Nucleosides from the Marine Sponge *Haliclona* sp.

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Three known nucleosides were isolated from the sponge *Haliclona* sp. The structures were established on the basis of NMR data and comparison with those reported, and chemotaxonomic relationships of the sponge nucleosides were discussed.

Key words: Marine Sponge, *Haliclona*, Nucleoside

Introduction

Sponges (phylum Porifera) are sessile marine filter feeders that have developed efficient defense mechanisms against foreign attackers such as viruses, bacteria, or eukaryotic organisms. Protected by a highly complex immune system, as well as by the capacity to produce efficient antiviral compounds (*e.g.*, nucleoside analogues), antimicrobial compounds (*e.g.*, polyketides), and cytostatic compounds (*e.g.*, avarol), they have not become extinct during the last 600 million years. It can be assumed that during this long period of time, bacteria and microorganisms coevolved with sponges, and thus acquired a complex common metabolism.

Marine sponges are a promising source of diverse chemical metabolites. Marine sponges belonging to the genus *Haliclona* have been the subject of extensive chemical studies (Blunt *et al.*, 2008; Faulkner, 2002). Recent investigations on *Haliclona* species have led to the isolation of alkaloids, macrolides, polyacetylenes, polyketides, steroids, and peptides (Aoki *et al.*, 2004; de Jesus and Faulkner, 2003; Erickson *et al.*, 1997; Fu *et al.*, 1999; Sperry *et al.*, 1998; Teruya *et al.*, 2006).

Results and Discussion

In our investigation, nucleosides **1–3** were isolated from the sponge *Haliclona* sp. They were identified as thymidine (**1**), adenosine (**2**), and 2′-deoxy-guanosine (**3**) by comparison of their spectral data with those reported (Cimino *et al.*, 1984; Kicha *et al.*, 1995; Komori *et al.*, 1980; Luyten *et al.*, 1997) (Fig. 1). The possibility that these nucleosides are the result of degradation of DNA during the work-up can be discarded because the resistance of DNA to selective hydrolysis is well known (Murray *et al.*, 2002). Marine nucleosides display antiviral, anticancer, vasodilator, muscle relaxant, and hypertensive activities. So far marine sponges have been the best source of nucleosides.

Sponges are divided into four classes: Demospongiae, Hyalospongiae, Calcispongiae, and Sclerospongiae. Demosponges are the most widespread and advanced class of sponges, as well as the largest and most diverse one. Some 90 to 95% of all sponge species belong to this class which is classified into three subclasses, the Homoscleromorpha, Tetractinomorpha, and Ceractinomorpha.

To the best of our knowledge, nucleosides have been reported from several species of sponges, *e.g.* from *Dysidea* sp. (family Dysideidae, order Dictyoceratida; Diaz-Marrero *et al.*, 2006; Shao *et al.*, 2004), *Isodictya erinacea* (family Isodictyidae, order Poecilosclerida; Moon *et al.*, 1998), *Aplysina* sp. (family Aplysinidae, order Verongida; Kondo *et al.*, 1992), *Tedania digitata* (family Tedaniidae, order Poecilosclerida; Bairdlambert *et al.*, 1980; Cook *et al.*, 1980; Goya and Martinez, 1988; Qui-

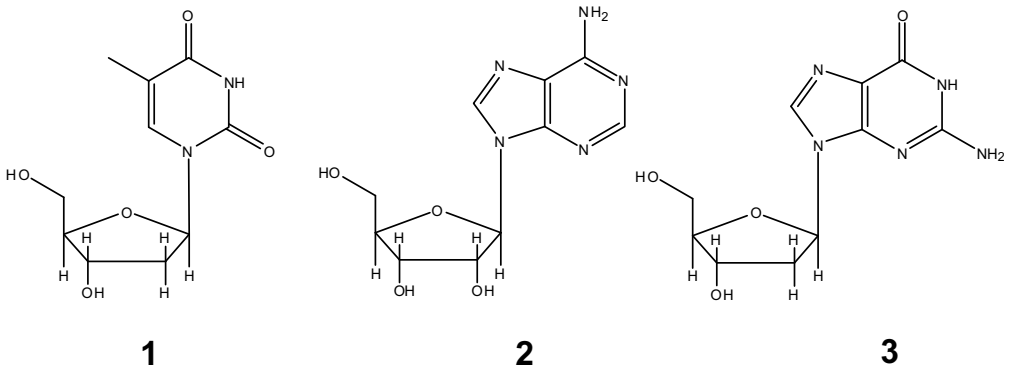

Fig. 1. Chemical structures of thymidine (**1**), adenosine (**2**) and 2'-deoxy guanosine (**3**).

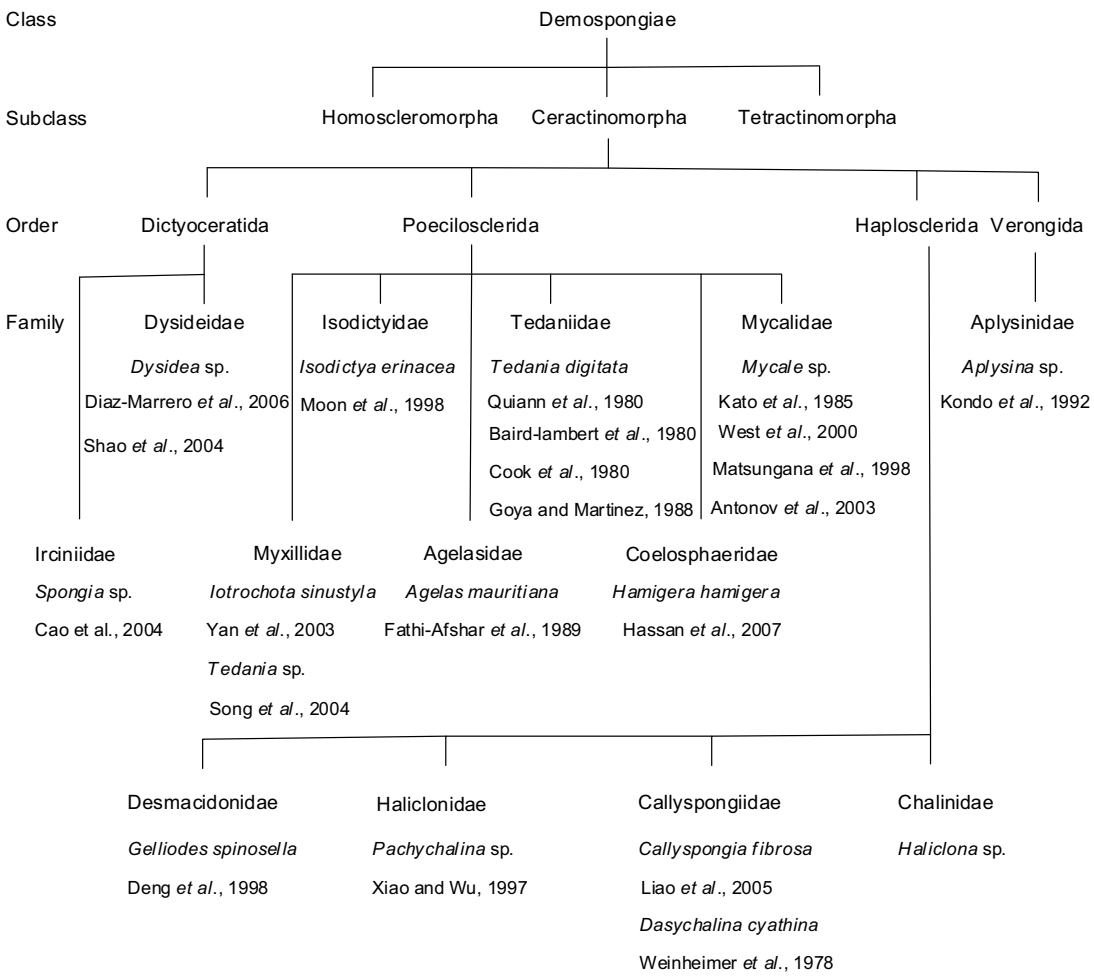

Fig. 2. Chemotaxonomic relationships of sponge nucleosides.

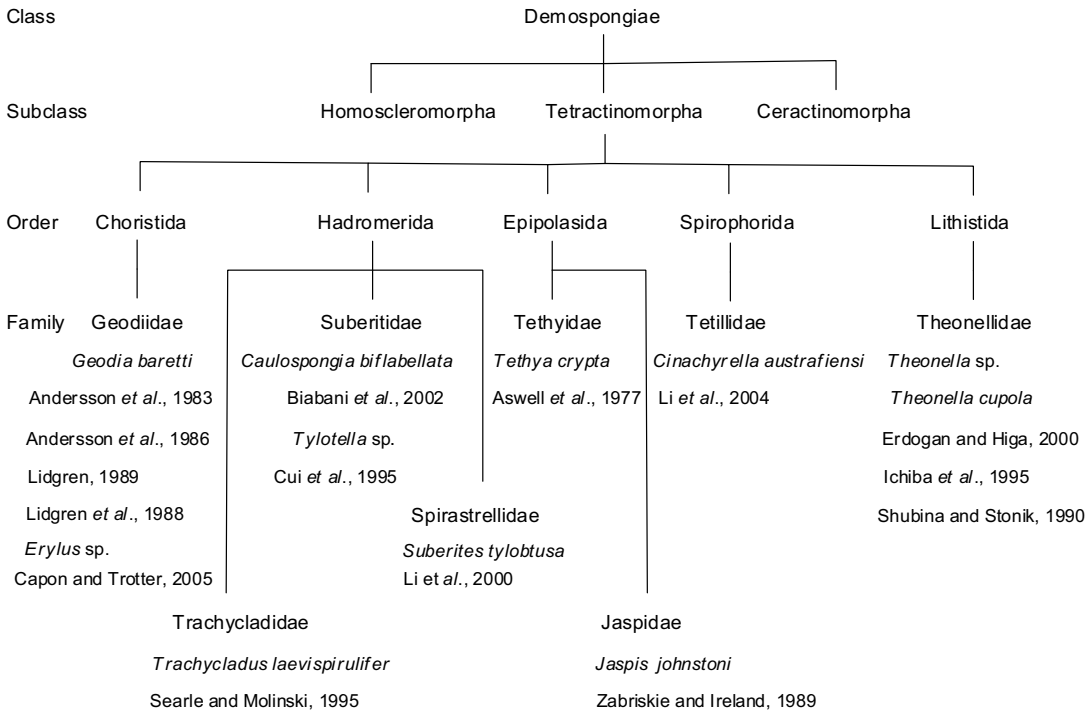

Fig. 3. Chemotaxonomic relationships of sponge nucleosides.

ann *et al.*, 1980; Tao *et al.*, 1993), *Mycale* sp. (family Mycalidae, order Poecilosclerida; Antonov *et al.*, 2003; Kato *et al.*, 1985; Matsunaga *et al.*, 1998; West *et al.*, 2000), *Dasychalina cyathina* (family Callyspongiidae, order Haplosclerida; Weinheimer *et al.*, 1978), *Hamigera hamigera* (family Coelosphaeridae, order Poecilosclerida; Hassan *et al.*, 2007), *Agelas mauritiana* (family Agelasidae, order Poecilosclerida; Fathi-Afshar *et al.*, 1989), *Spongia* sp. (family Irciniidae, order Dictyoceratida; Cao *et al.*, 2004), *Gelliodes spinosella* Thielle (family Desmacidonidae, order Haplosclerida; Deng *et al.*, 1998), *Callyspongia fibrosa* (family Callyspongiidae, order Haplosclerida; Liao *et al.*, 2005), *Tedania* sp. (family Myxillidae, order Poecilosclerida; Song *et al.*, 2004), *Pachychalina* sp. (family Haliclonidae, order Haplosclerida; Xiao and Wu, 1997), and *Iotrochota sinustyla* (family Myxillidae, order Poecilosclerida; Yan *et al.*, 2003), respectively. The three compounds isolated from *Haliclona* sp. belong to the family Chalinidae, order Haplosclerida. The orders Dictyoceratida, Poecilosclerida, Verongida, and Haplosclerida belong to the same subclass, namely Ceractinomorpha (class Demospongiae) (Fig. 2).

Nucleosides have also been reported from *Tethya crypta* (family Tethyidae, order Epipolasida; Aswell *et al.*, 1977), Geodia baretti (family Geodiidae, order Choristida; Andersson *et al.*, 1983, 1986; Lidgren, 1989; Lidgren *et al.*, 1988), *Caulospongia biflabellata* (family Suberitidae, order Hadromerida; Biabani *et al.*, 2002), *Erylus* sp. (family Geodiidae, order Choristida; Capon and Trotter, 2005), *Jaspis johnstoni* (family Jaspidae, order Epipolasida; Zabriskie and Ireland, 1989), *Theonella* sp. and *Theonella cupola* (family Theonellidae, order Lithistida; Erdogan and Higa, 2000; Ichiba *et al.*, 1995; Shubina and Stonik, 1990), *Trachycladus laevispirulifer* (family Trachycladidae, order Hadromerida; Searle and Molinski, 1995), *Tylotella* sp. (family Suberitidae, order Hadromerida; Cui *et al.*, 1995), *Cinachyrella austrafiensis* (family Tetillidae, order Spirophorida; Li *et al.*, 2004), and *Suberites tylobtusa* Levi (family Spirastrellidae, order Hadromerida; Li *et al.*, 2000). The orders Epipolasida, Choristida, Lithistida, Spirophorida, and Hadromerida belong to the same subclass, namely Tetractinomorpha (class Demospongiae) (Fig. 3).

There have been numerous reports on the isolation of nucleosides from marine sponges of the subclasses Tetractinomorpha and Ceractinomorpha. The sponge *Haliclona* sp. is classified under the subclass Ceractinomorpha, which is taxonomically distant from the subclass Tetractinomorpha. We may conclude that the presence of nucleosides has been the result of independent evolution in these species and is of great importance in the context of chemotaxonomical relationship between sponges of two different subclasses showing presence of the same class of compounds.

Experimental

General experimental procedures

^{1}H and ^{13}C NMR spectra were recorded on a Bruker AC-500 spectrometer. Chemical shifts were reported with reference to the respective residual solvent peaks (δ_H 2.49 and δ_C 40.0 for DMSO-d_6).

Animal material

The sponge was collected by hand in July 2005, off the coast of Hainan Island, China. The specimen was identified as *Haliclona* sp. by Dr. Kyung Jin Lee. A voucher specimen (0507003) was deposited in the Natural History Museum, Hannam University, Daejon, Korea and Guangdong Key Laboratory of Marine Materia Medica, South China Sea Institute of Oceanology, Guangzhou, China.

Extraction and isolation

The sponge (20 kg) was extracted with EtOH at room temperature. The EtOH extract was partitioned between $CHCl_3$ and water. The aqueous layer was further partitioned between water and *n*-BuOH. The *n*-BuOH fraction was subjected to reversed-phase flash column chromatography (YMC Gel ODS-A, 60 Å, 230 mesh) eluted with a stepped gradient solvent system of 10 → 80% EtOH/H_2O to afford 24 fractions. Fraction B3 (6.2 g, desalinated) was separated by silica gel flash column chromatography eluted with a gradient of 5 → 30% MeOH in $CHCl_3$, affording 22 subfractions. Fractions B3–5 (640 mg), B3–7 (150 mg), and B3–10 (215.5 mg) were separated by repeated silica gel flash column chromatography using a gradient of 0 → 20% MeOH in $CHCl_3$. Compounds **1** (24.9 mg), **2** (6.1 mg), and **3** (2.1 mg) were obtained in subfractions B3–5–9, B3–7–5, and B3–10–5, respectively.

Thymidine (**1**): Colourless crystals. – ^{1}H NMR (500 MHz, DMSO-d_6): δ = 11.26 (1H, s, 1-NH), 7.96 (1H, s, H-4), 6.16 (1H, t, H-1′, J = 6.5 Hz), 5.22 (1H, d, 3′-OH, J = 4.2 Hz), 5.01 (1H, d, 5′-OH, J = 5.2 Hz), 4.23 (1H, m, H-4′), 3.76 (1H, q, H-3′, J = 3.6, 6.8 Hz), 3.56 (2H, m, H-5′), 2.10 (2H, m, H-2′), 1.76 (3H, d, 5-CH_3). – ^{13}C NMR (125 MHz, DMSO-d_6): δ = 163.5 (C-6), 150.3 (C-2), 135.9 (C-4), 109.2 (C-5), 87.1 (C-1′), 83.6 (C-4′), 70.3 (C-3′), 61.2 (C-5′), 39.4 (C-2′), 12.0 (5-CH_3). –ESIMS (negative mode): m/z = 241 $[M–H]^-$.

Adenosine (**2**): White solid. – ^{1}H NMR (500 MHz, DMSO-d_6): δ = 8.34 (1H, s, H-8), 8.10 (1H, s, H-2), 7.35 (2H, s, 6-NH_2), 5.87 (1H, d, H-1′, J = 6.2 Hz), 4.60 (1H, m, H-3′), 4.14 (1H, m, H-4′), 3.96 (1H, m, H-2′a), 3.67(1H, m, H-2′b), 3.55 (1H, m, H-5′). – ^{13}C NMR (125 MHz, DMSO-d_6): δ = 156.1 (C-6), 152.3 (C-2), 149.0 (C-4), 139.8 (C-8), 119.3 (C-5), 87.8 (C-1′), 85.8 (C-4′), 73.3 (C-3′), 70.5 (C-2′), 61.6 (C-5′).

2′-Deoxy-guanosine (**3**): White solid. – ^{1}H NMR (500 MHz, DMSO-d_6): δ = 7.91 (1H, s, H-8), 6.47 (2H, s, 2-NH_2), 6.11 (1H, dd, H-1′, J = 6.5, 7.7 Hz), 4.34 (1H, m, H-4′), 3.80 (1H, m, H-3′), 3.55 (1H, m, H-5′a), 3.51 (1H, m, H-5′b), 2.49 (1H, m, H-2′a), 2.20 (1H, m, H-2′b). – ^{13}C NMR (125 MHz, DMSO-d_6): δ = 156.8 (C-6), 153.6 (C-2), 150.9 (C-4), 135.4 (C-8), 116.75 (C-5), 87.6 (C-1′), 82.7 (C-4′), 70.8 (C-3′), 61.7 (C-5′), 39.5 (C-2′).

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