Tetrahedron. Vol. 30, pp. 3611 to 3614. Pergamon Press 1974. Printed in Great Britain

METHYLATION OF ZOANTHOXANTHINS

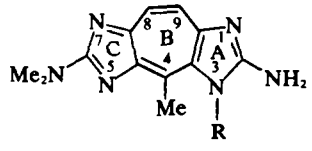
L. CARIELLO, S. CRESCENZI, G. PROTA* and L. ZANETTI

Stazione Zoologica, Napoli and Istituto di Chimica Organica dell'Università, Via Mezzocannone 16, Napoli, Italy

(*Received in the UK* 20 *May* 1974; *Accepted for publication* 7 *June* 1974)

Abstract—The methylation of parazoanthoxanthin D (**1**) and zoanthoxanthin (**2**), two natural tetrazacyclopentazulene pigments from *Parazoanthus axinellae*, has been examined under various conditions. As a rule, methylation in neutral or acidic conditions proceeds preferentially at N-1, while in liquid ammonia zoanthoxanthins are attacked by methyl iodide exclusively at the amino group at C-2. In addition to providing information on zoanthoxanthin tautomerism, the availability of various synthetic derivatives (**3–10**) led to the identification of the methyl derivatives **4** and **9** in *Parazoanthus axinellae*, and of **5** in *Epizoanthus arenaceus*, another zoanthid commonly found in the Bay of Naples.

We have recently reported[1,2] on the occurrence in colonial anthozoans belonging to the order Zoanthidea of a novel group of nitrogenous fluorescent pigments, named zoanthoxanthins, which are characterized by a tetrazacyclopentazulene chromophore. As the pigments so far identified differ only in the number and position of N-Me groups linked to the chromophore,[3] the availability of two typical representatives of the series, namely parazoanthoxanthin D (**1**) and zoanthoxanthin (**2**), led us to examine their behaviour on methylation with various alkylating agents. We expected that the results so obtained would assist the identification of other natural pigments in zoanthids and would provide information on the tautomerism of those zoanthoxanthins, as parazoanthoxanthin D (**1**), having an imino hydrogen which may be located at any of the nitrogens of the ring-system.



1: R = H
2: R = Me

The major difficulty encountered in the methylation of the natural pigments was their exceedingly low solubility in water and common organic solvents, which limited the choice of reaction conditions and often made it difficult to carry reactions to completion and to recover the methylated products.

Using methyl iodide in dimethyl sulphoxide or 2-methoxyethanol at room temperature, parazoanthoxanthin D (**1**) gave, as main product, an isomer of zoanthoxanthin (**2**), identified as **3**, along with trace amounts of the dimethyl-derivative **4** (Scheme 1). No other methylation products were detectable. The assignment of structure **4** for the minor product, and hence that of **3**, was established by its conversion into the known oxo-derivative **11** by acid hydrolysis. Thus, under these conditions the first methylation of **1** occurs exclusively at N-1, and a similar methylation of zoanthoxanthin (**2**) gave, as sole product, the 1-methyl derivative **4**.

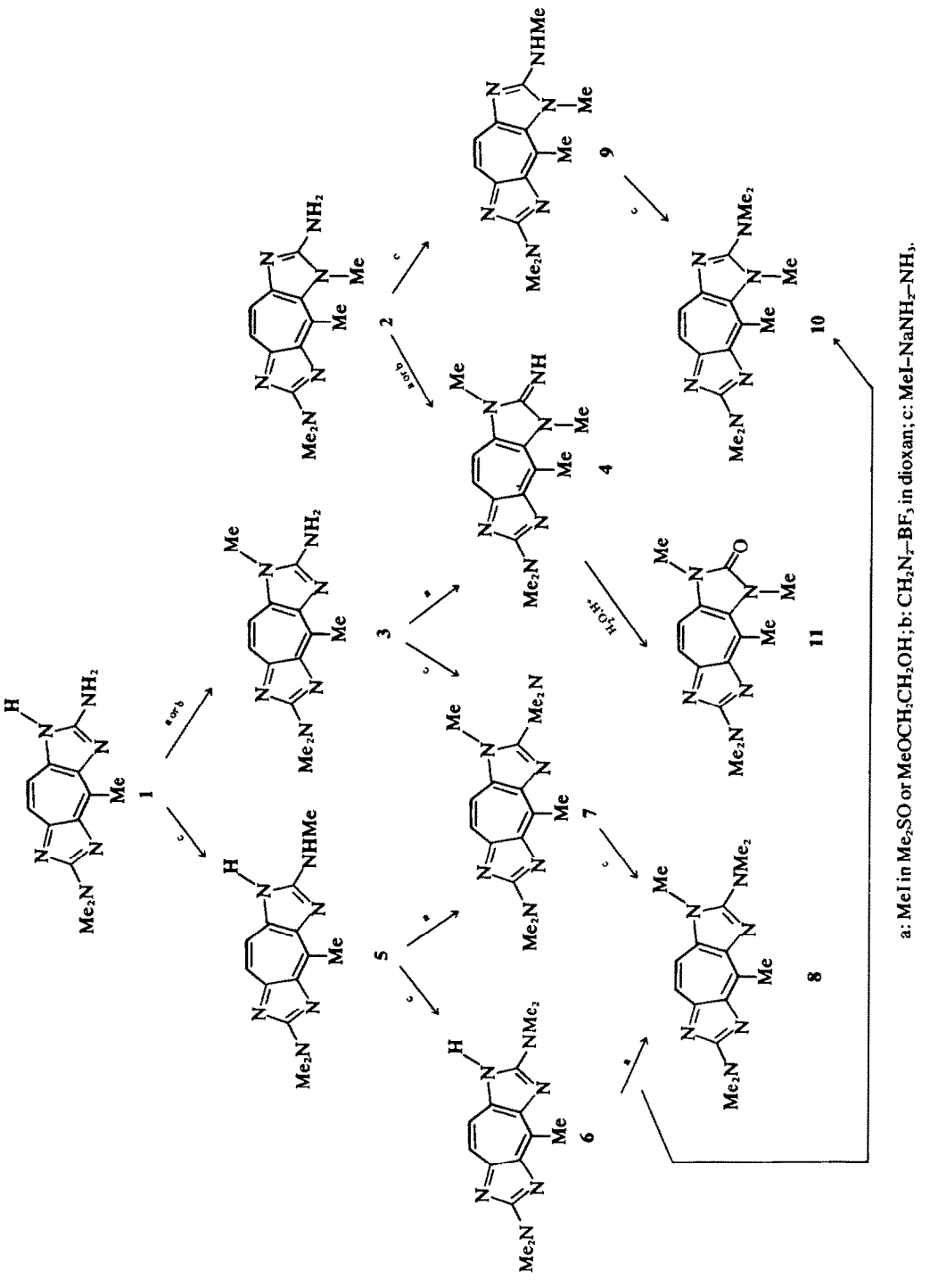
When diazomethane in the presence of boron trifluoride-ether complex was used as alkylating agent, **1** and **2** reacted similarly to give the corresponding 1-methyl derivatives **3** and **4**, respectively.

In contrast to methylation in neutral and acidic conditions, treatment of parazoanthoxanthin D (**1**) with methyl iodide and sodamide in liquid ammonia took place exclusively on the amino group at C-2, giving **5** and **6** in a ratio depending upon the experimental conditions; likewise the 1-methyl derivative **3** gave the mono- and dimethyl derivatives **7** and **8**, and zoanthoxanthin (**2**) afforded the corresponding derivatives **9** and **10**.

The availability of the two new zoanthoxanthins **5** and **6**, containing a tautomeric imino hydrogen, allowed us to explore further the course of methylation with methyl iodide in dimethyl sulphoxide or 2-methoxyethanol. While **5** gave, as expected, exclusively the 1-methyl derivative **7**, the compound **6** yielded a mixture of the 1-methyl and 3-methyl derivatives **8** and **10**.

From these results it can be concluded that under the various alkylating conditions used substitution is restricted to the nitrogens forming the guanidine moiety in ring A of the zoanthoxanthin skeleton, thus suggesting that the other two rings B and C form a fully aromatic diazazulene system. This implies that the tautomerism of the imino hydrogen in parazoanthoxanthin D (**1**) and its analogues **5** and **6** is probably limited to N-1 or N-3.

Examination of the UV spectra (in methanol) of



a: MeI in Me_2SO or $MeOCH_2CH_2OH$; b: CH_2N_2–BF_3 in dioxan; c: MeI–$NaNH_2$–NH_3.

natural and synthetic zoanthoxanthins (Table 1), revealed that all the pigments having a Me group at N-3, including **4**, exhibited an intense absorption maximum around 293 nm, while in 1-methylzoanthoxanthins (**3**, **7** and **8**) this band is shifted to the range 314–316 nm. A similar bathochromic shift is observed in the UV spectra of parazoanthoxanthin D (**1**) and its analogues **5** and **6**, suggesting that they occur mainly in the tautomeric forms with the imino hydrogen at N-1. Notably, the presence of a Me group at N-3 changes the fluorescence of zoanthoxanthins from blue to yellowish green. Moreover, as shown in Table 2, the PMR signal of the C-Me group in 1-H or 1-Me-zoanthoxanthins has an average value of δ $3{\cdot}27 \pm 0{\cdot}01$, while in 3-methylzoanthoxanthins, including **4**, it is shifted slightly to low field at δ $3{\cdot}41 \pm 0{\cdot}01$, except in the case of **9** in which the C-Me group resonates at an intermediate value of δ 3·33.

As expected, the characterization of the various methyl derivatives of parazoanthoxanthin D (**1**) and zoanthoxanthin (**2**) proved to be actually useful for the study of the distribution of zoanthoxanthins in zoanthids, especially for the identification of trace constituents. A chromatographic re-examination of the ethanolic extracts of *Parazoanthus axinellae* allow us to identify two new minor zoanthoxanthins, corresponding to the methyl derivatives **9** and **4**, for which we propose the names parazoanthoxanthin E and F. Furthermore, it was found that *Epizoanthus arenaceus*, another zoanthid available

Table 1. Some properties of zoanthoxanthins

Compound	Cryst. from	M.p. (°C)	R_f[a] (fluorescence)	λ_{max} nm (log ϵ) MeOH	MeOH–H$^+$
1	H_2O	303–304(dec)	0·33[b] (light blue)	415(4·24),306(4·56), 296 infl (4·46)	394(4·18),300(4·60), 255(4·12)
2	MeOH	275–276(dec)	0·65[b] (yellowish green)	427(4·35),293(4·52)	392(3·94),293(4·25), 259(3·87)
3	MeOH	>310	0·53[b] (light blue)	414(4·17),390br(4·09), 314(4·91),250(4·01)	394(4·34),303(4·80), 260(4·19)
4	EtOH	*d*	0·78[b] (yellowish green)	391(4·16),309sh(4·26), 293(4·52)	403(4·24),306(4·67), 261(4·13),245(4·01)
5	H_2O	191–192	0·59[b] (light blue)	419(4·37),310(4·65), 295 infl (4·51)	396(4·31),303(4·69)
6	EtOH	*d*	0·73[b] (light blue)	424(4·29),392br(4·02), 317(4·65),244(4·02)	397(4·27),308(4·61)
7	EtOH	*d*	0·69[b] (blue)	418(3·99),317(4·74)	399(4·19),306(4·65), 257(4·37)
8	EtOH/H_2O	234–235	0·27[c] (blue)	421(3·97),391br(3·79), 315(4·60),250(3·84)	399(4·05),312(4·50), 270br(3·90)
9	EtOH	*d*	0·76[b] (yellowish green)	429(4·43),306sh(4·48), 296(4·55)	404(4·24),309(4·66), 260(4·01)
10	EtOH	180–181	0·30[c] (yellowish green)	431(4·52),409br(4·36), 293(4·61)	412br(4·34),309(4·61), 265(4·35)

[a]Spots were located by UV irradiation at 366 nm; [b]In $CHCl_3$–MeOH–25% NH_4OH (90:10:1, v/v); [c]In $CHCl_3$–MeOH (90:10, v/v); [d]Darkens above 220° without melting below 310°.

Table 2. Mass and PMR data of zoanthoxanthins

Compound	*m/e* (relative intensities) M$^+$	M$^+$–Me	M$^+$–NMe	M$^+$–NCNMe$_2$	8 and 9-H	δ (ppm)[a] 1-Me	3-Me	4-Me	NHMe	NMe$_2$
1	242(100)	227(78)	213(38)	172(17)	8·82 and 8·91[b]			3·29		3·65
2	256(100)	241(91)	227(71)	186(9)	8·80[c]		4·26	3·42		3·62
3	256(70)	241(100)	227(62)	186(16)	8·72 and 8·85[b]	4·03		3·26		3·60
4	270(100)	255(80)	241(56)	200(9)	8·65 and 8·81[b]	4·08	4·26	3·40		3·60
5	256(100)	241(55)	227(30)	186(5)	8·91[d]			3·26	3·46	3·62
6	270(84)	255(100)	241(75)	200(7)	8·89[d]			3·28		3·63 (12 H)
7	270(100)	255(100)	241(77)	—	8·63[e]	3·99		3·28	3·51	3·59
8	284(100)	269(90)	255(69)	—	8·55 and 8·71[b]	4·15		3·23		3·57 and 3·62
9	270(100)	255(86)	241(100)	—	8·87[d]		4·21	3·40	3·50[f]	3·62
10	284(100)	269(75)	255(52)	—	8·85[c]		4·17	3·33		3·61 and 3·64

[a]All measurements in CF_3COOH at room temperature; [b]Doublets, J = 11 Hz; [c]Singlet; [d]Broadened singlet; [e]Broadened AB quartet; [f]Broadened signal, sharpened on addition of D_2O.

Table 3. Methylation of zoanthoxanthins

Zoanthoxanthin used	Product(s) (%)[a] MeI–Me_2SO	MeI–$MeOCH_2CH_2OH$	CH_2N_2–BF_3	MeI–$NaNH_2$–NH_3
1	3(35)	3(41)	3(27)	5(19)
	4(~1)	4(trace)		6(56)
2	4(35)	4(40)	4(80)	9(10)
				10(30)
3	4(3)	4(trace)	4(~1)	7(13)
				8(70)
5	7(33)	7(60)	no reaction	
6	8(22)	8(26)	8(trace)	
	10(8)	10(26)		

[a]In all reactions the other products were mostly starting materials.

in the Bay of Naples, contains a quite different pattern of fluorescent pigments, one of which was identified as **5**, while the others were found to belong to a new group of zoanthoxanthins with an isomeric tetrazacyclopentazulene chromophore, which will be described in a forthcoming paper.

EXPERIMENTAL

M.ps were determined with Kofler-hotstage apparatus and are uncorrected. UV spectra were recorded with an Optica CF4R spectrophotometer and PMR spectra with a Varian HA-100 or with Perkin-Elmer R-12 A Spectrometer; chemical shifts are expressed in ppm from TMS. Mass spectra were obtained by direct insertion technique with an AEI MS-902 spectrometer. Dowex 50 W (100–200 mesh; 2 per cent cross linkage; H^+ form) and basic alumina Fluka, standard grade, Type 5016 A (Brockmann activity 1) were used for column chromatography. 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.

Methylation of zoanthoxanthins

(a) *By methyl iodide in neutral solvents—General method.* Excess of MeI (0·1 ml) was added to a soln of the zoanthoxanthin (10–30 mg) in DMSO (or 2-methoxyethanol) in the presence of anhyd K_2CO_3. The mixture was stirred for 6 h at room temp, filtered, and evaporated under reduced pressure. After addition of water, the residue was extracted with butan-1-ol and the extract fractionated by preparative TLC with $CHCl_3$–MeOH– 25% NH_4OH (80:20:2 or 85:15:2). Properties and yields of the products are given in Tables 1–3.

(b) *By diazomethane—General method.* Excess of ethereal CH_2N_2 was added dropwise to a soln of the zoanthoxanthin (10–20 mg) in dioxan containing BF_3 etherate (0·1 ml). After 5 h at room temp, volatile constituents were removed *in vacuo* and the residue was fractionated as described above.

(c) *By methyl iodide in liquid ammonia—General method.* An excess of MeI (0·1–0·3 ml) was added portionwise to a soln of the zoanthoxanthin (30–50 mg) in liquid ammonia (5 ml) containing $NaNH_2$, prepared *in situ* by addition of 70 mg of Na. The mixture was stirred at −50° for 2 h. Removal of the solvent left a residue which was freed from inorganic constituents by column chromatography on alumina with $CHCl_3$–MeOH (90:10). The fraction containing the methylated products and unchanged starting material was evaporated and fractionated by preparative TLC on silica as described in (a).

Conversion of **4** *into* **11**. A soln of **4** (20 mg) in 0·1 N HCl (1 ml) was heated in a sealed tube at 130° for 2 h. After cooling, the mixture was basified with 2N NaOH and extracted with butan-1-ol. The residue obtained after evaporating the organic layer was purified by preparative TLC on silica with $CHCl_3$–MeOH (90:10) to give 12 mg of a product, yellow-orange prisms from aqueous EtOH, decomposing at 192°, which was identified as **11** by comparison of its chromatographic and PMR properties with those of an authentic sample obtained from **2** as previously described.[2]

Isolation of parazoanthoxanthins E (**9**) *and F* (**4**) *from Parazoanthus axinellae.* Whole colonies of *P. axinellae* (1 kg, wet weight) were exhaustedly extracted with EtOH as previously described.[2] The resulting yellow extracts were concentrated *in vacuo*, diluted with water, and extracted with ether to remove lipochromes and other impurities. After concentration to a small volume (*ca* 100 ml), the clear aqueous layer was basified with conc ammonia soln and left overnight at 4°. The brownish yellow ppt which formed, consisting of crude **2** and **1**, was removed by filtration and the filtrate was extracted with chloroform. The residue obtained evaporating the organic layer was fractionated by preparative TLC on silica gel with $CHCl_3$–MeOH–25% NH_4OH (85:15:2) to give, besides some **2** (R_f 0·52), 4 mg of parazoanthoxanthin E (R_f 0·65) and 3 mg of parazoanthoxanthin F (R_f 0·71) corresponding, respectively, to **9** and **4** (TLC, UV and MS).

Occurrence of **5** *in Epizoanthus arenaceus.* The experimental details concerning the occurrence and the identification of **5** in *E. arenaceus* will be described in a forthcoming paper in connection with the isolation and structure elucidation of the other related metabolites occurring in this species.

Acknowledgements—We would like to thank Prof. R. H. Thomson for discussions and kind revision of the manuscript. We also thank *Laboratorio per la Chimica e Fisica di Molecole di Interesse Biologico del C.N.R.*, Naples (Italy) for partial financial support.

REFERENCES

[1]L. Cariello, S. Crescenzi, G. Prota, F. Giordano and L. Mazzarella, *Chem. Comm.* 99 (1973)
[2]L. Cariello, S. Crescenzi, G. Prota, S. Capasso, F. Giordano and L. Mazzarella, *Tetrahedron* **30**, 3281 (1974)
[3]L. Cariello, S. Crescenzi, G. Prota and L. Zanetti, *Experientia* in press