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Sponge secondary metabolites: new results

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Abstract

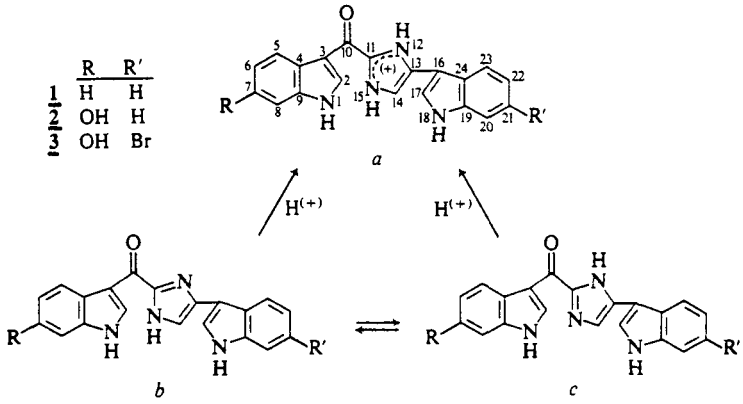
The methanolic extract of several marine sponges (Topsentia genitrix, Dysidea tupha, Acanthella acuta, Didiscus oxeata and two Agelas species) have been found to be ichthyotoxic. The metabolites responsible for this toxicity have been isolated and their structures determined on the basis of their chemical and spectral properties. The total synthesis of some of these metabolites is presented.

Among marine invertebrates, sponges appear to be one of the richest phyla in toxicogenic species. In an increasing number of cases it has been demonstrated that this toxicity is associated with the presence of specific secondary metabolites which may act to minimize predation by mobile animals and (or) to serve as weapons in spatial competition [1,2]. During the last few years our laboratory, in collaboration with spongologists, has been involved in a research program whose objective is to isolate and identify sponge toxic metabolites and to evaluate their role in the defense mechanism of these animals, as well as their use as chemotaxonomic markers. To this end about 100 different species of sponges have been collected in the Mediterranean sea and more recently in the Southern Caribbean. They have been extracted and their extracts evaluated for their ichthyo- and spongiotoxicities. Several species have been found to be toxic and we will present here some of the recent chemical results we have obtained.

TRYPTOPHAN DERIVATIVES FROM *Topsentia genitrix*

A large specimen of Topsentia genitrix, a rarely encountered bright yellow Mediterranean sponge, was collected near Banyuls (France) by Scuba diving. The toxic methanolic extract contained 3 major derivatives named topsentin-A, -B1 and -B2[3]. These derivatives which are responsible, at least in part, for the ichthyotoxicity of the extract (LD for Lebistes reticulatus ◻ 15 mg/L), could be only efficiently separated by droplet counter current chromatography, followed by flash chromatography on silica gel.

The spectral properties of topsentin-A ($C_{20}H_{14}N_4O$ by HRMS), the most abundant derivative, clearly indicate that it has an aromatic skeleton having no sp^3 carbon atom. Although the behaviour of topsentin-A, both in tlc and hplc, suggests that it is a pure compound, its ^1H NMR spectrum recorded in CD_3COCD_3 shows 28 protons (2 x 14 H) attributable to a mixture of two closely related derivatives in a ratio 60 : 40.



Four peaks amounting to a total of 6 H (3 H per derivative) disappear on treatment with D_2O, and the addition of a few drops of CF_3COOD induces a dramatic simplification of the spectrum, which under these conditions shows signals integrating for 11 protons only. Such behaviour is reminiscent of the existence of two slowly interconverting isomers whose protonated forms are identical. HRMS measurements of characteristic fragment ions, together with an extensive NMR study including homo- and hetero- correlated 2D-NMR spectra and SPI experiments, led to propose structure 1 for topsentin-A. The presence of an imidazole ring explains its behaviour in ^1H NMR. Indeed, in neutral solution 1 exists as a mixture of two slowly interconverting tautomers which on protonation by CF_3COOD yield the same imidazolium cation 1a.

Structure 1 has been confirmed by synthesis of topsentin-A starting from 3-acetylindole (figure 1)[4]. This synthesis is based on the rearrangement[5] of 1,1-dimethyl-1-phenacylhydrazinium bromide (4) into 2-[phenyl-1H-imidazol-4-yl] phenylmethanone (5). The formation of 5 can be best rationalized in terms of a N to N migration of the phenacyl to give 1,1-dimethyl-2-phenacylhydrazine (6) and subsequent decomposition of 6 into dimethylamine and phenylglyoxaldimine (7), followed by dehydrative self-condensation of the latter. It is obvious, that such a rearrangement performed on 8, in which the phenyl group is replaced by a 3-indolyl moiety, should lead to topsentin-A. Indeed, a solution of 8 in n-propanol heated to reflux in the dark yielded a compound identical in all respects with the natural derivative (yield : 27 %). Compound 8 could be obtained with a good yield (82 %) by treatment of 3-bromoacetylindole with 1,1-dimethylhydrazine, while 3-bromoacetylindole was synthetized by direct bromination of 3-acetylindole.

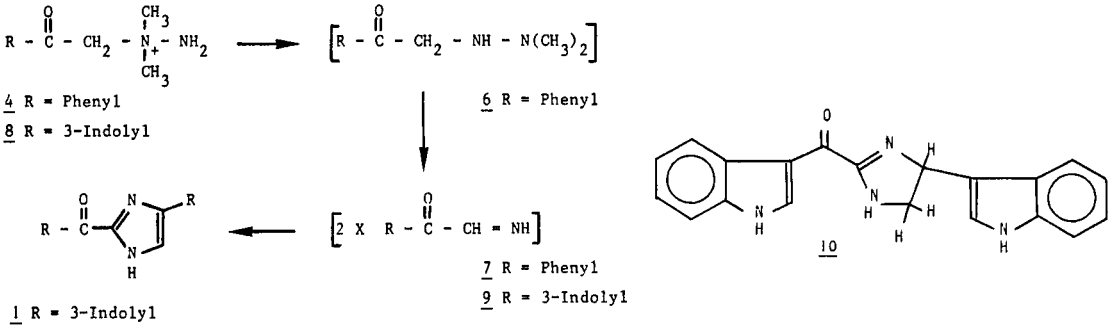


Figure 1 : Synthesis of topsentin-A

The spectroscopic properties of topsentin-B1 and -B2 are closely related to those of topsentin-A and their comparison indicates that topsentin-B1 is the corresponding 7-hydroxy derivative 2 while topsentin-B2 is the 21-bromo-7-hydroxy derivative 3. The positioning of the substituents on the indole rings rests mainly on NMR data and on the shifts to higher masses observed for characteristic fragment ions in mass spectrometry. In CD_3COCD_3, topsentin-B1 and -B2 behave as topsentin-A, existing as a mixture of two tautomeric forms ($\sim$ 60:40) in slow interconversion.

More recently, a fourth derivative, topsentin-D, was isolated from the methanolic extract of T. genitrix. This derivative is sensitive to air and is transformed into topsentin-A on standing. The EI.MS of topsentin-D shows an intense molecular ion at m/z 328 and high resolution measurements provide the molecular formula $C_{20}H_{16}N_4O$ indicating that it is a dihydro derivative of topsentin-A. This is corroborated by the presence : a) in the ^{13}C NMR spectrum, besides the signals expected for the two indole chromophores, of signals at δ 59.3 (CH) and 58.6 (CH_2), b) in the ^1H NMR of three 1H double doublets at δ 5.41, 4.25 and 3.87 respectively, assignable to a CH_2-CH group. All these data strongly support structure 10 for topsentin-D.

From a biogenetic point of view, the topsentins most probably derive from the condensation of two tryptamine units. In figure 1, the α-ketoimine 9 is assumed to be the key intermediate which dimerizes to form the imidazole ring[4]. By analogy such an intermediate, or at least a derivative having an analogous oxidation stage and derived from tryptophan,

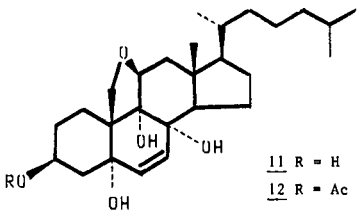
may be the precursor of the topsentins. It is interesting to mention that a similar reaction is proposed to explain the cross linking of proteins by glucose, leading to the formation of brown fluorescent pigments[6]. This reaction is thought to be implicated in the protein changes noted to occur during ageing, as well as in the appearance of brown pigments during the cooking of food (Maillard reaction).

A further striking feature of this interesting sponge is the presence, in the most polar fractions of the methanolic extract, of an important amount (0.45 % dry weight) of 3-methyladenine identified on the basis of its spectral properties[7]. This identification was further proved by direct comparison with an authentic sample prepared by methylation of adenine with methyl iodide in dimethylformamide. As far as we know, 3-methyladenine has never been reported from natural sources as a free base. But, 3-methyladenine-DNA and -RNA are formed when cells or cell extracts are treated with methylating agents[8].

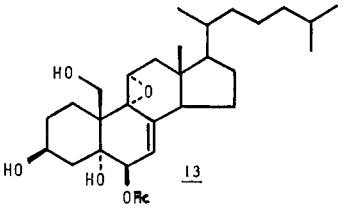
A POLYOXYGENATED STEROID FROM *Dysidea tupha*

For a sample of D. tupha, collected also near Banyuls, the toxicity was found to be located in the dichloromethane-soluble fraction of the methanol extract[9]. Successive chromatographies of this fraction monitored by testing the toxicity, led to the isolation of a subfraction, the major compound of which was found to be a polyoxygenated steroid (LD = 20 mg/L). Its structure (11) was deduced from the spectral properties of its monoacetate derivative (12). Polyoxygenated steroids are frequently encountered within many classes of marine invertebrates but rarely in sponges[10,11]. Moreover, oxidation at C-19, C-11 and C-9 is unusual and this rare pattern of oxidation has only been encountered in a steroid (13) isolated [12] from an undetermined Dysidea sp from Guam.

Species of the Dysidea have proved to be remarkably consistent in producing furanoterpenes and/or terpenoid (hydro)quinones derived from the linear isoprenoid precursors 14 and 15 by different cyclizations[10,13]. Two such terpenoids have been recently reported from a specimen of D. tupha also collected around Banyuls [14]. These terpenoids have been considered to be reliable chemotaxonomic markers for the genus[13]. It would be interesting to investigate if the rare pattern of oxidation found in 11 and 13 can be considered as a characteristic sterol oxidation process of the Dysidea and thus as a further taxonomic marker for the genus.



11 R = H
12 R = Ac



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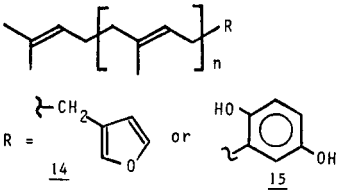


14 15

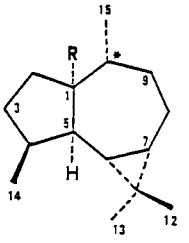
ISONITRILE SESQUITERPENES FROM *Acanthella acuta*

A. acuta is a bright orange sponge commonly found inside semi-dark caves of the Mediterranean sea. The toxicity of this sponge was found to be associated with a non polar fraction from which two major isonitrile sesquiterpenes were isolated[15]. One was found to be identical with axisonitrile-3 a derivative already known from Axinella cannabina[16]. The structure of 1-isocyanoaromadendrane(16) was established on the basis of its spectral properties and by chemical correlation with palustrol (17), a compound we had isolated a few years ago from the soft coral Cespitularia sp aff subviridis[17]. Two further aromadendrane derivatives were isolated : 1-isocyanatearomadendrane (18) and 1-isothiocyanatearomadendrane (19). Interestingly, these two latter derivatives, in contrast with the isonitrile 16 (LD = 30 mg/L), are not toxic for the fish Lebistes reticulatus suggesting that they could be the result of a detoxification process. Two other chemical analyses of A. acuta have been reported elsewhere[18,19]. In each case a mixture of sesquiterpene isonitriles and related derivatives have been evidenced, but these mixtures differ greatly in the nature and proportions of their constituents. Such intraspecific variations are not uncommon in sponges but their biological significance is not yet clear and it is still not known if these are due to individual, geographical or seasonal variations.

SESQUITERPENE PHENOLS FROM *Didiscus oxeata* AND ORNITHINE DERIVATIVES FROM *Agelas* SPECIES

The methanolic extract of several sponges collected in the Southern Caribbean have been found to be toxic for Lebistes reticulatus. As a result, two toxic sesquiterpene phenols : (+)curcuphenol and (+)curcudiol were isolated from Didiscus oxeata[20], and two toxic ornithine derivatives namely sceptrin[21] and hymenidin[22] were isolated from Agelas conifera and Agelas clathrodes respectively. All these compounds are already known sponge derivatives which were identified by comparison of their spectral properties with literature data.

In conclusion, these examples are a clear illustration of the great structural diversity of sponge toxins. This diversity must reflect the fact that chemical defense mechanisms have independently evolved several times in these primitive and sessile animals during evolution, due to the extreme diversity of predators and competitors that they had and still have to face.



17 R = -OH
16 R = -N=Cl
18 R = -NCO
19 R = -NCS

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