MARINE NATURAL PRODUCTS FROM THE SEYCHELLES

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Résumé : *127 échantillons d'organismes marins sessiles ont été récoltés à la main en plongée en scaphandre autonome le long des côtes des îles granitiques autour de Mahé aux Seychelles, archipel de l'Océan Indien équatorial. L'extrait brut méthanolique de chaque organisme a été évalué par essais antimicrobiens et par examen du spectre de ^1H RMN. Les données d'activités biologiques n'ont rien d'exceptionnel, mais plusieurs composés inhabituels ont été mis en évidence par spectrométrie de ^1H RMN.*

Un tunicier vert foncé, Eudistoma sp. *a été récolté à l'île Pralin. Le métabolite majoritaire de ce tunicier est l'asciddemin qui a été isolé précedemment d'une ascidie du genre* Didemnum. *Parmi les produits minoritaires figurent deux alcaloïdes octacycliques, les eudistones A et B. La détermination structurale des eudistones A et B est basée sur l'analyse détaillée des données de ^1H et de ^{13}C RMN.*

Une éponge du genre Smenospongia *a été récoltée à l'île Thérèse. Un extrait brut de l'éponge monte un spectre de ^1H RMN particulier. Un fractionnement guidé par RMN du ^1H conduit à l'isolement des smenochromenes A - D, qui sont des chromènes macrocycliques pouvant être des dérivés cyclisés de la farnesyl-hydroquinone. Si la structure du smenochromene A aurait pû être déterminée par l'interprétation de ses données spectrales, celle-ci a toutefois été confirmée par l'analyse aux rayons X, car une géométrie inhabituelle de ce composé conduit à des données spectrales anormales. Ces données spectrales et les conformations des smenochromenes sont discutées ici en détail.*

Abstract : *127 Specimens of sessile marine organisms were collected by hand using SCUBA along the coastlines of granite islands around Mahé in the Seychelles, which are a diverse group of islands located in the western equatorial Indian Ocean. The crude methanolic extract of each organism was evaluated in antimicrobial assays and by examination of the ^1H NMR spectrum. The bioactivity data was unexceptional but the ^1H NMR spectra revealed several unusual compounds.*

A dark-green tunicate, Eudistoma *sp., was collected at Praslin Island. The major metabolite of the tunicate was asciddemin, which had previously been isolated from a tunicate of the genus Didemnum. Among the minor constituents were two octacyclic alkaloids, eudistones A and B. The structural elucidation of eudistones A and B was based on a detailed analysis of ^1H and ^{13}C NMR data.*

A sponge of the genus Smenospongia *was collected at Thérèse Island. A crude extract of the sponge gave a distinctive ^1H NMR spectrum. A ^1H NMR guided fractionation of the extract resulted in the isolation of smenochromenes A - D, which are macrocyclic chromenes than can be derived by cyclization of farnesyl hydroquinone. Although the structure of smenochromene A could have been determined by interpretation of spectral data, the structure was confirmed by X-ray analysis because the unusual geometry of the compound led to unexpected spectral data. The spectral data and conformations of the smenochromenes are discussed in detail.*

The Seychelles are a widely scattered group islands situated in the tropical western Indian Ocean, between 3°40'S and 7°10'S, and 52°40'E and 57°00'E, with an international airport on the island of Mahé. Our collections, which consisted of 127 individual specimens, were made during the period April 26 to May 14, 1990 with the help of a commercial dive operation and were therefore limited to locations around Mahé, Praslin, and La Digue Islands. The islands around Mahé are granitic and thus we did not collect at any true coral atolls.

TABLE : Known compounds identified from the Seychelles collection.

Sample	Organism	Compound(s) or Class of Compound
90-047	dictyoceratid sponge	heteronemin
90-048	soft coral	furanosesquiterpene acid
90-051	*Haliclona* sp.	pyridinium salts
90-052	*Xestospongia* sp.	helenaquinones
90-057	*Jaspis* sp.	jaspamide
90-058	verongid sponge	brominated tyrosine derivatives
90-059	verongid sponge	aerothionins and 11,19-dideoxyfistularin-3
90-061	axinellid sponge	oroidin and dibromophakellin
90-063	*Jaspis* sp.	jaspamide
90-068	*Jaspis* sp.	jaspamide
90-069	dictyoceratid sponge	scalarins
90-070	haplosclerid sponge	manzamines
90-072	*Aaptos aaptos*	aaptamines (1 new)
90-074	Plakinidae	chondrillins
90-075	*Acanthella cavernosa*	bromophakellin
90-084	dictyoceratid sponge	heteronemin
90-085	verongid sponge	brominated tyrosine derivatives
90-087	*Smenospongia* sp.	(see below)
90-095	dictyoceratid sponge	ilimaquinone
90-096	sponge	cytosine, 5-methylcytosine
90-097	*Liosina* sp.	fatty acids
90-098	*Jaspis* sp.	bengamides
90-101	dictyoceratid sponge	ilimaquinone
90-104	*Druinella* sp. 1	bromotyrosine derivatives
90-113	*Druinella* sp. 2	araplysillins
90-114	dictyoceratid sponge	scalarins
90-118	verongid sponge	brominated tyrosine derivatives
90-121	*Tubastrea* sp.	aplysinopsin
90-127	*Aaptos aaptos*	aaptamines
90-132	*Druinella* sp. 3	psammaplysin
90-133	dictyoceratid sponge	ilimaquinone0
90-137	verongid sponge	brominated tyrosine derivatives
90-141	*Lobophytum crassum*	diterpene alcohols (1 new)
90-154	*Xestospongia* sp.	brominated acetylenic acids (2 new)
90-155	soft coral	cembranoids (3 new)

The 127 samples consisted of 93 sponges, 14 soft corals, 10 tunicates, 4 zoanthids, 3 hard corals, 2 algae, and an anemone. The crude methanolic extracts of 124 samples were screened against the Gram+ve bacteria *Staphylococcus aureus* and *Bacillus subtilis*, the Gram negative bacteria *Escherichia coli* and *Pseudomonas aeruginosa*, and the fungus *Candida albicans*. Seven extracts were very active against all microbes but unfortunately all of these extracts were from organisms that were very familiar to us from prior studies. A further 12 extracts showed broad spectrum activity while 11 extracts had only antifungal activity, 8 had broad antibacterial activity, 34 inhibited only Gram positive bacteria and 52 were inactive. These are typical antimicrobial screening results.

The next stage in our dereplication scheme was to partition the MeOH extracts between EtOAc and water. The EtOAc and aqueous extracts from active MeOH extracts were each screened for antimicrobial activity, a process that usually results in the antimicrobial activity being cleanly partitioned. A ^{1}H NMR spectrum was recorded for all EtOAC and aqueous extracts and many known marine natural products were identified at this stage. Finally, all of the sponge samples were examined microscopically and a preliminary taxonomic classification was made.

The results of the dereplication procedure are shown in the Table. Some samples that were stored separately in the field because of differences in shape, color or texture are obviously identical or closely related and have the same general chemistry. It is, however, much better to combine samples after dereplication than to combine samples in the field and then have to separate mixed samples after freezing and transport. Some of the samples are so small that they may never be studied. However, our preliminary evaluation of small samples will allow us to know which should be recollected in the future.

The most interesting of the samples that have been studied are a tunicate of the genus *Eudistoma* (1) and a sponge of the genus *Smenospongia* (2). Although the compounds described below are not biologically active, their structural complexity provided challenging problems of structural elucidation.

The dark-green tunicate, ***Eudistoma*** **sp.**, was collected at Praslin Island. The frozen animal was extracted with MeOH-CH_2Cl_2 and the extract was partitioned between *n*-BuOH and water. Successive chromatography of the *n*-BuOH-soluble material on Sephadex LH-20 and Spectral 40S yielded ascididemin (**1**, 0.26% dry wt.), eudistone A (**2**, 0.0023% dry wt.) and eudistone B (**3**, 0.0018% dry wt.). Ascididemin (**1**) was previously isolated from the tunicate *Didemnum* sp. by Kobayashi *et al.* (3) and has been synthetized (4).

Eudistone A (**2**) was obtained as an amorphous yellow powder. The molecular formula, $C_{27}H_{19}N_5O$, which was determined by high resolution mass measurement, implied twenty-one degree of unsaturation. The structural elucidation was based on interpretation of spectral data, predominantly NMT experiments. Comparison of these data and selected ^{13}C NMR data with literature values suggested the presence of the familiar moiety **a**, which is common to many of the pentacyclic aromatic alkaloids such as segolines (5), varamines (6), and ascididemin (**1**) (3), the major component of this tunicate. The fragment **b** is a 2-aminophenone moiety that is clearly defined by NMR experiments. The ^{1}H and ^{13}C NMR data for 2-amino-acetophenone (7) are very similar to those assigned to fragment **b**.

Analysis of the aliphatic portion of the ^{1}H NMR spectrum reveals the presence of two isolated spin systems that are assigned to -CH_2-CH_2- and -CH_2-CH< groups ; the coupling constants associated with these signals indicate that both groups are contained in six-membered rings. The chemical shifts of the H-14 and H-17 signals require that both C-14 and C-17 be adjacent to nitrogen atoms. In DMSO-d_6/$CDCl_3$ solution, the H-17 signals is directly coupled to the NH-18 signal. An HMBC experiment (J = 8 Hz) provided crucial information about the connectivities defined in fragment **c**. Cross peaks between both C-13 and C-14 and H-13a

indicate the attachment of C-13a to the carbonyl of the 2-aminophenone moiety. The signal assigned to an imine carbon (C-15a) shows two and three bond couplings to H-14, H-16 and H-17 signals, indicating that the two isolated system are linked through an imine bond. Furthermore, a long-range correlation between the $H\text{-}17_{eq}$ and C-18a signals suggests that N-18 is attached to an olefinic carbon and a similar correlation between H-14 and the only aliphatic quaternary carbon requires the aliphatic carbon atom (C-7b) be adjacent to C-13a and to a nitrogen atom, presumably N-8. The identity of fragment **c** and its connection to fragment **b** are therefore defined.

The structure of eudistone A (**2**) was elucidated by combining partial structures **a**, **b** and **c**, with two remaining quaternary aromatic carbon atoms which were not correlated to any proton signals in the HMBC spectra and which were assigned to C-15b and C-18b respectively. However, the data in hand did not eliminate the possibility that C-7a was connected to C-18a and C-7b was attached to C-18b. A second HMBC experiment, optimized for J = 6 Hz, clearly showed a weak correlation between H-13a and C-7a, eliminating the alternate structure.

There are two possible isomers of eudistone A (**2**), one having a *cis* ring junction at C-7b and C-13a and the other with a *trans*-diequatorial ring junction. Comparison of the observed spectral data with those predicted for the two isomers by usinbg a computer modeling program (8) clearly supported a *cis* ring junction. The coupling constant between H-13a and C-7a was determined by a heteronuclear proton-decoupling experiment : irradiation of the H-6 signal caused the C-7a signal to appear as a doublet with J = 1.5 Hz. The calculated values (8,9) for this coupling constant are J = 2.6 Hz for the *cis* ring junction and J = 8.4 Hz for the *trans* ring junction. Furthermore, the observed coupling constants between the H-13a signal and the axial and equatorial H-14 signals agree well with those predicted for the *cis* ring junction.

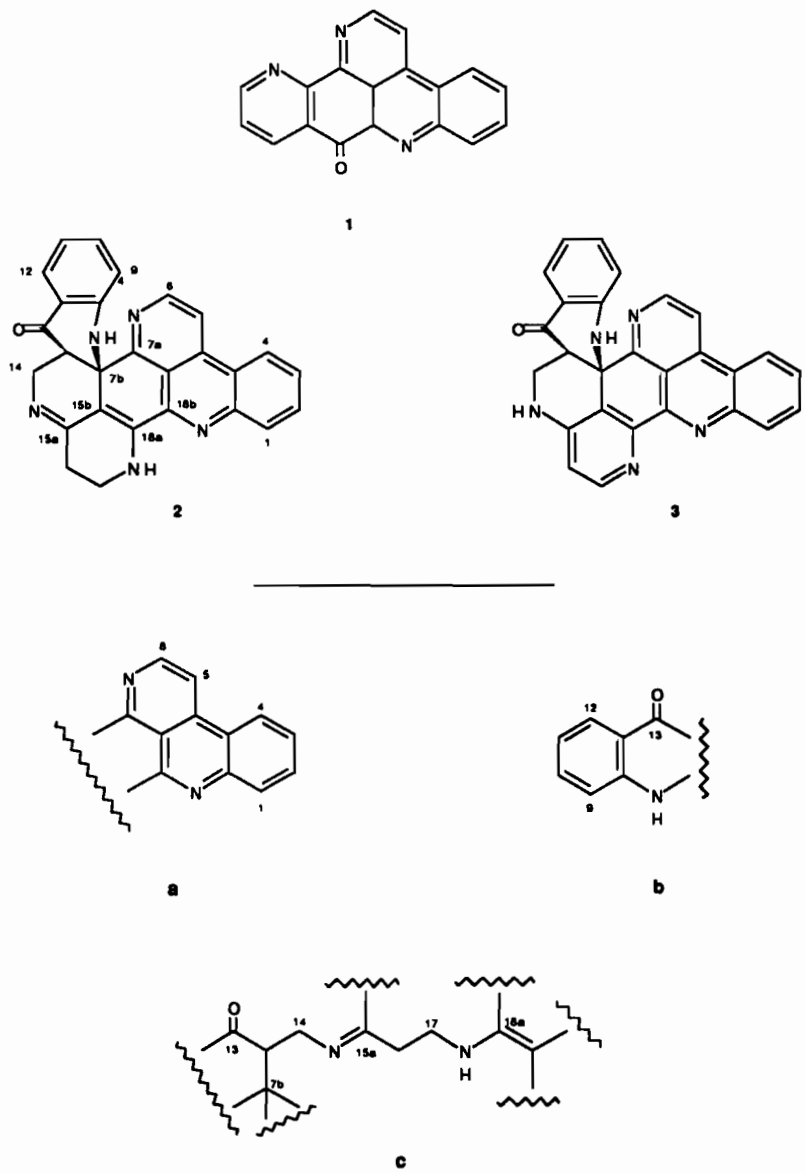
Eudistone B **3** was obtained as a white amorphous powder. The molecular formula, $C_{27}H_{17}N_5O$, required one more degree of unsaturation than was present in eudistone A (**2**). Both the ^1H and ^{13}C NMR data contained two new aromatic signals in place of the C-16 and C-17 methylene signals of **2**. Eudistone B **3** is therefore a dehydrogenation product of eudistone A (**2**). The synthesis of eudistone B **3** by air oxidation of eudistone A **2** confirmed its structure and stereochemistry.

In summary, we have isolated and identified two novel octacyclic aromatic alkaloids, eudistones A **2** and B **3**, as minor metabolites from tunicate *Eudistoma* sp. The major metabolite, ascididemin **1**, had previously been isolated from a tunicate of the genus *Didemnum* that is taxonomically distinct from *Eudistoma*. Specimens of a *Eudistoma* species from the Red Sea contained a quite different group of polycyclic aromatic alkaloids (5). Examples of polycyclic aromatic alkaloids have been isolated from several invertebrate phyla (10) ; one compound, dercitamide, has even been isolated from both the sponge *Stelletta* sp. (11a) and an unidentified tunicate (11b). The broad distribution of related polyclyclic aromatic alkaloids has led to the suspicion that they might be produced by symbiotic micro-organisms but there is no direct evidence to support this hypothesis. We could not detect the photosynthetic pigments associated with symbiotic algae in our specimen of *Eudistoma* sp. but we could not rule out the presence of non-photosynthetic symbionts.

The sponge ***Smenospongia*** **sp.** was collected at Thérèse Island. This sponge contained a bizarre group of macrocycyclic chromenes, smenochromenes A-D **4-7**, that are related to farnesyl hydroquinone (a biosynthetic precursor of many compounds from related sponges) (10) by unusual cyclizations.

Smenochromene A **4** was obtained as optically inactive, colorless crystals, mp. 98°C. The molecular formula, $C_{22}H_{26}O_3$, requires 10 unsaturation equivalents. Six unsaturations are assigned to the chromene ring system, one to a methylenedioxy ring, and two to isolated trisubstituted olefinic bonds, leaving the final unsaturation equivalent to be accommodated as a

macrocyclic ring. The UV spectrum is typical of a chromene chromophore and the ^{13}C NMR spectrum supported this assignment. The ^{1}H NMR spectrum was difficult to assign because of some very unusual chemical shift values. Analysis of the ^{1}H and ^{13}C NMR spectra clearly defined the chromene ring system, the attached methylenedioxy ring, the substitution pattern around the aromatic ring, and the point of attachment for the macrocyclic ring. The COSY, HMQC and HMBC experiments suggested that an isoprenoid chain linked the aromatic ring and C-10 on the chromene ring. Although both the ^{13}C NMR spectrum and biosynthetic considerations correctly led us to the conclusion that smenochromene A **4** contained a regular terpenoid chain as part of macrocyclic ring, it was difficult to reconcile the chemical shift value of the ^{1}H NMR signal at δ 1.19 (s, 3 H) with a methyl group on a trisubstituted olefin. We therefore chose to perform an X-ray crystallographic study of this unusual molecule.



Fragments identified by simple NMR experiments

A computer generated perspective drawing of the final X-ray model of smenochromene A **4** is given in Figure 1. One structural feature is noteworthy : Me-20 is directly under the

center of the aromatic rings and is only 3.61 Å away. This places the vinyl methyl group (Me-20) well inside the ring current of the aromatic ring system and accounts for the unusual upfield shift of the corresponding ^{1}H NMR signal to 1.19 ppm. A molecular model generated using the PC Model program was almost identical to the X-ray model and gave a distance of 3.64 Å between Me-20 and the plane of the aromatic rings. Examination of a Dreiding model of **4** did not exclude a conformation in which the Δ (7) double bond is rotated by about 180° so that H-7 lies within the ring current and Me-20 is outside : remarkably, empirical force-field calculations predict that both conformations are of approximately equal energy but the energy barrier for interconversion is very high. Another interesting feature of smenochromene A is that, unlike other compounds of this series, it is racemic.

Smenochromene B **5**, $[\alpha]_D$+6.4°, is an isomer of smenochromene A **4**. The ^{1}H and ^{13}C NMR data for smenochromene B **5** were assigned by interpretation of COSY and XHCORR experiments. Comparison of the ^{13}C NMR data with those of smenochromene A revealed that the C-21 methyl signal was at δ 14.3 (q) in **5** instead of 26.4 (q) in **4**, indicating the (*2E*) geometry in smenochromene B. The chemical shift of the Me-20 signal at δ 1.39 (s, 3 H) indicates that the methyl group is located within the ring current of the chromene system but is further away from the plane of the aromatic ring system. This conclusion is supported by empirical force-field calculations that indicate a distance of 5.23 Å between Me-20 and the plane of the aromatic ring system.

Smenochromene C **6**, $[\alpha]_D$-217°, was obtained as a colorless crystalline solid, mp. 52°C. The molecular formula, $C_{22}H_{28}O_3$, required 9 unsaturation equivalents. An initial examination of the NMR spectrum of **6** suggested that it was quite closely related to **4**. The "missing" unsaturation equivalent is that due to the methylenedioxy ring which is "replaced by a methoxyl group in smenochromene C **6**. The presence of two *para*-substituted aromatic protons required the second aromatic proton to be at the position occupied by the terpenoid chain in **4**. The terpenoid chain was in turn attached to oxygen through a methylene group. Since the NMR data indicated that the terpenoid chain and chromene ring were intact, there were two possible structures for **6**, one as drawn and the other with O-methyl and 0-methylene groups reversed. Three NOEDS measurements clearly defined the structure ; irradiation of the methoxyl signal caused a 9.5% enhancement of the H-17 signal and irradiation of the H-14 signal caused enhancements of the H-12 and H-2 signals. The (*2Z,6E*) geometry was deduced from the ^{13}C chemical.shifts of the methyl signals.

Smenochromene D **7**, $[\alpha]_D$-68.5°, is an isomer of smenochromene C **6** and has an identical structure except that the ^{13}C chemical shifts of the C-20 and C-21 signals require the (*2E, 6E*) geometry.

In addition to the compounds described above, we have also found some new cembranoids from an as yet unidentified soft coral, a new aaptamine from *Aaptos aaptos*, two new brominated acetylenic acids from *Xestospongia* sp., and a new diterpene alcohol from *Lobophytum crassum*.

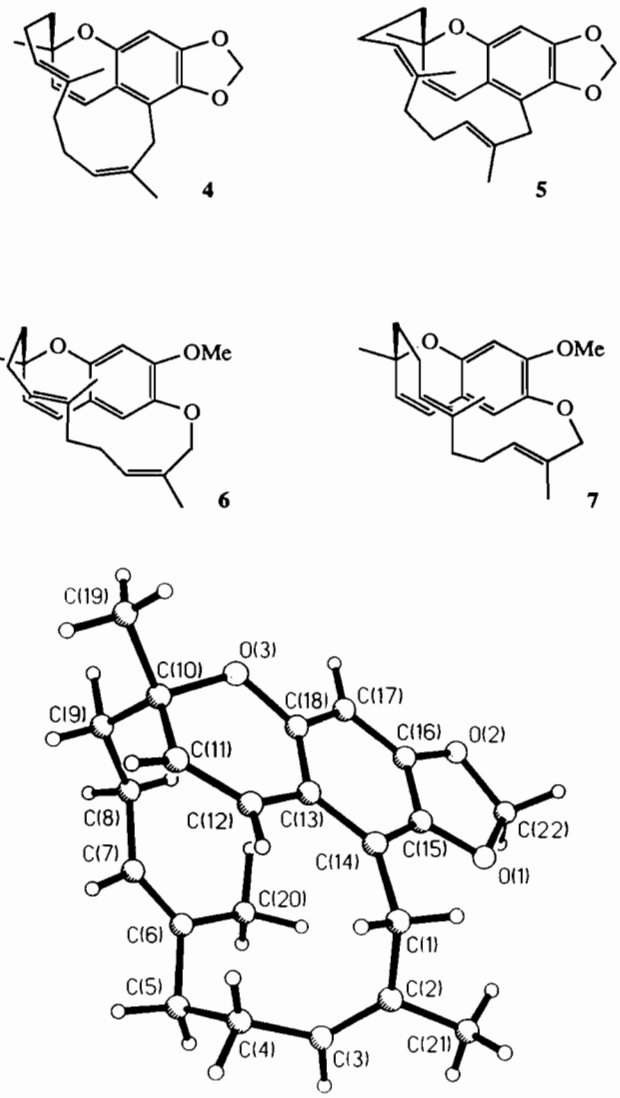


Figure 1 : A computer generated perspective drawing of the final X-ray model of smenochromene A 4. The naturally occuring material is racemic.

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[**Note.** The structure of dercitamide reported in reference 11a is incorrect (unpublished data from this an other laboratories) : the correct structure has been reported as kuanoniamine C in reference 11b]

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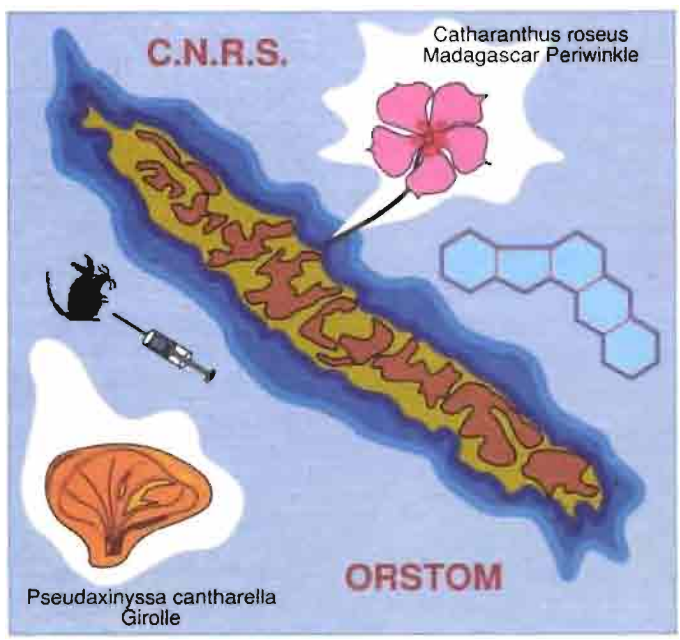

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