87. Novel Aplysinopsin-Type Alkaloids from Scleractinian Corals of the Family Dendrophylliidae of the Mediterranean and the Philippines. Configurational-Assignment Criteria, Stereospecific Synthesis, and Photoisomerization

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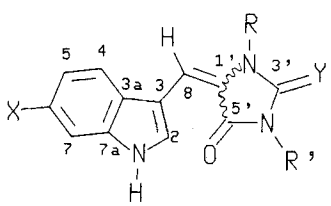
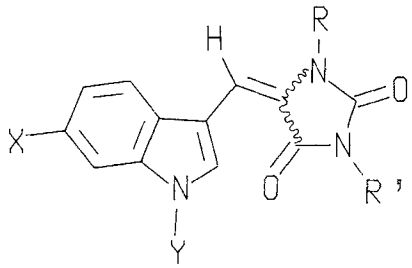
From the scleractinian coral *Tubastraea* sp. (Dendrophylliidae) collected at Palawan, Philippines, 3′-deimino-3′-oxoaplysinopsin (**4**) and 6-bromo-3′-deimino-3′-oxoaplysinopsin (**6**) are now isolated as 5:2 mixtures of (*E*/*Z*) stereoisomers. The 3′-deimino-2′,4′-bis(demethyl)-3′-oxoaplysinopsin (**7**) and 6-bromo-3′-deimino-2′,4′-bis(demethyl)-3′-oxoaplysinopsin (**5**) are isolated as 2:3 and 1:1 (*E*/*Z*) mixtures, respectively, from another dendrophyllid, *Leptopsammia pruvoti*, collected near Marseille, Mediterranean coast of France. Larger amounts of these and related compounds, needed for a full structural determination, are obtained by synthesis. Thus, condensations of indol-3-carboxaldehyde (**9**) or of its 6-bromo derivative **14** with hydantoin (**15**), 3-methylhydantoin (**11**), or 1,3-dimethylhydantoin (**10**) give the prevalent natural aplysinopsins with high stereospecificity. The minor stereoisomers (*Z*)-**4**, (*Z*)-**6**, (*E*)-**7**, and (*E*)-**5** are obtained by (*E*/*Z*) photoisomerization under UV light of the condensation mixtures. The configuration is assigned from larger H–C(8)/C(5′) $^{1}H,^{13}C$ couplings in the (*E*) than in the (*Z*) isomer, and, in the case of **4** and **6**, from NOE enhancement at Me–N(2′) on irradiation at H–C(8). The stereospecificity of the condensations is attributed to steric inhibition to planarity in the rate-limiting transition states, due to N(2′)/H–C(2) repulsion with (*Z*)-**4** and (*Z*)-**6**, or to C(5′)=O/H–C(2) repulsion with (*E*)-**7** or (*E*)-**5**. As the aplysinopsins undergo (*E*/*Z*) photoisomerization also under the daylight conditions of the laboratory, their isomeric composition in nature can not be presently assessed.

1. Introduction. – Aplysinopsin (**1**) was first isolated from the sponge *Aplysinopsis reticulata* (Dictyoceratida) of Lizard Island, the Great Barrier Reef [1a] [1b]. Later, it was





Compd.[a])	X	R = R′	Y	Conf. at C(8)	Ref.
1	H	Me	NH	(*E*/*Z*) = 9:1	[1a]
1	H	Me	NH	(*E*)	[1b–e]
2	Br	Me	NH	(*E*)	[1f]
3	H	Me	NMe	(*E*/*Z*) high	[1g]
4	H	Me	O	not stated	[1a]
5	Br	H	O	(*E*)	[1h]

Compd.[a])	X	R = R′	Y
4	H	Me	H
5	Br	H	H
6	Br	Me	H
7	H	H	H
8	H	Me	MeCO

[a]) Arbitrary numbering according to [1d] [1g]. Systematic names for **4**–**7** in the *Exper. Part.*

also reported from taxonomically unrelated sponges such as *Verongia spengelii* (Verongida) of Florida Keys [1c] and *Dercitus* sp. (Choristida) of Belize [1d] as well as from scleractinian corals of the family Dendrophylliidae such as *Tubastraea aurea* of Sagami Bay, Japan [1e], and *Astroides calycularis* of the Bay of Naples in the Mediterranean [1f].

In addition, other compounds of the same class have also been isolated from both dendrophylliids and sponges. Thus, the dendrophylliids *A. calycularis* [1f] and *Tubastraea coccinea* of Hawaii [1g] proved to contain also 6-bromoaplysinopsin (**2**), whereas the sponge *Smenospongia [=Polyfibrospongia] echina* (Dictyoceratida) of Belize was found to contain the 3'-oxo compound **5** [1h] and the sponge *A. reticulata* [1b] also $N^{3'}$-methylaplysinopsin (**3**).

These compounds have aroused considerable interest as potentially useful drugs. Aplysinopsin (**1**) shows specific cytotoxicity for cancer cells [1c], whereas $N^{3'}$-methylaplysinopsin (**3**) is most effective in affecting neurotransmission [1b]; in these properties, they have not yet been surpassed by any synthetic analogue [1b].

Reportedly, the (8*E*) stereoisomer largely prevails in **1** from [1a] [1b] and **3** [1g], and it is the only stereoisomer in **1** from [1c–e], **2** [1f], and **5** [1h]. In the case of the 3'-oxo analogue **4** of aplysinopsin, which was considered as a possible artifact of the extraction [1a], the configurational problem has not been addressed [1a] and, in general, although isomerization around the C(8)=C(1') bond has been taken into consideration [1b], the process has remained most obscure.

We report here on novel 3'-oxo analogues of these compounds from two scleractinian corals of the family Dendrophylliidae[1]), *i.e.* **4** and **6** and **5** and **7**, respectively, provide stereospecific syntheses of them, prove that they undergo (*E*/*Z*) photoisomerization at the C(8)=C(1') bond under daylight conditions, and furnish a general key for the configurational assignment.

2. Results and Discussion. – A polar pigment isolated in a 13-mg amount from a *Tubastraea* sp. (see *Exper. Part*), 3'-deimino-3'-oxoaplysinopsin (**4**), has a ^{13}C-NMR spectrum consisting of 14 signals, each one accompanied by a 2.5-fold weaker signal of the same multiplicity (*Table 1*). This suggests that the compound occurs as a 5:2 mixture of isomers.

The whole pattern of ^{13}C-NMR signals for **4** is similar to that for synthetic aplysinopsin (**1**; *Table 1*), except for the chemical shift of C(8), C(3'), and C(5'). The $M^{+\cdot}$ of **4** occurs at 1 *m*/*z* unit higher than that of **1** (*Exper. Part*). As fragmentation at N(2')–C(3') and C(1')–C(5') gives the same tryptophan fragment as from **1**, the extra mass must be located at the C(3')–C(5') portion of the molecule. Also the ^{1}H-NMR spectrum of **4** resembles much that of **1**, differences being limited to the chemical shift of H–C(8), H–C(2), and H–C(4) (*Table 2*). In harmony with the ^{13}C-NMR spectrum, the ^{1}H-NMR spectrum of **4** reveals 2 *d* in a *ca.* 5:2 integration ratio for H–C(2), and the same is true, though with less marked chemical shift differences, for H–C(8) (2 *s*) and for H–C(4) (2 br. *d*).

These data suggest that **4** has the structure of aplysinopsin (**1**) with C(3')=NH replaced by C(3')=O. This is confirmed by unambiguous synthesis of **4** *via* condensation of indol-3-carboxaldehyde (**9**) with 1,3-dimethylhydantoin (**10**) (*Scheme 1*)[2]). The reac-

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[1]) While the genus *Tubastraea* awaits for taxonomic assessment, detailed information on Mediterranean and Atlantic dendrophylliids is available [2].

[2]) The synthesis of **10** is not straightforward as MeI in basic media (KOH in DMSO or NaH in C_6H_6) leads to only methylation of the imidic N-atom of hydantoin [3a]. The problem was finally solved by methylation of hydantoin with MeI/KF on alumina [3b] to give a 4:1 mixture of 1,3-dimethylhydantoin (**10**) and 3-methylhydantoin (**11**).

Table 1. *^{13}C-NMR Data for the (E) and (Z) Stereoisomers of Aplysinopsin* (**1**), *3'-Deimino-3'-oxoaplysinopsin* (**4**), *6-Bromo-3'-deimino-2',4'-bis(demethyl)-3-oxoaplysinopsin* (**5**), *6-Bromo-3'-deimino-3'-oxoaplysinopsin* (**6**), *3'-Deimino-2',4'-bis(demethyl)-3'-oxoaplysinopsin* (**7**), *and for the Synthetic Analogues* **12** *and* **16** *in* $(CD_3)_2SO$

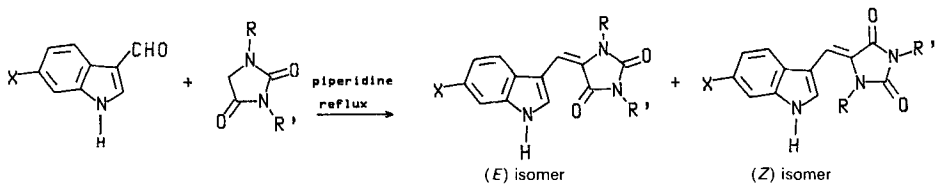
C-Atom[a])	**1**		**4**		**5**		**6**[b])
	(*E*) isomer	(*Z*) isomer	(*E*) isomer	(*Z*) isomer	(*E*) isomer	(*Z*) isomer	(*E*) isomer
C(2)	127.2 (*d*)	125.9	128.7 (*d*)	127.6	130.1 (*d*)	127.5	129.3 (*d*)
C(3)	108.8 (*s*)	107.6	108.3 (*s*)	107.0	108.9 (*s*)	108.7	108.7 (*s*)
C(3a)	127.7 (*s*)	127.3	127.7 (*s*)	127.5	127.3 (*s*)	126.3	127.5 (*s*)
C(4)	118.1 (*d*)	118.6	118.1 (*d*)	118.5	119.2 (*d*)	120.1	120.2 (*d*)
C(5)	119.4 (*d*)	119.7	119.9 (*d*)	120.1	122.8 (*d*)	122.9	122.6 (*d*)
C(6)	121.7 (*d*)	121.9	122.1 (*d*)	122.2	114.6 (*s*)	115.0	114.0 (*s*)
C(7)	111.7 (*d*)	111.8	112.0 (*d*)	112.1	115.0 (*d*)	114.4	114.6 (*d*)
C(7a)	135.5 (*s*)	135.8	135.7 (*s*)	135.9	136.5 (*s*)	136.6	136.5 (*s*)
C(8)	102.6 (*d*)	99.4	108.6 (*d*)	104.7	106.5 (*d*)	101.0	107.5 (*d*)
C(1')	126.4 (*s*)	128.5	124.4 (*s*)	126.7	125.4 (*s*)	124.9	125.1 (*s*)
C(3')	150.5 (*s*)	152.9	152.8 (*s*)	155.7	153.4 (*s*)	155.4	152.8 (*s*)
C(5')	162.2 (*s*)	162.8	162.0 (*s*)	163.6	163.9 (*s*)	167.3	162.0 (*s*)
R'	24.4 (*q*)	25.0	24.3 (*q*)	24.7	–	–	24.3 (*q*)
R	26.9 (*q*)	28.9	26.2 (*q*)	29.6	–	–	26.3 (*q*)

C-Atom[a])	**7**		**12**		**16**	
	(*E*) isomer	(*Z*) isomer	(*E*) isomer	(*Z*) isomer	(*E*) isomer	(*Z*) isomer
C(2)	128.5 (*d*)	126.8	128.0 (*d*)	127.0	128.7 (*d*)	127.4
C(3)	108.7 (*s*)	108.5	108.5 (*s*)	108.3	109.0 (*s*)	107.4
C(3a)	127.5 (*s*)	127.0	127.4 (*s*)	126.9	127.8 (*s*)	126.8
C(4)	117.4 (*d*)	118.1	117.4 (*d*)	118.1	118.0 (*d*)	118.6
C(5)	120.1 (*d*)	120.2	120.2 (*d*)	120.3	119.5 (*d*)	119.8
C(6)	122.1 (*d*)	122.4	122.1 (*d*)	122.4	121.7 (*d*)	122.0
C(7)	112.2 (*d*)	112.0	112.0 (*d*)	111.9	111.8 (*d*)	112.0
C(7a)	135.8 (*s*)	135.9	135.8 (*s*)	135.8	135.6 (*s*)	135.9
C(8)	107.6 (*d*)	101.8	105.6 (*d*)	102.8	104.8 (*d*)	102.2
C(1')	124.8 (*s*)	123.8	123.5 (*s*)	122.3	130.8 (*s*)	132.5
C(3')	153.7 (*s*)	155.7	152.6 (*s*)	155.0	164.9 (*s*)	169.7
C(5')	164.2 (*s*)	165.6	161.5 (*s*)	164.1	175.5 (*s*)	171.4
R'	–	–	24.0 (*q*)	24.2	–	–
R	–	–	–	–	27.9 (*q*)	31.4

[a]) Assignments are based on selective heteronuclear decoupling for compounds **1**, **4**, **7**, and **16**. Comparison with these data led to the assignment for the remaining compounds.

[b]) Signals for (*Z*)-**6** were too weak to be assigned confidently.

Scheme 1



9 X = H
14 X = Br

10 R = R' = Me
11 R = H, R' = Me
15 R = R' = H

4 X = H, R = R' = Me, (*E*)/(*Z*) > 95:5
5 X = Br, R = R' = H, (*Z*)/(*E*) > 95:5
6 X = Br, R = R' = Me, (*E*)/(*Z*) > 95:5
7 X = R = R' = H, (*Z*)/(*E*) > 95:5
12 X = R = H, R' = Me, (*Z*)/(*E*) > 95:5

Table 2. *^{1}H-NMR Data for the (E) and (Z) Stereoisomers of Aplysinopsin* (**1**), *3'-Deimino-3'-oxoaplysinopsin* (**4**), *6-Bromo-3'-deimino-2',4'-bis(demethyl)-3'-oxoaplysinopsin* (**5**), *6-Bromo-3'-deimino-3'-oxoaplysinopsin* (**6**), *3'-Deimino-2',4'-bis(demethyl)-3'-oxoaplysinopsin* (**7**), *and for the Synthetic Analogues* **12** *and* **16** *in* $(CD_3)_2SO$

H-Atom	**1**		**4**	
	(*E*) isomer	(*Z*) isomer	(*E*) isomer	(*Z*) isomer
H–C(2)	8.69 (*d*, *J* = 2.4)	7.57	8.84 (*d*, *J* = 2.8)	7.70
H–C(4)	7.87 (*d*, *J* = 7.5)	7.54	7.95 (br. *d*, *J* = 7.3)	7.62
H–C(5)	7.09 (*t*, *J* = 7.5)	7.09	7.13 (*t*, *J* = 7.3)	7.13
H–C(6)	7.15 (*t*, *J* = 7.5)	7.15	7.18 (*t*, *J* = 7.3)	7.18
H–C(7)	7.41 (*d*, *J* = 7.5)	7.40	7.45 (*d*, *J* = 7.3)	7.43
H–C(8)	6.43 (*s*)	6.57	6.77 (*s*)	6.89
R'	3.05 (*s*)	3.04	3.00 (*s*)	2.99
R	3.25 (*s*)	3.06	3.24 (*s*)	3.10
H–N(1)	11.50 (br. *s*)	11.50	11.68 (br. *s*)	11.55

	5		**6**	
	(*E*) isomer	(*Z*) isomer	(*E*) isomer	(*Z*) isomer
H–C(2)	8.71 (br. *s*)	8.15 (br. *s*)	8.83 (br. *s*)	7.72
H–C(4)	7.57 (*d*, *J* = 8.4)	7.75 (*d*, *J* = 8.4)	7.95 (*d*, *J* = 8.0)	7.60
H–C(5)	7.26 (*dd*, *J* = 8.4, 1.7)	7.24 (*dd*, *J* = 8.4, 1.7)	7.29 (*dd*, *J* = 8.0, 1.5)	7.30
H–C(6)	–	–	–	–
H–C(7)	7.63 (*d*, *J* = 1.7)	7.61 (*d*, *J* = 1.7)	7.66 (*d*, *J* = 1.5)	7.64
H–C(8)	6.63 (*s*)	6.71 (*s*)	6.76 (*s*)	6.85
R'	10.15 (br. *s*)[a]	10.20[b]	3.00 (*s*)	2.99
R	11.05 (br. *s*)[a]	11.10[b]	3.24 (*s*)	3.07
H–N(1)	11.72 (br. *s*)	11.98	11.85 (br. *s*)	11.85

	7		**12**	
	(*E*) isomer	(*Z*) isomer	(*E*) isomer	(*Z*) isomer
H–C(2)	8.75 (br. *s*)	8.13	8.79 (*d*, *J* = 2.0)	8.14
H–C(4)	7.60 (*d*, *J* = 7.5)	7.75	7.63 (*d*, *J* = 7.8)	7.77
H–C(5)	7.11 (*t*, *J* = 7.5)	7.11	7.11 (*t*, *J* = 7.8)	7.11
H–C(6)	7.18 (*t*, *J* = 7.5)	7.18	7.18 (*t*, *J* = 7.8)	7.18
H–C(7)	7.44 (*d*, *J* = 7.5)	7.42	7.44 (*d*, *J* = 7.8)	7.42
H–C(8)	6.69 (*s*)	6.75	6.75 (*s*)	6.85
R'	9.40 (br. *s*)	9.40	2.96 (*s*)	2.96
R	9.40 (br. *s*)	9.40	10.30 (br. *s*)	10.35
H–N(1)	11.82 (br. *s*)	11.82	11.68 (br. *s*)	11.85

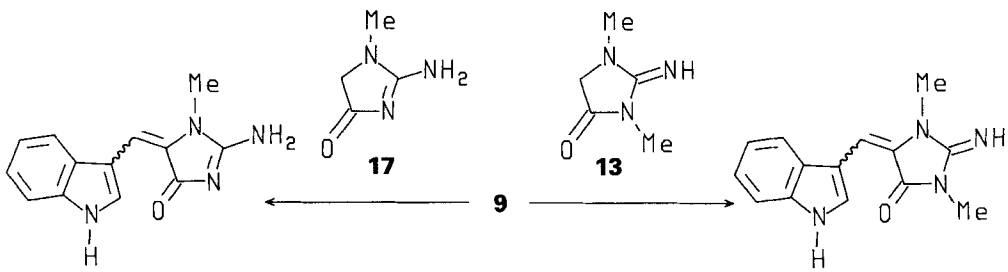
	16	
	(*E*) isomer	(*Z*) isomer
H–C(2)	9.08 (*d*, *J* = 2.0)	7.60
H–C(4)	7.89 (*d*, *J* = 7.3)	7.57
H–C(5)	7.10 (*t*, *J* = 7.3)	7.10
H–C(6)	7.15 (*t*, *J* = 7.3)	7.15
H–C(7)	7.42 (br. *d*, *J* = 7.3)	7.42
H–C(8)	6.54 (*s*)	6.68
R'	7.62 (br. *s*)	7.62
R	3.28 (*s*)	3.16
H–N(1)	11.49 (br. *s*)	11.52

[a])[b]) These assignments can be interchanged.

tion proves highly stereospecific, giving a mixture of stereoisomers **4** in a ratio > 95:5[3]). To assign the configuration, **4** is acetylated to **8** which is soluble enough in nonpolar solvents, such as $CDCl_3$, suitable for NOE studies. It can thus be established that with the major isomer of **4**, there is +20 and +6% differential NOE effect on H–C(4) and Me–N(2′), respectively, on irradiation at H–C(8); this supports the (*E*) configuration at the C(8)=C(1′) bond for the major isomer.

Aplysinopsin (**1**), obtained in a (*E*)/(*Z*) ratio > 95:5 on condensation of methylcreatinine **13** with indol-3-carboxaldehyde (**9**, *Scheme 2*), or isolated in a small amount from the *Tubastraea* sp. (see *Exper. Part*) when subjected to the isolation/purification conditions of **4**, proves perfectly stable; this rules out that **4** originates from **1** during the workup.

Scheme 2



16 (*E*)/(*Z*) > 95:5 **1** (*E*)/(*Z*) > 95:5

As (*E*)-**1** has stronger effects on neurotransmission than the (*Z*) isomer [1b], the (*E*) stereospecificity of the above condensations may turn useful for pharmacological studies.

Relevant to the behaviour and structural analysis of aplysinopsins is the finding that the synthetic 95:5 (*E*)/(*Z*) mixtures **1** or **4** undergo photoisomerization in solution under either UV irradiation or merely under the conditions of laboratory daylight, to become appreciably richer in the (*Z*) isomer[4]). With larger amounts of both isomers at hand, it is now possible to establish that there is a larger H–C(8),C(5′)=O heteronuclear coupling constant [5] in the (*E*) than in the (*Z*) isomer, and the same is true for aplysinopsin (**1**), as shown in *Table 3*.

Table 3. H–*C(8),C(5′) Heteronuclear Coupling Constants* (in Hz) *for the (Z) and (E) Isomers of* **1**, **4**, **7**, *and for the Synthetic Analogue* **16** *in* $(CD_3)_2SO$

	(*Z*) Isomer $^3J(H$–C(8),C(5′))	(*E*) Isomer $^3J(H$–C(8),C(5′))
1	5.1	10.5
4	5.2	11.0
7	5.0	9.8
16	4.2	10.0

[3]) Accompanied by 25% of **12** deriving from 3-methylhydantoin (**11**) present in our sample of **10**.

[4]) This recalls the photochemical behaviour of echinulin-type indole alkaloids which are structurally similar to **4** except for having a dioxopiperazine ring in place of the methylhydantoin ring [4].

Another polar pigment isolated in only 1-mg amount from the *Tubastraea* sp., 6-bromo-3′-deimino-3′-oxoaplysinopsin (**6**), contains a Br-atom according to MS *(Exper. Part)*. The MS fragmentation, following the above reasoning for **4**, indicates that the Br-atom is at the indole moiety. The ^{1}H-NMR spectra *(Table 2)* indicate a mixture of stereoisomers in a *ca.* 5:2 ratio, as in the case of **4**; however, the signal for H–C(5) is sharper than in the case of **4**, which allows us to locate the Br-atom at C(6).

Due to the scarce availability from natural sources, also **6** is synthesized in view of a complete structural study. Condensation of 6-bromoindol-3-carboxaldehyde (**14**) with 1,3-dimethylhydantoin (**10**) affords **6** as a 95:5 mixture of stereoisomers with a ^{1}H-NMR spectrum nearly superimposable to that of (*E*)-**4**, in particular with identical chemical shifts for H–C(2) and H–C(8). This allows us to assign the (*E*) configuration to the more abundant stereoisomer of **6**. As in the case of **4**, the 95:5 (*E*)/(*Z*) mixture **6** undergoes photoisomerization to become richer in the (*Z*) isomer; this allows us to obtain NMR data for both stereoisomers (*Tables 1* and *2*) and to confirm the configurational assignments.

The other dendrophylliid, *Leptopsammia pruvoti*, contains two main polar pigments, 3′-deimino-2′,4′-bis(demethyl)-3′-oxoaplysinopsin (**7**) and 6-bromo-3′-deimino-2′,4′-bis-(demethyl)-3′-oxoaplysinopsin (**5**), isolated in 10-mg and 4-mg amounts[5]). MS fragmentation at C(2′)–C(3′) and C(5′)–C(1′) gives the same indole tryptophan fragment as with **4**. In accordance, NMR spectra of **7** (*Tables 1* and *2*) resemble closely those for **4**, except for lack of the Me groups, indicating a 3:2 mixture of stereoisomers.

However, because of the lack of a Me group at N(2′), NOE studies can not provide any configurational information. A larger amount of **7** is needed in order to carry out heteronuclear NMR experiments. To this end, **9** is condensed with **15** to give **7** as a nearly pure stereoisomer. Following expectations from the above results, this sample undergoes photoisomerization to give a mixture appreciably richer in the other stereoisomer and thus suitable for heteronuclear coupling experiments. Surprisingly, these reveal that the condensation has afforded mainly (*Z*)-**7**, which is thus also established to be the prevalent stereoisomer isolated from *L. pruvoti*.

The stereospecificity of the condensations must result from steric effects. According to crystal diffraction data for (*Z*)-**1**, steric repulsions between H–C(2) and Me–N(2′) force the five-membered ring out of the plane of the indole nucleus, making N(2′) tetrahedral [1b]. The analogous conformation can now be suggested also for (*Z*)-**4** on the basis of molecular-mechanics calculations [9]. It is thus conceivable that, owing to H–C(2)/Me–N(2′) repulsions in the rate-limiting transition state of the condensation/elimination leading to (*Z*)-**4**, the route to the (*E*) isomer, which avoids such repulsions, is followed. When N(2′) bears a H-atom, as in the case of **7**, molecular-mechanics calculations [9] for the planar forms indicate the reverse situation; (*Z*)-**7** is calculated to be less strained than (*E*)-**7** owing to C(5′)=O/H–C(2) repulsions. This is reflected in the rate-

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[5]) A previous study of *L. pruvoti*, collected around Marseille and in other Mediterranean areas, vaguely alludes to two unstable phenolic pigments, leptopsammin A and B [6]. From the data reported [6], they cannot be either **5** or **7**, and it is also unclear whether they are aplysinopsin-type compounds or rather anthraquinones, anthrones, or chalcones, as isolated from *Tubastraea micranthus* (EHRENBERG) collected in Palau, West Caroline Islands [7]. In contrast, phenolic guanidiniostyrenes, as isolated from *Tubastraea aurea* of Okinawa [8], seem to be ruled out as structural possibilities.

limiting transition state of the condensation/elimination which leads preferentially to (*Z*)-**7** with gain of conjugation in the fully planar form.

Analogous reasoning and conclusions hold for **5**, which is obtained from *L. pruvoti* as a 1:1 (*E*)/(*Z*) mixture, and it is also secured by its synthesis as the (*Z*) isomer *(Scheme 1)*; the latter undergoes photoisomerization to give (*E*)-**7**.

On the basis of these configurational criteria, the literature needs some revision. Thus, **5** which has been isolated from the sponge *Smenospongia [= Polyfibrospongia] echina* and drawn as the (*E*) stereoisomer [1h] must have the (*Z*) configuration. In fact, its ^{1}H-NMR data exactly match those for our (*Z*)-**5**. Subsequently, the misassigned compound (*E*)-**5** of *S. echina* [1h] has served [10] in a comparison concluding that a Me group in the place of a H-atom at N(2′) induces a deshielding of H–C(2), irrespective of the configuration at C(8)–C(1′), as implicitely suggested [10]. Actually, our study shows that deshielding of H–C(2) always occurs with the (*E*) stereoisomer of each (*E*/*Z*) couple of isomers *(Table 2)*, independently of the presence or not of a Me group at N(2′). On the basis of data presently available, the C(5′)=O group seems to be the cause of such a large (*ca.* 1 ppm) deshielding.

One may wonder, whether photoisomerization of aplysinopsins, as a non-destructive process for entrapping radiant energy, protects from sunburning dendrophylliids and sponges of exposed shallow-water areas of the tropics. To this concern, it has been shown that coral reef epifauna can be damaged by direct sun radiation and that only species protected by pigments can grow well in exposed areas [11] of shallow clear tropical waters which do not filter the solar UV radiation [12]. In the case of the reef-forming coral *Acropora formosa* it has been proposed that UV-absorbing mycosporine-like amino acids act as protective filters [13].

However, just because the photochemical isomerization of aplysinopsin-type compounds occurs as easily as under the daylight conditions of the laboratory, neither the (*E*)/(*Z*) stereoisomeric ratio for the aplysinopsins in nature nor its possible dependence on the wavelength of solar radiation filtered by the sea can be assessed from our study. Moreover, whereas our shallow-water tropical *Tubastraea* sp. may need protection against solar radiation, *Leptopsammia pruvoti* and *Astroides calycularis* [1f], living in caves of temperate areas, do not seem to need protection from solar radiation[6]).

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Experimental Part

1. *General.* UV irradiations were carried out with a *RS55* semimicro photochemical reactor from *Applied Photophysics*, London, at 350 nm. Yields for the condensations are given with respect to reacted compounds. Reverse-phase HPLC: 25 × 1-cm column filled with *Merck-LiChroprep RP-18* (7 mμ), UV monitoring at λ 254 nm, solvent flux 5 ml·min^{-1}. Flash chromatography (FC): *Merck silica gel 60*, 20–50 mμ. TLC: *Merck-Si$_{F254}$* plates. M.p.: *Kofler* hot-stage microscope. UV spectra (λ_{max} in nm, ε in mol^{-1}·l·cm^{-1}): *Perkin-Elmer Lambda-3* spectrophotometer. IR spectra ($\tilde{\nu}_{max}$ in cm^{-1}): *Perkin-Elmer 337* spectrometer. NMR spectra (calibrated with respect to

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[6]) Even the cited case of reef corals containing mycosporine-like pigments presents conflicting problems in our view. In fact, such pigments are also contained in marine organisms which live in shadowed areas in temperate waters. The extreme case is of that of bivalve mollusks [14].

either δ(H) 2.49 and δ(C) 39.50; $(CD_3)_2SO$): *Varian XL-300* spectrometer; ^1H at 300 MHz, ^{13}C at 75.4 MHz; *J* in Hz, multiplicities from APT [15]. MS (EI; *m/z* (%)): home-built spectrometer based on the *ELFS-4-162-8-Extranuclear* quadrupole [16]. Molecular mechanics calculations were carried out with the MMPMI program by Serena Software, Bloomington, Indiana.

2. *Collection and Isolation.* The *Tubastraea* sp. was collected in May 1985 as a rather cryptic species under small overhangs on the reef, depth *ca.* 3 m, in the small island ('Manu-Manou'), south of Capsalon Island, off Danlig village in Dumaran Passage (*ca.* 10°33'N–119°42.5'E), NE of Palawan, Philippines. Colonies up to 5-cm high, bushy to subdendroid (not plocoid). Live tissues green-brownish (not dark coloured). According to general aspect and calicular structure, these could be young colonies of *Tubastraea micranthus* (EHRENBERG, 1834) [= *T. nigrescens* (DANA, 1846)]. Reference specimens are deposited in the National Museum of Natural History, Washington (USNM 80822) and the British Museum (Natural History) (BMNH 1987.12.23.1–4). Eighteen colonies, each comprising up to 30 polyps, were immediately immersed in 95% EtOH, transported in the dark at r.t. and stored at –20° in December 1985. In May 1987, the liquid was decanted and evaporated, and the aqueous residue was extracted first with petroleum ether and then with AcOEt. Evaporation of the two extracts gave 0.5 and 0.2 g of dark, oily residues, resp. The latter residue was subjected to reverse-phase HPLC with MeCN/H_2O 45:55 to give indole-3-carboxaldehyde (**9**, t_R 4 min, 5 mg), (*Z*)-**4** (t_R 7.5 min, 5 mg), (*E*)-**4** (t_R 9.3 min, 13 mg), (*Z*)-**6** (t_R 15.8 min, trace amount), and (*E*)-**6** (t_R 24.0 min, 1 mg). With MeCN/H_2O 4:1, (*E*)-**1** (t_R 8.0 min, 8 mg) was also isolated. However, in the aqueous medium under daylight conditions, the pure isomers **4** and **6** were found to convert rapidly in (*E*)/(*Z*) mixtures.

Leptopsammia pruvoti (LACAZE-DUTHIERS, 1897), polyp tissues bright yellow, was collected on 15 July 1981 in a cave, depth 10 m, presqu'ile de Morgiou, near Marseille. The corals were immediately immersed in 95% EtOH and, after a week, the solvent was evaporated, and the residue was extracted first with petroleum ether (2.5 g after solvent evaporation) and then with AcOEt (0.5 g after solvent evaporation) (dry corals after extraction, 1200 g). The residue from the AcOEt extraction was subjected to flash chromatography, gradient elution hexane/AcOEt. Fractions more polar than sterols, eluted with hexane/AcOEt 1:1 and giving charring spots on TLC, were subjected to reverse-phase HPLC with MeCN/H_2O 2:3 to give **7** (t_R 4.0 min, 10 mg) as a (2:3 (*E*)/(*Z*) mixture), 6-bromoindole-3-carboxylic acid (t_R 5.9, 1 mg) and **5** (t_R 7.5 min, 4 mg, as a 1:1 (*E*)/(*Z*) mixture). Separation of the stereoisomeric pairs was carried out by prep. TLC with $CHCl_3$/MeOH 9:1 ((*E*)-**7** R_f 0.50; (*Z*)-**7** R_f 0.45; (*E*)-**5** R_f 0.50; (*Z*)-**5** R_f 0.43). A 2nd and a 3rd collection of *L. pruvoti* of 24 July 1987 at Impériaux de terre, overhang 17–20 m, immersed in denaturated MeOH, or at Cape Rederis, near Banyuls-sur-Mer, east Pyrenean waters, proved to contain the same compounds.

3. *Synthesis of 1,3-Dimethylhydantoin* (= *1,3-Dimethylimidazolidine-2,4-dione;* **10**). A mixture of hydantoin (0.5 g, 5 mmol), MeI (0.95 ml, 15 mmol), and KF/Al_2O_3 (3.75 g, 25 mmol of KF) in 50 ml of MeCN was stirred at r.t. during 24 h. The mixture was filtered, washed with MeCN, and evaporated to give 0.55 g (85%) of **10**/**11** 4:1 as a white solid. IR (nujol): 1715vs. ^1H-NMR ($CDCl_3$): 6.25 (br. *s*, 1 H); 3.93 (*s*, 2 H); 2.96 (*s*, 3 H). ^{13}C-NMR ($CDCl_3$): 171.43 (*s*); 158.36 (*s*); 46.4 (*t*); 24.43 (*q*). MS: 128 (60), 114 (14), 58 (15), 43 (58), 42 (100).

10: ^1H-NMR ($CDCl_3$): 3.82 (*s*, 2 H); 2.96 (*s*, 6 H). ^{13}C-NMR ($CDCl_3$): 169.94 (*s*); 156.94 (*s*); 51.65 (*t*); 29.57 (*q*); 24.83 (*q*).

4. *Synthesis of Aplysinopsin-Type Compounds by Condensation.* Equimolar amounts of the aldehyde and the imidazolidinone were either *a)* stirred under N_2 and then heated over the *Bunsen* flame during several minutes or *b)* heated at reflux for 3 h in piperidine [17] with the same results. In case *a)*, except for **4** and **12**, the products were used as such, whereas in case *b)*, piperidine was first evaporated. In all cases, yields were practically quantitative.

5. *Photoisomerization of Aplysinopsin-Type Compounds.* Irradiations of *ca.* 0.2M solns. in $(CD_3)_2SO$ were carried out in a 5-mm NMR tube for 2 h, monitoring the transformations by ^1H- and ^{13}C-NMR. Clean (*E*)→(*Z*) isomerization was observed, without by-products.

6. *3'-Deimino-3'-oxoaplysinopsin* (= *5-[(1H-Indol-3-yl)methylidene]-1,3-dimethylimidazolidine-2,4-dione;* **4**). ^1H- and ^{13}C-NMR (from the 2.5:1 (*E*)/(*Z*) mixture isolated from the *Tubastraea* sp.): *Tables 1* and *2*.

Reaction of indole-3-carboxaldehyde (*Fluka*, 99%; **9**) with **10**/**11** 4:1 led to crystalline **4**/**12** 4:1 (NMR: (*E*)/(*Z*)-**4** > 95:5 and (*Z*)/(*E*)-**12** > 95:5). Part of this mixture was subjected to reverse-phase HPLC under the above conditions for isolation from the *Tubastraea* sp., leading to (*Z*)/(*E*)-**12** 4:1 (t_R 5 min) and (*E*)/(*Z*)-**4** 5:2 (t_R *ca.* 8 min) as deduced by ^1H-NMR analysis of the crystalline residues after evaporation. Another portion of **4**/**12** 4:1 was recrystallized from MeOH to give (*E*)/(*Z*)-**4** > 95:5. Yellow solid. M.p. 281–282°. UV (MeOH): 373 (14600), 276 (6100), 233 (13800). IR (KBr): 3300*s*, 1740*s*, 1690*s*, 1620. MS: 255 (100, M^+), 169 (11), 155 (81).

A portion (4 mg) of (*E*)/(*Z*)-**4** 5:2 isolated from the *Tubastraea* sp. was stirred with Ac_2O/pyridine 1:1 (0.4 ml) at r.t. for 3 days. Prep. TLC with $CHCl_3$/MeOH 95:5 led to yellow solid (*E*)/(*Z*)-**8** 5:2 (by ^{1}H-NMR ($CD_3)_2SO$; 3 mg, 65%) and to recovery of 1 mg of the starting **4**. (*E*)/(*Z*)-**8**: Yellow solid decomposing at 300° before melting. ^{1}H-NMR ($CDCl_3$; only signals of the more soluble (*E*)-**8** visible): 9.04 (br. *s*, H–C(2)); 8.34 (*dd*, *J* = 7.5, 1.8, H–C(7)); 7.55 (*dd*, *J* = 7.5, 1.8, H–C(4)); 7.25–7.20 (*m*, H–C(5), H–C(6)); 6.28 (*s*, H–C(8)); 3.18 (*s*, Me–N(2′)); 3.03 (*s*, Me–N(4′)); 2.60 (*s*, MeCO). ^{1}H-NMR ($(CD_3)_2SO$): 9.14, 8.03 (H–C(2) of (*E*)- and (*Z*)-**8**, resp.); 6.64, 6.70 (H–C(8) of (*E*)- and (*Z*)-**8**, resp.). ^{13}C-NMR ($CDCl_3$): 168.9 (*s*, Me*C*O); 161.9 (*s*, C(5′)); 153.0 (*s*, C(3′)); 134.9 (*s*, C(7a)); 129.7 (*s*, C(1′)); 128.3 (*s*, C(3a)); 127.5 (*d*, C(2)); 125.3 (*d*, C(6)); 123.6 (*d*, C(5)); 117.3 (*d*, C(4) or C(7)); 116.5 (*d*, C(7) or C(4)); 113.6 (*s*, C(3)); 104.6 (*d*, C(8)); 26.1 (*q*, Me–N(2′)); 24.4 (*q*, Me–N(4′)). MS: 297 (25, M^+), 255 (100, M^+ – MeCO), 169 (11), 155 (36).

UV irradiation of (*E*)/(*Z*)-**4** > 95:5 gave (*E*)/(*Z*)-**4** 3:1.

UV irradiation of (*Z*)/(*E*)-**12** 4:1 (MS: 241 (100, M^+), 156 (30), 155 (55)) gave (*Z*)/(*E*)-**12** *ca.* 1:**4**.

7. *6-Bromo-3′-deimino-3′-oxoaplysinopsin* (= *5-[(6-Bromo-1*H*-indol-3-yl)methylidene]-1,3-dimethylimidazolidine-2,4-dione;* **6**). ^{1}H- and ^{13}C-NMR (from the 2.5:1 (*E*)/(*Z*) mixture isolated from the *Tubastraea* sp. and from the purified synthetic isomers): *Tables 1* and *2*.

Reaction of 6-bromoindole-3-carboxaldehyde (**14**) [18] with **10**/**11** 4:1 mainly led to crystalline (*E*)/(*Z*)-**6** 95:5. A portion was purified by reverse-phase HPLC with MeCN/H_2O 1:1 (t_R 10 min) to give yellow crystals decomposing at 300° before melting. UV (MeOH): 380 (7400), 283 (3500), 230 (11700). MS: 333 and 335 (100, M^+), 254 (52), 247 and 249 (**4**), 233 and 235 (50), 169 (13), 155 (20).

UV irradiation of (*E*)/(*Z*)-**6** 95:5 gave (*E*)/(*Z*)-**6** *ca.* 5:1.

8. *3′-Deimino-2′,4′-bis(demethyl)-3′-oxoaplysinopsin* (= *5-[(1*H*-Indol-3-yl)methylidene]imidazolidine-2,4-dione;* **7**). ^{1}H- and ^{13}C-NMR (from 2:3 (*E*)/(*Z*) mixture isolated from *L. pruvoti*): *Tables 1* and *2*.

Reaction of **9** with hydantoin (**15**) led to (*Z*)/(*E*)-**7** 95:5. TLC ($CHCl_3$/MeOH 95:5) gave pure (*Z*)-**7** as yellow crystals. A portion of (*Z*)/(*E*)-**7** 95:5 was subjected to UV irradiation to give (*Z*)/(*E*)-**7** 3:2. TLC of the latter mixture led to pure (*E*).**7**.

(*Z*)-**7**: Yellow crystals decomposing at 300° before melting. UV (MeOH): 365 (25000), 277 (13000), 223 (34000). MS: 227 (100, M^+), 156 (41), 155 (58).

(*E*)-7. UV (MeOH): 381 (26000), 276 (13000), 223 (44000).

9. *6-Bromo-3′-deimino-2′,4′-bis(demethyl)-3′-oxoaplysinopsin* (= *5-[(6-Bromo-1*H*-indol-3-yl)methylidene]-imidazolidine-2,4-dione;* **5**). ^{1}H- and ^{13}C-NMR (from the 1:1 (*E*)/(*Z*) mixture isolated from *L. pruvoti*): *Tables 1* and *2*. This mixture was purified by TLC ($CHCl_3$/MeOH 95:5). *Purified natural* (*Z*)-**5**: Yellow solid decomposing at 300° before melting. UV (MeOH): 368 (14200), 282 (8100), 226 (25800). MS: 305 and 307 (100, M^+), 234 and 236 (25), 208 and 210 (4), 155 (48).

Reaction of 6-bromoindole-3-carboxaldehyde (**14**) [18] with **15** led to (*Z*)/(*E*)-**5** > 95:5.

10. *Synthesis of Aplysinopsin* (= *2-Imino-5-[(1*H*-indol-3-yl)methylidene]-1,3-dimethylimidazolidin-4-one;* **1**) *and of 2′-Demethylaplysinopsin* (= *2-Amino-4,5-dihydro-5-[(1*H*-indol-3-yl)methylidene]-1-methyl-1*H*-imidazol-4-one;* **16**). Creatinine (**17**; *Fluka*) was methylated with MeI in EtOH at reflux [19] to give methylcreatinine **13** (93%) which was subjected to chromatography on *Amberlite IRA-68* (*Fluka*) with MeOH to give **13**·H_2O.

Reaction of **9** with **13**·H_2O led to (*E*)/(*Z*)-**1** > 95:5. A portion of the latter was subjected to UV irradiation to give (*E*)/(*Z*)-**1** 4:1.

Reaction of **9** with creatinine (**17**) led to (*E*)/(*Z*)-**16** > 95:5. A portion of the latter was subjected to UV irradiation to give (*E*)/(*Z*)-**16** 5:1.

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