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Chemotaxonomy of *Agelas* (Porifera: Demospongiae)

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Key Word Index—*Agelas*; Agelasidae; Axinellidae; Halichondriidae; secondary metabolites; pyrrole-2-carboxylic acid derivatives; isonitriles; chemotaxonomy.

Abstract—The secondary metabolite content of four different species of *Agelas* (Porifera) from the West Indies has been studied. All the compounds isolated are already known metabolites whose identification was confirmed by comparison of their spectral properties with those reported in the literature. They pertain to two different classes of compounds: terpenoids and pyrrole-2-carboxylic acid derivatives. The chemotaxonomic value of these secondary metabolites has been evaluated. Their distribution amongst the Porifera, as well as that of isocyanide derivatives, suggests a close relationship between the Agelasidae, the Axinellidae and the Halichondriidae.

Introduction

The genus *Agelas* is an interesting and enigmatic genus of sponges both from a systematic and from a biogeographical point of view. There are 12 well-established species occurring commonly in tropical and subtropical shallow water environments. All these species seem to be fairly closely related judging from their morphological characters, but the relationships with other sponge genera, families and even orders remain obscure. Contrary to existing biogeographic patterns there seem to be more *Agelas* species in the West Indies than in the whole of the Indopacific region.

Chemotaxonomic studies integrate chemical and biological data and, in many groups of organisms, secondary metabolite distribution has been found to provide reliable new input to taxonomic problems and has led to re-evaluation of existing taxonomic conclusions. Despite the fact that sponges are a rich source of secondary metabolites, it is only in a few cases that these compounds have been used as taxonomical characters [for a recent review see Bergquist and Wells (1983)].

Our aim is to evaluate if chemical data could reveal some relationships between *Agelas* and other groups of sponges such as the Halichondria, the Poecilosclerida, the Haplosclerida or the Keratosa [for a general discussion of the classification of the demosponge higher taxa see van Soest (1991)]. For this purpose several samples of *Agelas* from the West Indies have been collected and their secondary metabolite content determined and compared to that of already chemically studied *Agelas* and sponges of the other groups.

Materials and Methods

After collection, each fresh sponge was preserved in MeOH and shipped by air to Brussels via Amsterdam. The sponges were cut into small pieces and extracted exhaustively with MeOH. The methanol extract was fractionated according to the standard procedure summarized in Fig. 1.

Extraction and isolation. Agelas clathrodes (sample no. XXX.1; massive, irregularly wall-shaped, bright orange sponge, provided with grooves and ridges). The methanol extract of the sponge (160 g dry weight) yielded 11.9 g of Fr A, 3.1 g of Fr B and 13.6 g of Fr C + Fr D. The fractions A and B were found to be toxic against *Lebistes reticulatus* at 50 mg/l, the only concentration tested. Fraction B (493 mg) was subjected to a silica gel chromatography with $CHCl_3$–MeOH 6/4 to afford a toxic fraction (152 mg), of which 98 mg was further chromatographed on a C_{18} reversed phase column with H_2O–MeOH 7/3. This yielded hymenidine (**27**; 65 mg)

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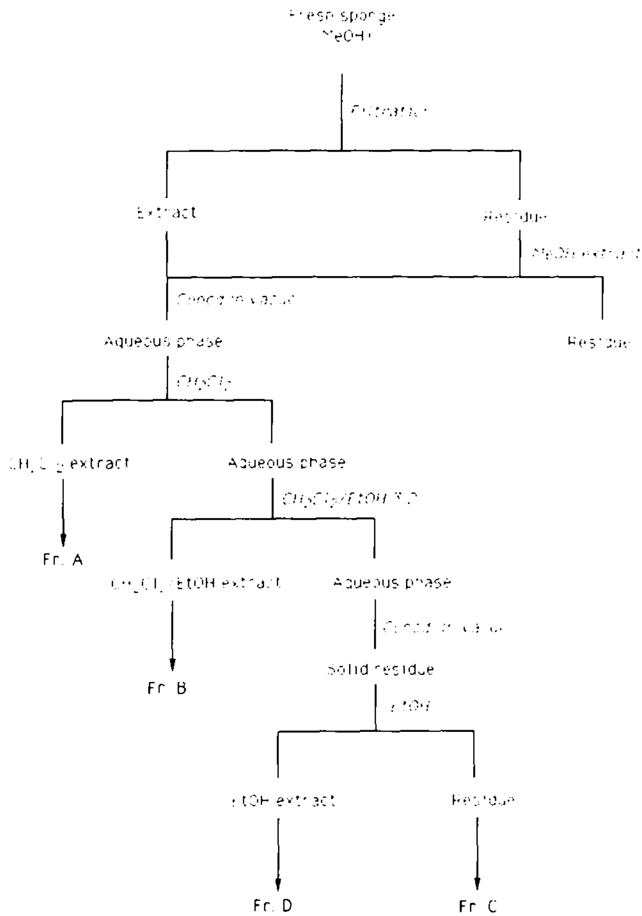


FIG. 1. EXTRACTION AND FRACTIONATION PROCEDURE.

homogeneous by TLC (silica gel CH_2Cl_2–MeOH–NH_3 25% 10/9/1) and identified on the basis of its spectral properties (UV, MS, ^1H and ^{13}C NMR) which are compatible with those reported (Kobayashi *et al.*, 1986a).

Agelas conifera (sample no. XXX.2; repent-ramose, orange sponge with oscules on large volcanoe-shaped elevations, with a smooth surface). The methanol extract of the sponge (109 g dry weight) yielded 3.1 g of Fr A, 6.1 g of Fr B, 18.2 g of Fr C and 7.33 g of Fr D. Fraction B was found to be toxic against *Lebistes reticulatus* at 50 mg/l. This fraction (212 mg) was subjected to a C_{18} reversed phase column with H_2O–MeOPH 5/5. The active fraction (140 mg) was further purified by chromatography on Sephadex G10 (eluent: H_2O). This yielded sceptrin (**29**; 50 mg) homogeneous by TLC (silica gel CH_2Cl_2–MeOH–NH_3 25% 10/3/1) and was identified on the basis of its spectral properties (FABMS$^+$ and FABMS$^-$, UV, ^1H and ^{13}C NMR) which are compatible with those reported (Walker *et al.*, 1981). Moreover, TLC of the active fraction showed the presence of oroidine (**28**) and dibromopyrrole (**16**).

Agelas dispar (sample no. XXX.3; globular, orange sponge with large key-hole shaped openings, next to slightly raised round oscules, with smooth surface. The methanol extract of the sponge (263 g dry weight) yielded 5.4 g of Fr A, 12.4 g of Fr B, 16.1 g of Fr C and 7.6 g of Fr D. Fraction B showed two major compounds by TLC (silica gel, CH_2Cl_2–MeOH 9/1); 1.38 g of this fraction was chromatographed successively on Sephadex LH-20 (eluent MeOH) and on a C_{18} reversed phase column (MeOH–H_2O 2/8), this yielded ageline-A (**4**; 25 mg) which was recrystallized from acetonitrile. The spectral properties of the crystals are compatible with those reported for ageline-A (Capon and Faulkner, 1984).

A second batch of Fr B (829 mg) was partitioned between the two phases of the mixture $CHCl_3$–MeOH–H_2O 13/7/8. The chloroform soluble material (326 mg) was chromatographed on a silica gel column using $CHCl_3$–MeOH–NH_3 25% 10/1/1 as eluent. This yielded the formamide **5** as a crude compound that was further purified by preparative TLC ($CHCl_3$–MeOH 97/3). The spectral properties of **5** (IR, UV, MS, ^1H NMR) were identical to those reported (Capon and Faulkner, 1984).

Fraction D was chromatographed on Sephadex LH-20 (eluent: MeOH) monitored by TLC (UV). This led to three UV-positive compounds, each of which was further purified by chromatography on a C_{18} reversed phase column (H_2O–MeOH 100/0 to 0/100). This yielded **27** and the bromopyrroles **16** and **17**, respectively. The spectral properties of **27** were identical to those reported for **27** (Kobayashi *et al.*, 1986a).

Spectral properties of 2-carbamido-5-bromopyrrole (**16**): EIMS: *m/z* 188–190 (M^+, 100), 171–173 (100), 144–146 (20), 117–119 (15); UV (MeOH, λ_{max}): 201 (6920), 229 (5210), 265 (6630); IR (KBr): 2000–3300, 1650 cm^{-1}; ^1H NMR (CD_3OD; TMS; *J*): 6.78 (1H, *d*, 1.6 Hz), 6.93 (1H, *d*, 1.6 Hz).

Spectral properties of 4-bromopyrrole-2-carboxylic acid (**17**): EIMS: *m/z* 189–191 (M^+, 50), 171–173 (100), 144–146 (10), 143–145 (10), 117–119 (10); UV (MeOH, λ_{max}): 202 (6170), 225 (3760), 268 (4190); IR (film): 3200 (broad), 1640 cm^{-1}; 1H NMR (CD_3OD; TMS; *J*): 6.80 (1H, *d*, 1.4 Hz), 6.91 (1H, *d*, 1.4 Hz).

Agelas conifera (sample no. XXXI.0; ramose-tubiform, orange sponge, with smooth surface. The methanol extract of the sponge yielded 0.1 g of Fr A, 1.7 g of Fr B, 5.5 g of Fr C and 3.2 g of Fr D. Fraction B (310 mg) was chromatographed on Sephadex LH-20 (MeOH), C_{18} reversed phase (MeOH–H_2O 0/100 to 100/0) and silica gel ($CHCl_3$–MeOH–NH_3 25% 10/3/1) successively. This yielded **28** (Nakamura *et al.*, 1984c; Kobayashi *et al.*, 1986b) and **16** (Forenza *et al.*, 1971) identified on the basis of their spectral properties (IR, UV, ^1H and ^{13}C NMR). Moreover, TLC of Fr B showed the presence of **29**.

Agelas clathrodes (sample no. XXXI.1; irregular, massive orange sponge, with grooved surface provided with many irregular openings). The methanol extract of the sponge (65 g) yielded 4.0 g of Fr A and 0.7 g of Fr B. Fraction B (700 mg) was partitioned between the two phases of the mixture $CHCl_3$–MeOH–H_2O 13/7/8. The water-soluble fraction (180 mg) was chromatographed on C_{18} reversed phase (MeOH–H_2O 5/5). This yielded **16** (IR, UV, MS, ^1H and ^{13}C NMR).

Agelas schmidti (sample no. XXXI.2; hollow, repent, dark orange-red sponge, heavily encrusted, with many small openings, as well as short tubular oscules). The methanol extract of the sponge (67 g) yielded 2.6 g of Fr A, 0.6 g of Fr B, 13.9 g of Fr C and 1.7 g of Fr D. Fraction B (100 mg) was treated as Fr B of sample XXXI.1. This yielded **16** identical in all respects with an authentic sample.

Results and Discussion

Six samples of *Agelas* pertaining to four different species were collected in the West Indies (Table 1), preserved in methanol and the methanol extracts fractionated according to the standard procedure summarized in Fig. 1. Each fraction was analyzed by thin layer chromatography in order to evaluate the presence of secondary metabolites. The compounds detected by this method were isolated using appropriate chromatographic techniques. This led to the isolation of several compounds which were found to be already known derivatives whose identification was confirmed by comparison of their spectral properties with those reported in the literature. The details of these results are described in Materials and Methods while the derivatives isolated in this study are listed in Table 2, together with the secondary metabolites of all the *Agelas* species that have been chemically investigated until now.

On the basis of their structure and taking into account their probable biogenetic origin, two types of *Agelas* secondary metabolites can be considered: terpenoid derivatives and pyrrole-2-carboxylic acid derivatives. The terpenoids are either sesquiterpenoid derivatives of hypotaurocyamine (**1–3**) or adenine derivatives of diterpenes (**4–15**). Only five *Agelas* specimens out of the 18 so far examined produce these unique terpenes, not found in any other group of sponges. Strictly taken, these terpenes could be considered, within *Agelas*, as a synapomorphy for the concerned species. But, to be of taxonomical value, this character should coincide with the other ones, since it is well known that a species producing certain secondary metabolites

TABLE 1. LIST OF THE *AGELAS* SAMPLES COLLECTED IN THE WEST INDIES AND STUDIED IN THIS WORK

Ref no.	Species	Collecting place
XXX.1	*A. clathrodes* (Schmidt, 1870)	Bonaire (12–20 m)
XXX.2	*A. conifera* (Schmidt, 1870)	Bonaire (12–20 m)
XXX.3	*A. dispar* (Duchassaing and Michelotti, 1864)	Bonaire (12–20 m)
XXXI.0	*A. conifera* (Schmidt, 1870)	Granate* (25 m)
XXXI.1	*A. clathrodes* (Schmidt, 1870)	Morro* (20 m)
XXXI.2	*A. schmidti* (Wilson, 1902)	Granate and Morro* (10–20 m)

*Colombian Caribbean.

TABLE 2. OCCURRENCE OF SECONDARY METABOLITES IN THE GENUS *AGELAS*

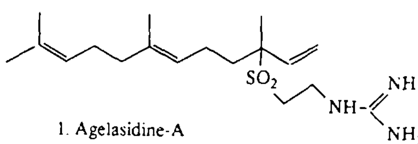
Species	Origin	Compounds TS	TD	P	PA	References
A. clathrodes	Bonaire	·	—	—	**27**	this paper
A. clathrodes	Morro	—	-	**16**		this paper
A. conifera	Bonaire		—	**16**	**28, 29**	this paper
A. conifera	Granate	—	–	**16**	**28, 29**	this paper
A. conifera	Caribbean	—			**29, 30–31, 32, 33–34**	Rinehart (1989)
A. dispar	Bonaire	—	**4, 5**	**17, 18**	**27**	this paper
A. mauritiana	Enewetak	—	**6**	-	–	Nakatsu *et al.* (1984)
A. mauritiana	Enewetak	—	**7, 8**	**19**	**25, 26**	Fathi-Afshar and Allen (1988)
A. cf. *mauritiana**	Caribbean	—	··	-	**29, 30–31, 32, 33–34**	Rinehart (1989)
A. nakamurai†	Okinawa	**1–3**	**4, 9–13**	—	—	Nakamura *et al.* (1983, 1984, 1985); Wu *et al.* (1984, 1986)
A. nemoechinata	Okinawa		–	—	**37**	Nakamura *et al.* (1984)
A. oroides	Napoli	·	—	**16, 20–22**	**28**	Forenza *et al.* (1971); Garcia *et al.* (1973); Kobayashi *et al.* (1986)
A. sceptrum	Belize		—	—	**28, 29**	Walker (1981)
A. schmidti	Granate	—	--	**16**	—	this paper
A. sp.	Palau	1	**4, 5, 14, 15**	—	—	Capon and Faulkner (1984)
A. sp.	?	·	-	**16, 19, 20, 22–26**	-	Tada and Tozyo (1988)
A. sp.	Okinawa	-			**32–34**	Kobayashi *et al.* (1988)
A. sp.	Tanzania	—	·	--	**38**	Fedoreyev *et al.* (1989)

TS = sesquiterpenoid derivatives of hypotaurocyamine; TD = adenine derivatives of diterpenes; P = pyrrole-2-carboxylic acid derivatives; PA = pyrroloaminopropylimidazole derivatives.

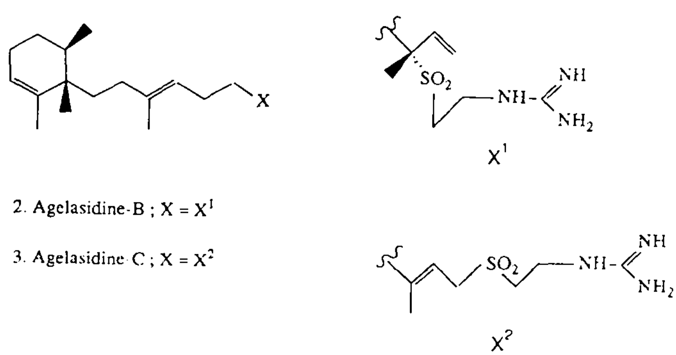
**Agelas mauritiana* has never been described from the Caribbean. This record probably refers to *A. clathrodes*.

†*Agelas nakamurai* is probably synonymous with *A. mauritiana*.

might still be related to others that do not, because the latter may have lost the ability to produce them or its ability is inhibited. Preliminary examination of morphological characters (e.g. *A. dispar* and *A. mauritiana*) brings little support to the hypothesis that terpenes are a synapomorphy for the *Agelas* species containing this type of compound. The latter are thus of limited chemotaxonomical value. Nevertheless, they could be utilized as characteristic fingerprints for some species, since some *Agelas*

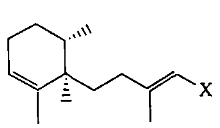


1. Agelasidine-A

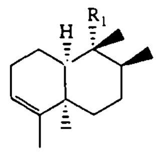


2. Agelasidine-B ; X = X[1]

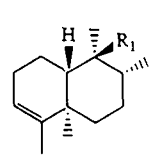
3. Agelasidine-C ; X = X[2]



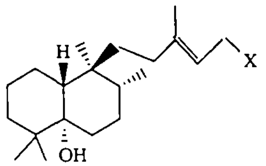
4. Ageline-A ; X = R_1
5. - Ageline-A ; X = R_2



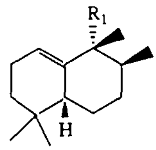
9. Agelasine-A



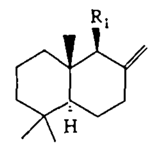
10. Agelasine-B



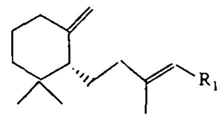
6. Unnamed ; X = R_5
7. Agelasimine-A ; X = R_3
8. Agelasimine-B ; X = R_4



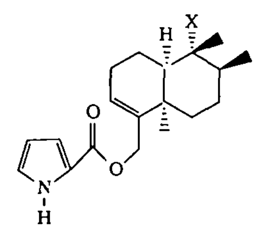
11. Agelasine-C



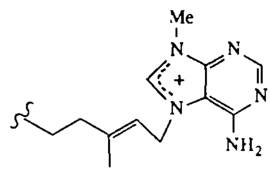
12. Agelasine-D



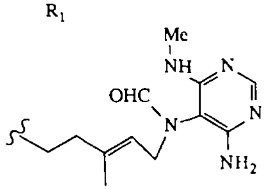
13. Agelasine-E



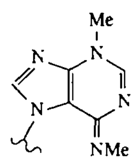
14. Ageline-B ; X = R_1
15. Unnamed ; X = R_2



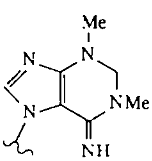
R_1



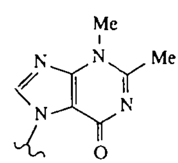
R_2



R_3



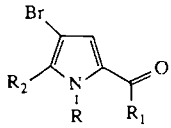
R_4



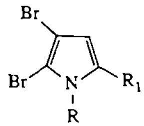
R_5

repeatedly possess them or are repeatedly devoid of them. Thus it is likely to happen that some of the provisionally identified specimens listed in Table 2 can be associated with certain species, e.g. *A.* sp. (Palau) and *A. nakamurai* with *A. mauritiana*; *A. mauritiana* (Caribbean) with *A. clathrodes* and perhaps *A.* sp. (?) with *A. oroides.* Examination of voucher specimens will reveal whether this is feasible.

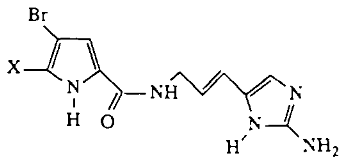
The pyrrole-2-carboxylic acid derivatives (**16–38**) are more generally distributed amongst the *Agelas.* Indeed, these compounds have been reported in 15 out of the 18 specimens examined. Moreover, it is possible that the remaining specimens may not have been screened for pyrrole derivatives. As far as the structure of these derivatives is concerned they may be divided into two main groups according to the fact that the



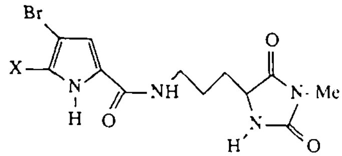
	R	R_1	R_2
16	H	OH	Br
17	H	NH_2	H
18	H	OH	H
19	Me	OMe	Br
20	H	OMe	Br
21	H	CN	Br
22	H	NH_2	Br
23	Me	OH	Br



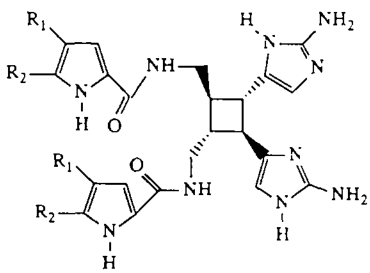
24 $R = R_1 = H$
25 $R = H$; $R_1 = CH_2OMe$
26 $R = Me$; $R1 = CH_2OMe$



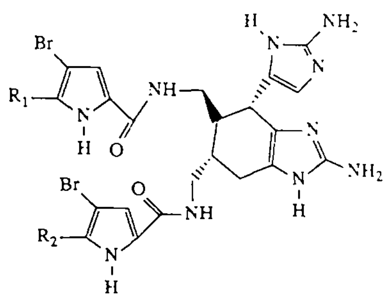
27 Hymenidine ; X = H
28 Oroidine ; X = Br



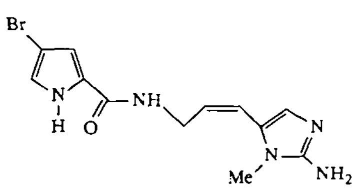
35 5-debromomidpacamide ; R = H
36 midpacamide ; R = Br



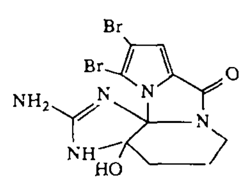
29 Sceptrin ; $R_1 = Br$ $R_2 = H$
30 Debromosceptrin ; $R_1 = R_2 = H$
31 Dibromosceptrin ; $R_1 = R_2 = Br$



32 Ageliferin ; $R_1 = R_2 = H$
33 Bromoageliferin ; $R_1 = Br$ $R_2 = H$
34 Dibromoageliferine ; $R_1 = R_2 = Br$

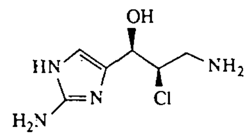


37 Keramadine



38 Dibromoagelaspongin

pyrrole-2-carboxylic acid moiety is linked or not to an aminopropylimidazole moiety. Although biosynthetic experiments have not yet been performed, it seems reasonable to admit that ornithine is the common precursor for all these nitrogenous derivatives. An hypothetical biogenetic pathway showing the relationships between these compounds is depicted in Fig. 2. It is supported by the following arguments: (a) proline and ornithine are two closely related amino acids of the glutamate group and the oxidation of proline into pyrrole-2-carboxylic acid is a general catabolic pathway (Michal, 1972); (b) an analogous aminopropylimidazole moiety is found in saxitoxin. In this particular case, it has been established that some of the atoms forming this moiety arise from ornithine (Shimizu *et al.*, 1984); (c) the isolation from the sponge "*Pseudaxinyssa*" *cantharella* (Ahond *et al.*, 1988) of the antitumoral compounds girolline (**39**) together with linear or cyclized pyrrole derivatives, supports the suggestion that the biosynthetic pathway of these alkaloids proceeds by formation of an amide bond between a pyrrole-2-carboxylic acid precursor and an aminopropylimidazole moiety.



39 Girolline

Interestingly, in contrast to the *Agelas* terpenoids, pyrrole-2-carboxylic acid derivatives have been reported in several sponges other than *Agelas*. Table 3 lists the variety of skeletons based on this system that have been isolated so far and their species of origin. A likely biogenetic relationship between all these skeletons is depicted in Fig. 3.

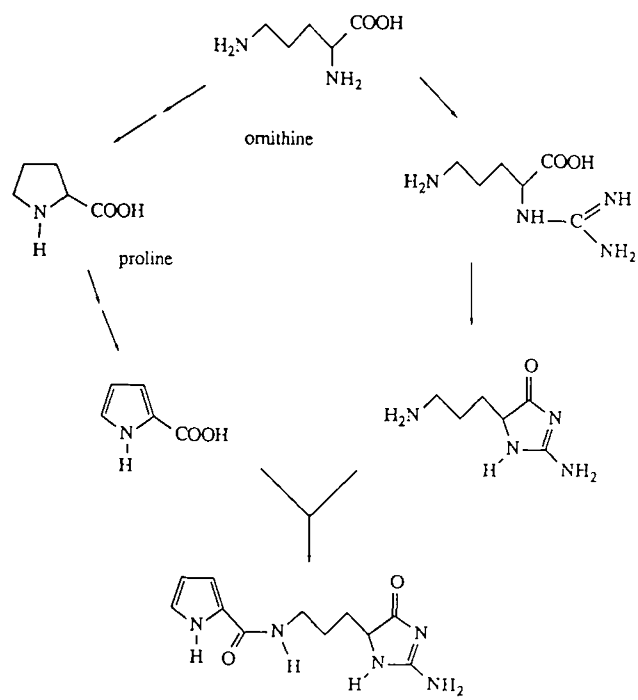


FIG. 2. HYPOTHETICAL BIOGENETIC ORIGIN OF THE PYRROLOAMINOPROPYLIMIDAZOLE DERIVATIVES.

TABLE 3. OCCURRENCE OF PYRROLE AND PYRROLOAMINOPROPYLIMIDAZOLE ALKALOIDS IN PORIFERA IN ADDITION TO AGELASIDAE

Species	Types of skeletons* P	P1	P2	P3	P4	P5	References
Axinellidae							
Acanthella aurantiaca†		X			X		Cimino *et al.* (1982)
A. carteri†			X		X	X	Fedoreyev *et al.* (1986); Outkina *et al.* (1984)
Axinella damicornis	X	X					Braekman and Daloze (1986); Cimino *et al.* (1975)
A. verrucosa		X			X		Cimino *et al.* (1982)
A. sp					X		Schaufelberger and Pettit (1989)
Phakellia flabellata				X	X		Sharma and Magdoff-Fairchild (1977); Sharma *et al.* (1980); Shimizu *et al.* (1984)
Pseudaxinella massa‡	X					X	Schmitz *et al.* (1985)
"Pseudaxinyssa" cantharella§		X	X	X	X	X	De Nanteuil *et al.* (1985)
Halichondriidae							
"Hymeniacidon aldis"‖						X	Schmitz *et al.* (1985)
"H. aldis"‖					X		Kitagawa *et al.* (1983)
H. sp.		X			X		Kobayashi *et al.* (1986a, 1986b, 1988)
H. sp.					X		Schaufelberger and Pettit (1989)
Ceratoporellida							
Goreauiella sp.		X			X		Rinehart (1989)

*P1–P5 skeletons are shown in Fig. 3. P = pyrrole.

†*Acanthella aurantiaca* syn. *A. carteri.*

‡Erroneously described as *Lissodendoryx* sp. in Fedoreyev *et al.* (1989)

§The genus *Pseudaxinyssa* is now considered to be a synonym of *Axinyssa* (Halichondriidae). However, *cantharella* is an Axinellid of uncertain generic assignment, provisionally assigned to *Axinella.*

‖*Hymeniacidon aldis* is a junior synonym of *Pseudaxinella massa.* The identifications are thus suspect Axinellidae.

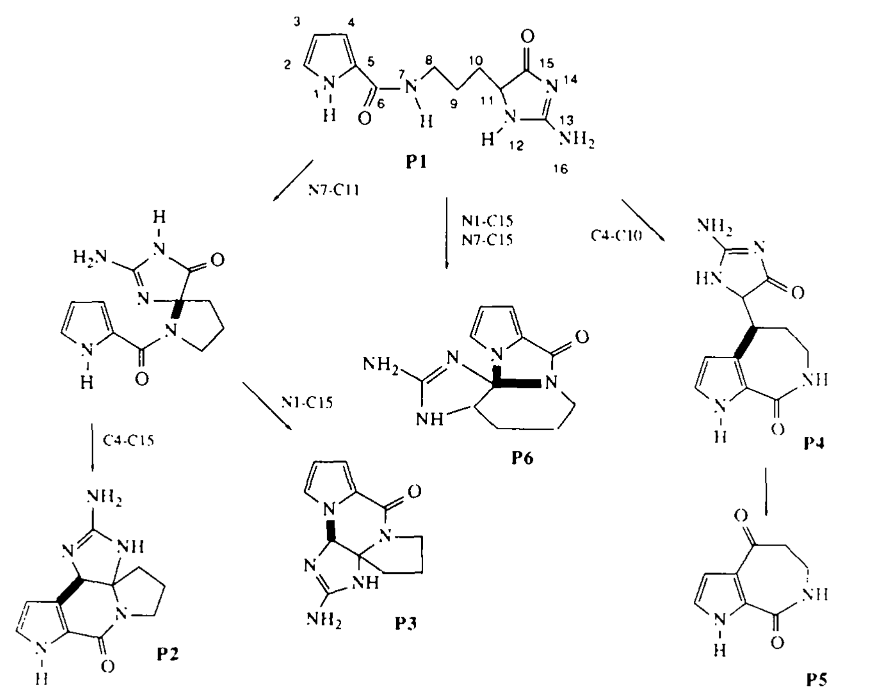


FIG. 3. A LIKELY BIOGENIC RELATIONSHIP BETWEEN THE VARIETY OF PYRROLOAMINOPROPYLIMIDAZOLE ALKALOIDS.

From Table 3, it is clear that the distribution of the alkaloid types found in *Agelas* is restricted to some species of the family Axinellidae (genera *Acanthella, Axinella, Phakellia, Pseudaxinella* and *"Pseudaxinyssa"*) and of the genus *Hymeniacidon* (Halichondriidae), as well as to a Ceratoporellida (*Goreauiella* sp.) collected in a deep-sea habitat (Rinehart, 1989).

It is likely that this limited occurrence of pyrrole derivatives is still more restricted than one would have thought, since the species reported as Hymeniacidons in Table 3 may have been incorrectly determined and are suspected to belong to the Axinellidae. Indeed, a piece of the type specimen of *Hymeniacidon aldis* De Laubenfels (1954) which has been examined by one of us (R.v.S.) turned out to be *Pseudaxinella massa.* Of course, this does not mean that the specimens used for the secondary metabolite studies are the same species but at least it makes it dubious. All this points rather strongly to a fairly limited occurrence of the pyrrole derivatives which have been found only in members of the Agelasidae, the Axinellidae and in the sole Sclerospongia that has been chemically investigated until now.

Many other species of these families have not been screened for their alkaloids. Thus, it is not known how general this chemical character is amongst them. However, it is certain that some members of these families have been found to be devoid of these alkaloids. If the pyrrole-2-carboxylic acid derivatives are to be considered a synapomorphy for this group of sponges, then we must assume a certain loss of this character amongst them. There is morphological evidence for a close relationship between the Agelasidae and the Ceratoporellida since they share the curious verticillate acanthostyles, but those two groups have little in common with the Axinellidae.

A further problem is the distribution amongst the Porifera of another type of secondary metabolites: the terpenoid isocyanides and related derivatives (e.g. isothiocyanates, formamides). These secondary metabolites occur mainly in members of the Axinellidae and the Halichondriidae (Table 4). There are two exceptions, the presence of diterpenoid isocyanides in an undetermined species of *Amphimedon*

TABLE 4. LIST OF THE PORIFERA SPECIES FROM WHICH ISOCYANIDES AND/OR RELATED DERIVATIVES HAVE BEEN ISOLATED

Axinellidae

Acanthella acuta [Braekman *et al.* (1987); Ciminiello *et al.* (1987); Mayol *et al.* (1987); Minale *et al.* (1974)] — *A. cavernosa* [Omar *et al.* (1988)] — *A. klethra* [Fusetani *et al.* (1990)] — *A. pulcherrima* [Capon and Macleod (1988)] — *A.* sp. [Chang *et al.* (1984, 1987); Patra *et al.* (1984)]

Axinella cannabina [Cafieri *et al.* (1973); Ciminiello *et al.* (1984, 1987); Di Blasio *et al.* (1976); Fattorusso *et al.* (1974, 1975); Iengo *et al.* (1977)

*Pseudaxinella amphilecta** [Wratten *et al.* (1978)]

Pseudaxinyssa pitys [Wratten and Faulkner (1977, 1978); Wratten *et al.* (1978)] — *P.* sp. [Karuso and Scheuer (1987)]

Halichondriidae

Axinyssa sp. [Marcus *et al.* (1989)] — *Axinyssa* sp.† [Sullivan *et al.* (1986)] — *Axinyssa* sp. [Molinski *et al* (1987)]

"Epipolasis" kushimotoensis [Tada and Yasuda (1985)]

Ciocalypta sp.‡ [Burreson *et al.* (1975); Gulavita *et al.* (1986); Hagadone *et al.* (1979); Karuso *et al.* (1989); Tada and Yasuda (1985)]

"Stylotella" sp.§ [Pais *et al.* (1987)] = *Axinyssa aplysinoides*

Trachyopsis aplysinoides [He *et al.* (1989)]

Halichondria sp. [Burreson *et al.* (1975)]

Other families

Amphimedon sp. [Kazlauskas *et al.* (1980)]

Theonella swinhoei [Nakamura *et al.* (1984)]

*Described as *Hymeniacidon amphilecta* (Wratten *et al.*, 1987b).

†Described as *Halichondria* sp. (Molinski *et al.*, 1987; Sullivan *et al.*, 1986).

‡Previously determined as *Hymeniacidon* sp. (Patra *et al.*, 1984).

§This genus is now considered a synonym of *Hymeniacidon.*

[previously determined as *Adocia* sp. (Kazlauskas *et al.*, 1980)] and of sesquiterpenoid isocyanides in a sample determined as *Theonella swinhoei* (Nakamura *et al.*, 1984b).

The data reported in Tables 3 and 4 indicate that most of the Axinellidae that have been chemically investigated until now contain isocyanide or proline-2-carboxylic acid derivatives. Only in a few species of Axinellidae have other types of compounds been reported. So, pregnane steroids have been isolated from *Axinella agnata* but this species does not appear a typical Axinellidae (Guella and Pietra, 1988). *Axinella polycapella* (Wratten and Meinwald, 1981) and *A. polypoides* (Cimino *et al.*, 1974) contain polyphenols, derivatives well known as being characteristic of the algal metabolism (Bergquist and Wells, 1983). Thus, it could be that these polyphenols originate from the microalgae contaminating the sponges. An undetermined species of *Axinella* (Herb *et al.*, 1990) contains unique derivatives whose structures are closely related to those found in two *Trikentrion*, namely *T. laeve* (Aknin *et al.*, 1990) and *T. flabelliforme* (Capon *et al.*, 1986); the identification could be wrong because, without detection of the characteristic *Trikentrion* spicules, it might easily be mistaken for *Axinella.* Finally, it has been reported that "*Pseudaxinyssa*" sp. from Papua New Guinea contains traces of cyclodepsipeptides (de Silva *et al.*, 1990) also found in an undetermined species of *Geodia* (Chan *et al.*, 1987), and which are suspected to be of microbial origin.

As far as the Halichondriidae are concerned, most of the specimens of the family that have been chemically investigated until now are characterized by the presence of isocyanides (Table 4). Exceptions have been reported, but again, as in the case of the Axinellidae, the identification of the sponge and/or the origin (symbiotic or contaminating microorganisms) of the secondary metabolites could be questioned. Thus, terpenes devoid of isocyanide functions have been reported in two *Epipolasis* [*E.* sp. (Fusetani *et al.*, 1987)—probably *E. novaezelandiae*, suspected to be a *Topsentia*— and *E. reiswigi* (Kashman *et al.*, 1987), now considered a synonym of *Myrmekioderma styx*] and two *Halichondria* [*H. panicea* (Cimino *et al.*, 1973) and *Halichondria* sp. (Capon *et al.*, 1982)]. Interestingly, the structures of the sesquiterpenes present in these latter are closely related to those of the sesquiterpenes found in *Didiscus oxeata* (Stoller, 1990) and *D. flavus* (Wright *et al.*, 1987), suggesting that the identification of these specimens should be confirmed. In a recent study, *Didiscus* was found to be morphologically similar and closely related to *Myrmekioderma* (van Soest *et al.*, 1990). Moreover, specimens of *Halichondria* sp. (Kernan *et al.*, 1988), *H. okadai* (Tachibana *et al.*, 1981) and *H. melanodocia* (Uemura *et al.*, 1985) have been found to possess cytotoxic polyethers (e.g. okadaic acid) which are known to be of dinoflagellate origin (Murakami *et al.*, 1982).

To summarize, the general trends that arise from the comparison of the secondary metabolite content of the Agelasidae, the Axinellidae, the Halichondriidae and the Ceratoporellida is that the Agelasidae share with some Axinellidae and *Goreauiella* sp., the ability to biosynthesize pyrrole-2-carboxylic acid derivatives, while the remaining Axinellidae share with the Halichondriidae the ability to biosynthesize isocyanide terpenoid derivatives.

There are two possible ways to integrate such a distribution in terms of phylogenetic relationships. One, is to admit that in these groups of sponge the secondary metabolites make poor synapomorphies because of the loss in some species of the ability to produce either of the two types of secondary metabolites (e.g. the absence of pyrrole-2-carboxylic acid derivatives in the Halichondriidae and in some Axinellidae). If this is the case, the phylogenetic relationships that derive from this evidence could be visualized as depicted in Fig. 4A. Another way to interpret the biochemical results is to consider that the Axinellidae do not form an homogeneous group and thus should be divided following the type of secondary metabolites they contain. In that case, Fig. 4B

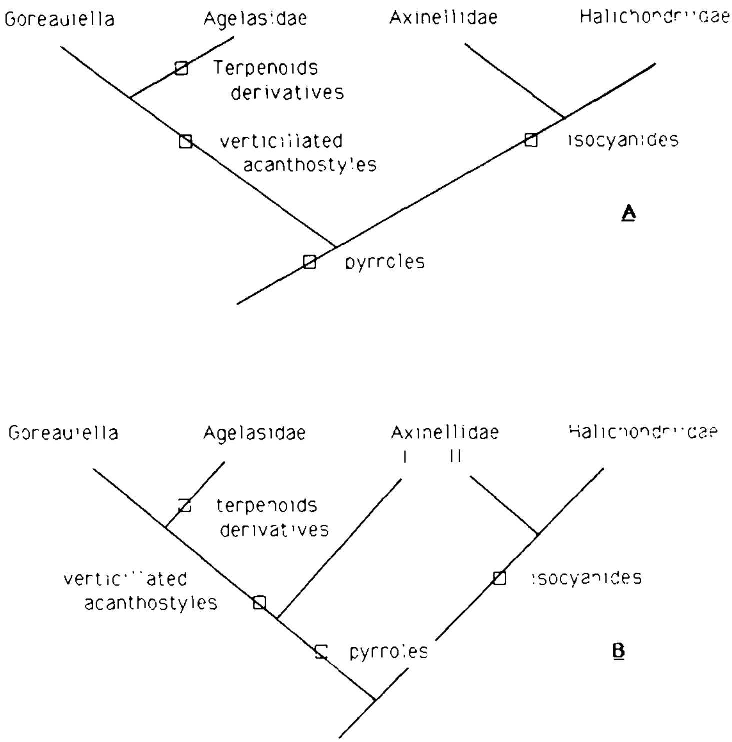


FIG. 4. PROPOSED PHYLOGENETIC RELATIONSHIPS BASED ON THE SECONDARY METABOLITE CONTENT.

could be proposed. Of course, these proposals are still prospective and need further detailed studies.

In conclusion, although the present results indicate that secondary metabolites could provide very useful additional characters for phylogenetic studies, the evaluation of the significance of their distribution is seriously hampered by the almost universal casual treatment in the current chemical literature of the identity of the studied material. Identities are often quite wrong and nomenclatorial changes are ignored, giving a false suggestion of secondary metabolite distribution. Often, no voucher specimens are kept and if they are, it is not stated where they can be re-examined. This precludes discovery of the true taxonomic distribution. Often the person(s) responsible for the identification are not named, preventing a discussion on identity assignments. Moreover, it is difficult to determine if a particular metabolite is produced by the sponge or by a potential microsymbiont.

Thus, we would suggest that reports on secondary metabolites found in sponges should be accompanied preferably by a short descriptive account of its habit and/or anatomical features given by the identifier. Such a description would make it possible to judge from the literature whether or not an identification would need to be verified by re-examination of the vouchers. At the very least, the name of the sponge involved should be accompanied by that of the identifier, the institution where the voucher specimens are kept and, if relevant, their collection number.

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