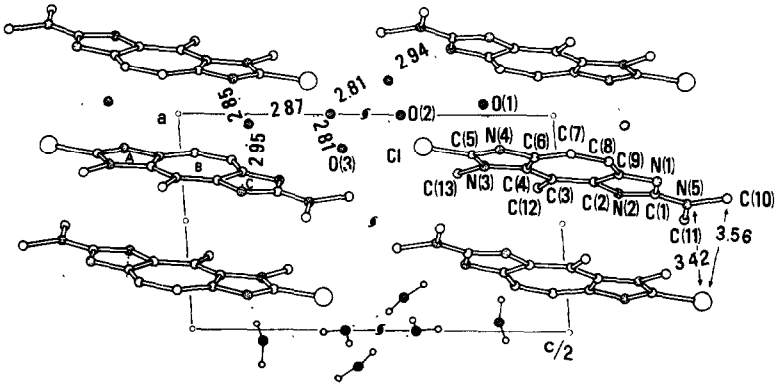


Fig. 3. Crystal structure of the transformed product (3) of zoanthoxanthin viewed along *b* axis. The shortest intermolecular distances and the hydrogen bonds are indicated. Hydrogen atoms of water molecules are also shown at bottom of figure.

In conclusion, the results of the X-ray analysis of **3** coupled with the simple chemical relationship between **3** and zoanthoxanthin allows to assign unambiguously structure **1** to the natural pigment. The most striking feature of this structure is the previously unknown heteroaromatic chromophore which may be described as 3H-1,3,5,7-tetrazacyclopent[*f*]azulene. The evidence so far available indicates that zoanthoxanthin is the first representative of a family of closely related pigments occurring in the Zoanthidae (a small group of marine animals related to sea anemones and stony corals). As the classification of these animals is difficult, even at genus levels, the distribution of these pigments appears to be of taxonomic value.

EXPERIMENTAL

Microanalyses were carried out by E. Thommen, Department of Organic Chemistry, University of Basel, Switzerland. M.ps were determined in capillaries or with a

Table 3. Displacment of the atoms from some least-squares planes of the molecule of **3**

(a) Least squares planes.
The equations of the planes are expressed in the form $Ax + By + Cz = D$ and referred to crystallographic axes.

Plane	I	II	III	IV	V	VI
A	6·886	6·947	6·899	6·849	6·872	6·914
B	−2·403	−2·053	−2·340	−2·585	−2·383	−2·331
C	2·040	2·504	2·111	1·780	2·448	1·828
D	6·188	6·439	6·235	6·008	6·409	6·116

(b) Deviations of atoms ($A° \times 10^3$).
Distances marked with asterisk refer to atoms defining the plane. The average estimated error for the distances is less than 0·005 A°.

Cl	−35	−37	−40	4	−134	2
N(1)	−24*	44	−19	−26	1*	−69
N(2)	5*	−32	−9	59	5*	−46
N(3)	−4*	−39	−15	52	−72	1*
N(4)	−18*	50	−11	−16	−74	2*
N(5)	−82	−86	−91	−46	−45	−155
C(1)	−25*	−16	−31	4	−4*	−82
C(2)	7*	1*	−1*	45	−4*	−28
C(3)	33*	−4*	21*	89	−1	9
C(4)	8*	1*	1*	48	−38	1*
C(5)	−22*	−10	−24	10	−94	−2*
C(6)	−6*	53	−1*	0*	−44	−2*
C(7)	22*	132	35	−1*	4	21
C(8)	25*	137	38	1*	27	9
C(9)	−3*	58	1*	0*	2*	−34
C(10)	81	124	79	90	140	1
C(11)	−182	−250	−203	−113	−150	−268
C(12)	108	1*	83	201	64	75
C(13)	−86	−109	−31	82	−94	−7

(c) Dihedral angles between planes.
II − III = 2·2°; III − IV = 1·9°; III − V = 0·8°; III − VI = 0·7°; V − VI = 1·5°.

Kofler hot-stage apparatus and are uncorrected. IR spectra were recorded with a Perkin–Elmer Infracord 157, UV spectra with an Optica CF4R spectrophotometer, and NMR spectra with a Perkin–Elmer R-12 A or with a Varian HA-100 instrument; chemical shifts are expressed in ppm from TMS; the fluorescence measurements were made with an Aminco–Bowman spectrophotofluorometer. 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 lowest temp which gave a definite spectrum (170–220°). The chromatographic column of Dowex 50 W (100–200 mesh; 2% cross linkage; H^+ form) was prepared according to standard procedure. Chromatograms were carried out on Whatman No. 1 paper (descending technique). Whatman 3MM paper, washed with 2% HCl, was used for preparative purpose. In any case the solvent system used was butan-1-ol-acetic acid-water (BAW, 60:20:20, v/v). Analytical and preparative TLC were carried out on precoated plates of F_{254} Kiesegel (E. Merck, A.G., Germany) 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 zoanthoxanthin (**1**). Whole tissue of living specimens of *Parazoanthus axinellae* (500 g, wet weight), collected from the littoral zone at Naples in April were macerated in a Waring blendor with EtOH (500 ml). The homogenate was centrifuged and the residue was re-extracted twice with EtOH. The combined extracts, adjusted to pH 1 by addition of 6N HCl and clarified by centrifuging, were concentrated *in vacuo* to a small volume (about 100 ml), diluted with water, and extracted with ether. The aqueous phase was passed through a column of Dowex 50 W (2 × 20 cm) which was then washed with 0·1 N HCl, water and 1N NH_4OH. The latter step resulting in the elution of zoanthoxanthin which precipitated in crystalline form on the resin. The crystals were separated from the resin by fractional sedimentation in 1N NH_4OH, washed with water and dried in a vacuum desiccator over P_2O_5 (yield 320 mg). Zoanthoxanthin crystallized from MeOH as yellow needles, m.p. 275–276° (dec), insoluble in water and in ordinary organic solvents, homogeneous on paper chromatography (BAW) and on TLC [$CHCl_3$–MeOH (80:20)]. In vacuum it sublimed above 160° without decomposition. Excitation of **1** in methanolic solution at 348 nm produced a short-lived emission at λ_{max} 479 nm. The IR spectrum is shown in Fig 1 while UV and NMR are described in the general part; *m/e* 256 (100%, M^+), 241 (91%, M–CH_3), 227 (71%, M–NCH_3), and 186 (9%, M–$NCNMe_2$). [Found: C, 59·87; H, 6·77; N, 31·52, C-Me, 4·75. Calc. for $C_{13}H_{16}N_6$: C, 60·92; H, 6·29; N, 32·79; for 1 C-Me, 5·86%].

Acetylation of zoanthoxanthin (**1**). A suspension of

zoanthoxanthin (100 mg) in Ac_2O (10 ml) was refluxed for 24 h. Volatile constituents were removed *in vacuo*, and the residue, containing considerable amount of unchanged starting material, was subjected to chromatography on Whatman 3MM paper, using BAW as the developing solvent. The yellow band at R_f 0·31 was cut out and eluted with EtOH yielding **2** (20 mg), amorphous, λ_{max}^{EtOH} 428, 402 sh, 302 sh, 290 and 263 sh nm; δ (DMSO-d_6) 2·21 (3H, s, NHAc), 3·24 (9H, s, C-Me and NMe_2), 3·88 (3H, s, >N–Me), 7·93 (2H, br s, aromatic protons). [Found: M^+, 298·1558. $C_{15}H_{18}N_6O$ requires M, 298·1542].

Diazotization of zoanthoxanthin (**1**)

Isolation and properties of **3** *and* **4**. To a cold soln of zoanthoxanthin hydrochloride (50 mg) in 0·7 N HCl (5 ml), NaCl (100 mg) and $NaNO_2$ (40 mg) were added and the mixture allowed to stand for 36 h at 4°. After addition of urea, the mixture was heated for 20 min on a steam bath, adjusted at pH 5, and extracted with chloroform. The residue obtained after evaporating the organic layer was purified by PLC on silica gel [$CHCl_3$–MeOH (90:10)] to give **3** (10 mg), yellow-orange prisms (from aqueous EtOH) decomposing at 192°, λ_{max}^{MeOH} 427, 405 br, 303, 288 and 258 nm (log ε 4·27, 4·29, 4·38, 4·56 and 4·45); δ ($CDCl_3$) 3·28 (3H, s, C-Me), 3·36 (6H, s, NMe_2), 4·15 (3H, s, >N–Me) and 8·02 (2H, s, aromatic protons). [Found: M^+, 275·0930. $C_{13}H_{14}N_5{}^{35}Cl$ requires M, 275·0938].

The bromo analogue **4** was obtained by dropwise addition of 0·7 N HBr (2 ml) to a cold aqueous solution of zoanthoxanthin hydrobromide (20 mg) containing NaBr (40 mg) and $NaNO_2$ (20 mg). After 2 h the mixture was heated for 20 min on a steam bath and then extracted with chloroform. Evaporation of the solvent gave a solide product (4 mg), homogeneous on TLC [$CHCl_3$–MeOH (97:3)], λ_{max}^{MeOH} 424, 400 br, 288 and 263 sh nm, which owing to its great instability, was analysed directly by mass spectrometry without further purification: *m/e* 321 (99%), 319 (100%), 306 (97%), 304 (98%), 292 (92%), 290 (93%), 251 (16%) and 249 (16%). [Found: M^+, 319·0445. $C_{13}H_{14}N_5{}^{79}Br$ requires M, 319·0433].

A soln of **3** (20 mg) in dioxan (10 ml) was saturated with anhyd ammonia at 80° for 4 h. After removal of the solvent, the residue was chromatographed on Whatman 3 MM paper to give mainly unchanged **3** (14 mg) along with 2 mg of **1**, identified by chromatographic, UV and MS comparison with authentic zoanthoxanthin.

To soln of **3** (20 mg) in dioxan, 5 ml of 2N NaOH were added and the mixture was heated at 60° for 1 h. The product was repeatedly extracted with butan-1-ol, and purified by paper chromatography to give 15 mg of **5**, yellow-orange prisms (from MeOH), m.p. 304–305° (dec), *m/e* 257 (M^+), 242 (M-CH_3), and 228 (M-NCH_3); λ_{max}^{MeOH} 420 br, 396 br, 298 and 243 nm (log ε 3·86, 3·80, 4·28 and 3·75); $\nu_{max}^{CHCl_3}$ 3140 (N-H), 1722 (C=O, 5-membered lactam) cm^{-1}; δ (CF_3COOH) 3·39 (3H, s, C-Me), 3·60 (6H, s, NMe_2), 4·13 (3H, s, >N–Me), 8·73 (2H, br s, aromatic protons). [Found: C, 60·25; H, 6·12; N, 26·87. Calc. for $C_{13}H_{15}N_5O$: C, 60·68; H, 5·88; N, 27·22%]. Under similar conditions, reaction of **4** (2 mg) with 2N NaOH gave **5**, identified by chromatographic and UV comparison with an authentic sample obtained as above.

A methanolic solution of **5** (25 mg) was treated with excess of ethereal diazomethane, allowed to stand for 30 min at room temp and then evaporated *in vacuo* to dryness. The residue was dissolved in chloroform and separated by PLC on silica with $CHCl_3$–MeOH (88:12) to give mainly two yellow products. The major band R_f 0·62 (**6**) formed prisms from MeOH (8 mg), m.p. 267–269°, showing M^+ at *m/e* 271 and four sharp Me singlets at δ ($CDCl_3$) 3·27 (3H, C-Me); 3·41 (6H, NMe_2), 3·58 (3H, >N-Me) and 3·92 (3H, >N-Me); λ_{max}^{MeOH} 411 br, 390 br, 299, 271 sh and 249 nm (log ε 4·09, 4·07, 4·52, 4·06 and 3·94); $\lambda_{max}^{MeOH-HCl}$ 405, 310 and 262 nm (log ε 4·18, 4·67 and 4·13; $\nu_{max}^{CHCl_3}$ 1701 (C=O). [Found: M^+, 271·1439. Calc. for $C_{14}H_{17}N_5O$: M, 271·1433]. The minor band R_f 0·45 gave 4 mg of **7**, the IR spectrum of which ($CHCl_3$) showed no absorption in the N–H/O–H and C=O stretching regions; λ_{max}^{MeOH} 413, 301 and 261 nm; $\lambda_{max}^{MeOH-HCl}$ 397, 300 and 261 nm. [Found: M^+, 271·1441. Calc. for $C_{14}H_{17}N_5O$: M, 271·1433].

The structure of **7** is further substantiated by the reaction of the chloro-containing derivative **3** with methanolic KOH which afforded a 80% yield of a product having chromatographic and spectral (UV and MS) properties identical with those of **7**.

X-ray data and structure determination of 2 - *chloro* - 3,4 - *dimethyl* - 6 - *dimethylamino* - 3H - 1,3,5,7 - *tetrazacyclopent*[*f*]*azulene* (**3**)

Crystals of the chloro derivative **3**, grown from aqueous EtOH are yellow-orange prisms elongated along the *a* axis. Precise lattice constants were obtained from a least-squares refinement of the setting angles of fourteen high angle reflections on a Siemens automatic diffractometer with Cu$K\alpha$ radiation (λ = 1·5418 A°). The absence of *h*0*l* reflections with *l* odd and 0*k*0 with *k* odd, indicated the space group $P2_1/c$. A summary of crystal data is given in Table 4.

A crystal of dimensions *ca* 0·6×0·3×0·2 mm was selected for data collection using the ϑ–2ϑ scan technique. A total of 2397 independent reflections ($\vartheta_{max} = 65°$) with intensity values larger than 2·5σ_I was collected. From the intensities the set of the structure factors were calculated, with no correction for absorption. The data were put on an absolute scale by the use of a Wilson plot. The normalized structure factor magnitudes $|E_h|$ were also evaluated; the statistical averages were 0·786 for $|E|$, 0·963 for $|E^2|$ and 0·959 for $|E^2-1|$.

The structure was solved by the multisolution method using the program Multan.[7] In all, 377 reflections having $|E|$ values greater than 1·40 were used in the sign determining procedure. The starting set is given in Table 5, which includes 5 reflections with the signs accepted

Table 4. Crystal data of the chloro-containing derivative (**3**), $C_{13}H_{14}N_5Cl \cdot 3H_2O$

System	Monoclinic
a	7·282 ± 0·008 A°
b	8·408 ± 0·004
c	25·422 ± 0·015
β	94°31′ ± 10′
U	1552 A^{o3}
Dm	1·40 g cm^{-3}
Dc	1·41 g cm^{-3}
M	329·8
Z	4
Space group	$P2_1/c$
F (000)	696
μ (Cu-$K\alpha$)	23·7 cm^{-1}

Table 5. Reflections used as starting set for phase generating process.

h	*k*	*l*	|*E*|	Sign
4	0	−20	3·67	+
2	0	−22	3·05	−
4	2	0	2·94	+
4	0	2	1·77	+
0	6	0	1·59	+
2	1	0	4·05	+
2	1	1	3·59	+
1	2	21	2·72	+
0	6	13	3·39	
1	3	11	2·46	
1	5	0	2·43	

from Σ_1 relationship, 3 origin-defining reflections and 3 variables. Thus, 8 sets of signs were generated. The most reliable set was used to calculated an *E* map, which showed clearly all the non-H atoms of the molecule and the presence of 3 water molecules.

The atomic coordinates thus obtained were refined by the block-diagonal matrix least-squares method. After 4 cycles of refinement the disagreement value $R = \Sigma||F_o| - |F_c||/\Sigma|F_o|$ was reduced to 0·13 for the observed reflections. The refinement was continued, introducing anisotropic temperature factors. After 3 cycles the *R* value was 0·09. On the difference electron density map, synthetized at this stage, all the H atoms of the molecule and of the water molecules could be located unambiguously. Further least-squares refinement included the H atoms, allowing their positional and isotropic thermal parameters to shift. At convergency the *R* value was 0·040.

The weighting scheme used during the refinement procedure was $w = [0{\cdot}1 + 0{\cdot}04|F_o| + 0{\cdot}0004|F_o|^2]^{-1}$ for the observed reflections.

A list of the structure factor is available on request from the authors.

Atomic scattering factors for C, N, O and Cl atoms were taken from Ref 8 and for H atoms from Ref 9.

Acknowledgements—The work was supported in part by grants from the Laboratorio per la Chimica e Fisica di molecole di interesse biologico del C.N.R., Naples, and the C.N.R., Rome. We also thank Dr. E. Trivelloni and Mr. C. Di Pinto for 100 MHz NMR spectra.

REFERENCES

[1]L. Cariello, S. Crescenzi, G. Prota, F. Giordano and L. Mazzarella, *Chem. Comm.* **99** (1973)
[2]J. C. N. Ma and E. W. Warnhoff, *Canada. J. Chem.* **43**, 1849 (1965)
[3]D. D. Bly and M. G. Mellon, *J. Org. Chem.* **27**, 2945 (1962)
[4]H. Shimanouchi and Y. Sasada, *Acta Cryst.* **B29**, 81 (1973)
[5]J. M. Robertson, H. M. M. Shearer, G. A. Sim and D. G. Watson, *Acta Cryst.* **15**, 1 (1962)
[6]A. W. Hanson, *Acta Cryst.* **19**, 19 (1965)
[7]G. Germain, P. Main and M. M. Woolfson, *Ibid.* **B26**, 274 (1970)
[8]D. T. Cromer and J. M. Mann, *Ibid.* **A24**, 321 (1968)
[9]F. H. Moore, *Ibid.* **16**, 1169 (1963)