

Review of saponin diversity in sea cucumbers belonging to the family Holothuriidae

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Abstract

Saponins are secondary metabolites produced by holothurians. Structurally, they are triterpene glycosides that play an important role in chemical defense and possess a wide spectrum of pharmacological effects. This review highlights the very high diversity of saponins detected in different species of the family Holothuriidae. No less than 59 triterpene glycosides are reported. Several saponins are shared by many species but others are very specific. Overall, most species appear to possess a specific congener mixture. The most evident inter-specific differences that can be highlighted among Holothuriidae are based on the presence or absence of a sulfate group attached to the carbohydrate chain of their saponins. Within a single animal, saponin mixtures also present different concentrations and compositions depending on the organ, with Cuvierian tubules showing the highest saponin concentrations. All of the data combined indicate a complex chemical defence mechanism with different sets of saponins originating from different body compartments and presenting different properties in relation to their ecological role(s).

Introduction

Saponins are an important class of natural products first discovered in higher plants where they are widely spread (Li et al. 2006). In the search for new pharmacologically active substances, saponins have also been isolated from marine organisms such as holothurians (Nigrelli 1952; Yamanouchi 1955), seastars (Mackie and Turner 1970) and sponges (Thompson et al. 1985). Structurally, holothurian saponins are described as triterpene glycosides composed of an oligosaccharide chain and an aglycone based on holostane-3b-ol (Fig. 1A) (Kornprobst 2005). Saponins of Holothuriidae (Fig. 1B,C) contain a D⁹⁽¹¹⁾ double bound in the aglycone and the carbohydrate chain encloses up to 6 sugars units, including xylose, glucose, 3-O-methylglucose and quinovose, and can be branched only once (Kalinin et al. 2005). Some of these saponins can be sulfated at the level of the sole xylose (Fig. 1C).

Holothurian triterpene glycosides present a high scientific interest in pharmacology and ecology. Indeed, these secondary metabolites have been reported to possess a wide spectrum of pharmacological effects including hemolytic, antitumoral, anti-inflammatory, antifungal, anti-bacterial, antiviral, ichthyotoxic, cytostatic and antineoplastic activities (Kerr and Chen 1995; Kalinin et al. 1996a, 1996b; Prokofieva et al. 2003). Many of these activities are the result of their tensioactive properties. In

ecology, saponins are deleterious for most organisms and probably function as a chemical defense to deter predation (Kalinin et al. 1996a, b; Van Dyck et al. unpubl. obs.).

Saponin diversity in Holothuriidae

This article reviews the diversity of saponins detected in different species of the family Holothuriidae. Table 1 presents all the saponins extracted and characterized from holothurians of the genera *Actinopyga*, *Bohadschia*, *Holothuria* and *Pearsonothuria* during the last 40 years. Saponins detailed in this table have been purified by different methods including liquid-liquid extractions with different solvents, solid phase extraction or chromatography (silica gel or resins), and high performance liquid chromatography. Mass spectrometry-based techniques and nuclear magnetic resonance combined with chemical reactions and chemical evidence were used to highlight the chemical structure of these saponins. Table 1 emphasizes the very high diversity of saponins in holothuriids. Indeed, no less than 59 triterpene glycosides are reported. When these data are gathered from the literature, some nomenclature problems can be identified. It happens that two names have been given independently to the same molecule. For instance, the structure of nobiliside 2a detailed by Wu et al. (2006c) corresponds exactly to desholothurin A described by Rodriguez et al. (1991). Also, authors should homogenize the

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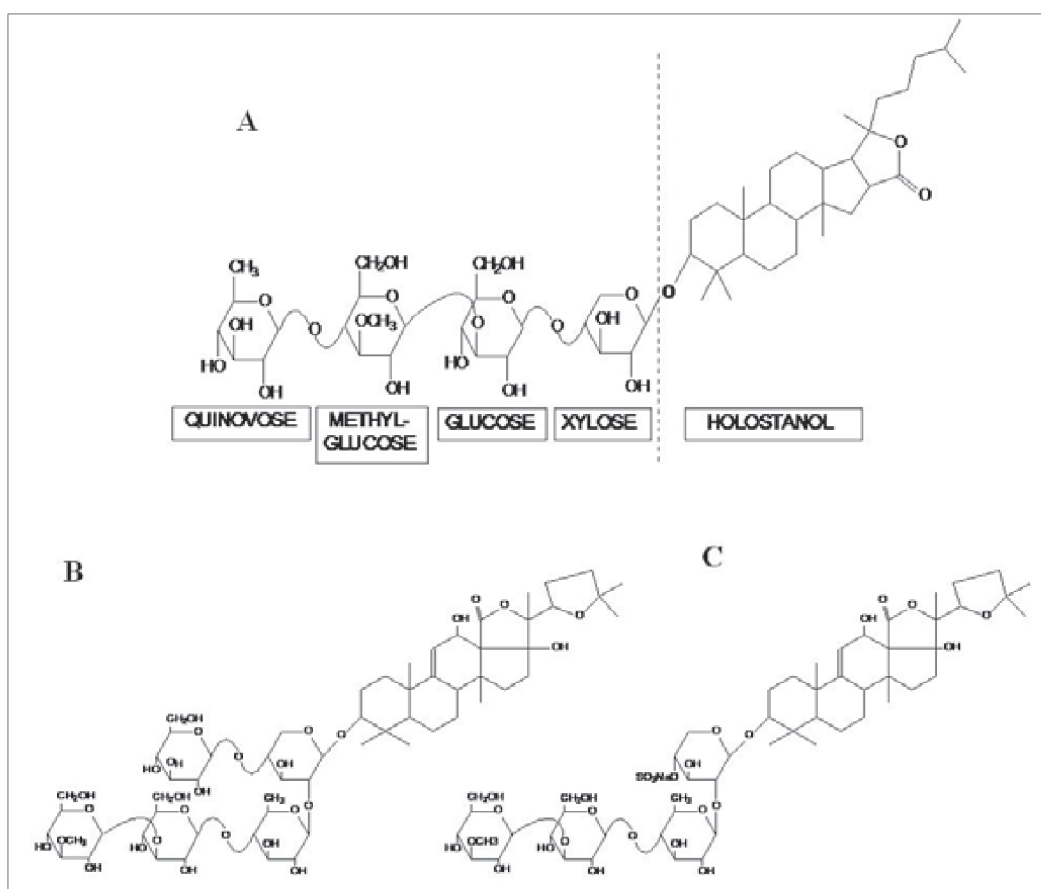


Figure 1. Molecular structure of (A) a hypothetical saponin composed of an aglycone of holostanol (according to the Comparative Toxicogenomic Database) and a linear glycosidic chain constituted by the four most frequent monosaccharides found in holothurian saponins; (B) holothurinoside A, a non-sulfated saponin; and (C) holothurin A, a sulfated saponin.

saponin nomenclature by giving logical names to new molecules, based on the structure of known congeners, rather than names based on the specific origin of the molecules.

The most evident inter-specific differences that can be highlighted among Holothuriidae are based on the presence or absence of sulfate group attached to the carbohydrate chain of their saponins (Kobayashi et al. 1991). The genus *Actinopyga* contains only sulfated saponins (in green in Table 1), the genus *Bohadschia* encloses only non-sulfated ones (in red in Table 1), and the genera *Pearsonothuria* and *Holothuria* present both saponin types. In this last genus, the situation is even more complex with several species containing only sulfated saponins, some others presenting the two types of congeners, and finally one species, *H. forskali*, enclosing exclusively non-sulfated saponin. Several saponins are shared by a lot of species, holothurins A and B for example, but others are very specific like griseaside A or argusides A-E. It must be noted that holothurins A and B were the first to be discovered (Yamanouchi 1955; Kitagawa et al. 1978, 1979) and were subsequently detected in many species of the genus *Holothuria*

(Elyakov et al. 1973, 1975). In the future, one should expect that new studies, using contemporary techniques, will detect additional saponins in these species. Indeed, Table 1 clearly shows that many new congeners have been described only recently. Most species therefore appear to possess a specific congener mixture, a valuable chemotaxonomic character allowing the assignment of a holothuriid species to a specific taxa according to its chemical signature. For example, the taxonomic position of *Bohadschia graeffei* was revised, following the isolation and characterization of its saponins, to a newly established genus *Pearsonothuria graeffei* (Kalinin et al. 2005).

Among the numerous studies dealing with saponins from holothuroids, very few make the distinction between their different body compartments (Table 1) although, within an animal, saponins may present different concentrations and compositions according to the organ considered. Matsuno and Ishida (1969) reported about the distribution of saponins in sea cucumber body compartments. Saponins were found in the digestive organs, the longitudinal retractor muscles, the epidermis, the

Table 1. Saponins of Holothuriidae by species.

Species	Saponins	BW	CT	References
<i>Actinopyga agassizi</i>	24-dehydroholothurin A ₁	x	x	Kitagawa et al. (1982)
	Holothurin A	x	x	Kitagawa et al. (1982)
	Holothurin A ₁			Kitagawa et al. (1980)
	Holothurin B			Elyakov et al. (1975)
	Holothurin B ₁			Kitagawa et al. (1980)
<i>Actinopyga echinites</i>	Fuscocineroside B/C*	x	x	Chapter 2
	Holothurin A	x	x	Elyakov et al. (1973)
	Holothurin A ₁	x	x	Kitagawa et al. (1980)
	Holothurin B			Elyakov et al. (1973)
	Holothurin B ₁ /B ₂ *			Chapter 2
	Holothurin B ₁	x	x	Kitagawa et al. (1980)
	Holothurin B ₂	x	x	Chapter 2
	Holothurin B ₃			Chapter 2
<i>Actinopyga flammea</i>	Holothurin A			Bhatnagar et al. (1985)
	Holothurin B			Bhatnagar et al. (1985)
<i>Actinopyga lecanora</i>	Holothurin A			Elyakov et al. (1973)
	Holothurin B			Elyakov et al. (1973)
<i>Actinopyga mauritana</i>	24-dehydroholothurin A ₁	x	x	Kobayashi et al. (1991)
	24-dehydroholothurin B ₁	x	x	Kobayashi et al. (1991)
	Holothurin A			Elyakov et al. (1973)
	Holothurin A ₁	x	x	Kobayashi et al. (1991)
	Holothurin B	x	x	Elyakov et al. (1973)
	Holothurin B ₁	x	x	Kobayashi et al. (1991)
<i>Actinopyga miliaris</i>	Holothurin A			Elyakov et al. (1973)
	Holothurin B			Elyakov et al. (1973)
<i>Bohadschia argus</i>	Arquaside A			Liu et al. (2007)
	Arquaside B			Liu et al. (2008a)
	Arquaside C	x	x	Liu et al. (2008a)
	Arquaside D	x	x	Liu et al. (2008b)
	Arquaside E (Desholothurin A ₁)**			Liu et al. (2008b)
<i>Bohadschia bivittata</i>	Bivittoside A	x	x	Ohta and Hikino (1981)
	Bivittoside B	x	x	Ohta and Hikino (1981)
	Bivittoside C	x	x	Ohta and Hikino (1981)
	Bivittoside D	x	x	Ohta and Hikino (1981)
<i>Bohadschia marmorata</i>	17-hydroxy impatienside A			Yuan et al. (2009)
	25-acetoxy bivittoside D			Yuan et al. (2009)
	Bivittoside C			Yuan et al. (2009)
	Bivittoside D			Yuan et al. (2009)
	Marmoratoside A (Impatienside A)**			Yuan et al. (2009)
	Marmoratoside B/Holothurinoside H*			Yuan et al. (2009)
<i>Bohadschia subrubra</i>	Arquaside C	x	x	Chapter 2
	Bivittoside B	x	x	Chapter 2
	Bivittoside C	x	x	Chapter 2
	Bivittoside D	x	x	Chapter 2
	Impatienside A (Marmoratoside A)**	x	x	Chapter 2
Species	Saponins	BW	CT	References
<i>Bohadschia subrubra</i>	Holothurinoside F	x	x	Chapter 2
(Continued)	Holothurinoside H/Marmoratoside B*	x	x	Chapter 2
	Holothurinoside H ₁	x	x	Chapter 2
	Holothurinoside I	x	x	Chapter 2
	Holothurinoside I ₁	x	x	Chapter 2
	Holothurinoside J ₁	x	x	Chapter 2
	Holothurinoside K ₁	x	x	Chapter 2
<i>Bohadschia tenuissima</i>	Bivittoside C			Radhika et al. (2002)
	Bivittoside D	x		Radhika et al. (2002)
<i>Bohadschia vitiensis</i>	Bivittoside C			Radhika et al. (2002)
	Bivittoside D			Radhika et al. (2002)
<i>Holothuria arenicola</i>	Holothurin A			Elyakov et al. (1973)
	Holothurin B			Elyakov et al. (1973)
<i>Holothuria atra</i>	Holothurin A			Kobayashi et al. (1991)
	Holothurin A ₁			Kobayashi et al. (1991)
	Holothurin B	x		Kobayashi et al. (1991)
	Holothurin B ₁ /B ₂ *			Chapter 2
	Holothurin B ₁	x		Kobayashi et al. (1991)
	Holothurin B ₂	x		Chapter 2
	Holothurin B ₃	x		Chapter 2
<i>Holothuria axilloqua</i> (<i>Microthele</i>)	Axilogoside A			Yuan et al. (2008)
	Holothurin A			Kobayashi et al. (1991)
	Holothurin A ₁			Kobayashi et al. (1991)
	Holothurin B			Yuan et al. (2008)
<i>Holothuria cinerascens</i>	Holothurin A			Elyakov et al. (1973)
<i>Holothuria coluber</i>	Holothurin A			Elyakov et al. (1973)
	Holothurin B			Elyakov et al. (1973)
<i>Holothuria cubana</i>	Holothurin A			Elyakov et al. (1975)
	Holothurin B			Elyakov et al. (1975)
<i>Holothuria difficilis</i>	Holothurin A			Elyakov et al. (1973)
	Holothurin B			Elyakov et al. (1973)
<i>Holothuria edulis</i>	Holothurin A	x		Kobayashi et al. (1991)
	Holothurin A ₁			Stonik (1986)
	Holothurin B			Stonik (1986)
<i>Holothuria floridana</i>	Holothurin A ₁			Stonik (1986)
	Holothurin A ₂			Stonik (1986)
	Holothurin B ₁			Elyakov et al. (1982)
<i>Holothuria forskali</i>	Desholothurin A (Nobiliside 2a)**	x	x	Rodriguez et al. (1991)
	Desholothurin A ₁ (Arquaside E)**			Chapter 1
	Holothurinoside A	x	x	Rodriguez et al. (1991)
	Holothurinoside A ₁	x	x	Chapter 1
	Holothurinoside B	x	x	Rodriguez et al. (1991)
	Holothurinoside C	x	x	Rodriguez et al. (1991)
	Holothurinoside C ₁			Chapter 1
	Holothurinoside D	x	x	Rodriguez et al. (1991)

Table 1 (continued)

Species	Saponins	BW	CT	References
<i>Holothuria forskali</i>	Holothurinoside E	x	x	Chapter 1
	Holothurinoside E ₁		x	Chapter 1
	Holothurinoside F	x	x	Chapter 1
	Holothurinoside F ₁		x	Chapter 1
	Holothurinoside G	x	x	Chapter 1
	Holothurinoside G ₁		x	Chapter 1
	Holothurinoside H/Marmoratoside B*	x	x	Chapter 1
	Holothurinoside H ₁		x	Chapter 1
	Holothurinoside I	x	x	Chapter 1
	Holothurinoside I ₁		x	Chapter 1
	Holothurinoside L***			Chapter 4
	Holothurinoside M***			Chapter 4
<i>Holothuria fuscocinerea</i>	Holothurin A			Zhang et al. (2006)
	Holothurin B			Elyakov et al. (1973)
	Fuscocineroside A			Zhang et al. (2006)
	Fuscocineroside B			Zhang et al. (2006)
	Fuscocineroside C			Zhang et al. (2006)
	Pervicoside C			Zhang et al. (2006)
<i>Holothuria gracilis</i>	Holothurin A			Elyakov et al. (1973)
	Holothurin B			Elyakov et al. (1973)
<i>Holothuria grisea</i>	17-dehydroxyholothurinoside A			Sun et al. (2008)
	Griseaside A			Sun et al. (2008)
	Holothurin A			Elyakov et al. (1975)
	Holothurin A ₁			Elyakov et al. (1975)
	Holothurin B			Elyakov et al. (1975)
<i>Holothuria hilla</i>	Hillaside C			Wu et al. (2006c)
	Holothurin A			Elyakov et al. (1973)
	Holothurin B			Elyakov et al. (1973)
<i>Holothuria impatiens</i>	Bvittoside D			Sun et al. (2007)
	Holothurin A			Elyakov et al. (1973)
<i>Holothuria leucospilota</i>	Impatienside A (Marmoratoside A)**			Sun et al. (2007)
	Bvittoside C		x	Chapter 2
	Bvittoside D	x	x	Chapter 2
	Desholothurin A (Nobiliside 2a)**		x	Chapter 2
	Fuscocineroside B/C*		x	Chapter 2
	Holothurin A		x	Kitagawa et al. (1979)
	Holothurin B	x	x	Kitagawa et al. (1978)
	Holothurin B/B4*			Chapter 2
	Holothurin B ₁	x		Chapter 2
	Holothurin B ₁	x		Chapter 2
	Holothurin B ₁	x		Chapter 2
	Holothurinoside E ₁		x	Chapter 2
	Leucospilotaside A			Han et al. (2007)
	Leucospilotaside C			Han et al. (2008)
Species	Saponins	BW	CT	References
<i>Holothuria lubrica</i>	Holothurin A			Yasumoto et al. (1967)
	Holothurin B			Yasumoto et al. (1967)
<i>Holothuria mexicana</i>	Holothurin A			Elyakov et al. (1975)
	Holothurin B			Elyakov et al. (1975)
<i>Holothuria moebi</i>	Holothurin A			Matsuno and Iba (1966)
<i>Holothuria monacaria</i>	Holothurin A			Matsuno and Iba (1966)
<i>Holothuria nobilis</i>	Holothurin A			Elyakov et al. (1973)
	Holothurin B	x		Radhika et al. (2002)
	Nobiliside 1a			Wu et al. (2006a)
	Nobiliside 2a (Desholothurin A)**			Wu et al. (2006a)
	Nobiliside A			Wu et al. (2006b)
	Nobiliside B			Wu et al. (2006b)
	Nobiliside C			Wu et al. (2006b)
<i>Holothuria pervicax</i>	Holothurin A			Elyakov et al. (1973)
	Holothurin B			Elyakov et al. (1973)
	Pervicoside A	x	x	Kitagawa et al. (1989)
	Pervicoside B	x		Kitagawa et al. (1989)
	Pervicoside C	x		Kitagawa et al. (1989)
<i>Holothuria polii</i>	Holothurin A	x		Silchenko et al. (2005)
	Holothurin B	x		Silchenko et al. (2005)
	Holothurin B ₁	x		Silchenko et al. (2005)
	Holothurin B ₁	x		Silchenko et al. (2005)
<i>Holothuria pulla</i>	Holothurin A			Elyakov et al. (1973)
	Holothurin B			Elyakov et al. (1973)
<i>Holothuria scabra</i>	24-dehydroholothurin A ₁	x		Kobayashi et al. (1991)
	Holothurin A	x		Dang et al. (2007)
	Holothurin A ₁	x		Dang et al. (2007)
	Holothurin A ₁			Dang et al. (2007)
	Holothurin A ₁			Dang et al. (2007)
<i>Holothuria squamifera</i>	Holothurin B			Elyakov et al. (1973)
<i>Holothuria surinamensis</i>	Holothurin A			Stonik (1986)
	Holothurin A			Elyakov et al. (1975)
<i>Holothuria tubulosa</i>	Holothurin B			Elyakov et al. (1975)
	Holothurin A			Silchenko et al. (2005)
	Holothurin B			Silchenko et al. (2005)
<i>Pearsonothuria graeffei</i>	Bvittoside D		x	Chapter 2
	Desholothurin A (Nobiliside 2a)**	x	x	Chapter 2
	Fuscocineroside B/C*	x	x	Chapter 2
	Holothurin A	x	x	Elyakov et al. (1973)
	Holothurin A ₁	x	x	Stonik (1986)
	Holothurin B	x	x	Elyakov et al. (1973)
	Holothurin B/B ₁ *			Chapter 2
	Holothurinoside C	x		Chapter 2

BW = body wall; CT = Cuvierian tubules; sulfated saponins in green; non-sulfated saponins in red.

* Isomeric saponins.

** Different names for the same structure.

*** Extracted from seawater in which the animal stayed.

intestinal hemal vessels, the ovaries, the testes and the Cuvierian tubules. Amounts of saponins (expressed by the hemolytic index) were different between body compartments and the body wall and Cuvierian tubules showed the highest values (ovaries also presented high saponin concentrations but they varied with the reproductive cycle of the animal; Matsuno and Ishida 1969). Van Dyck et al. (2010) also highlighted a variation of saponin quantities between the Cuvierian tubules and the body wall in several species of Holothuriidae. Triterpene glycosides appear to be particularly concentrated in the Cuvierian tubules, a specialized defense system developed by some species within the family Holothuriidae (Matsuno and Ishida 1969; Elyakov et al. 1973; Kobayashi et al. 1991). This organ located in the posterior part of the animal consists of multiple tubules that can, in some species, be expelled by the individual after stimulation (Bingham and Braithwaite 1986; Hamel and Mercier 2000; Becker and Flammang in press).

In terms of composition of the congener mixture, although for a same species many saponins are common to both body wall and Cuvierian tubules, some congeners seem to be organ-specific (Table 1). Some species possess more saponin congeners in the Cuvierian tubules than in the body wall (e.g. *H. leucospilota*), some less (e.g. *A. echinites*), and some have roughly the same number of saponins in the two organs (e.g. *B. subrubra* and *P. graeffei*) (Kobayashi et al. 1991; Van Dyck et al. 2009, 2010).

The large number of different saponins within a species as well as the intra-individual variations in saponin mixtures raises the question of the specific functions of these molecules. One holothuriid species can indeed contain numerous different saponins (more than 20 in *H. forskali*), the different congeners varying in the number, position and nature of the monosaccharide units and also in the number and position of double bonds, hydroxyl, acetate, sulfate and other functional groups on the aglycone and the carbohydrate chain (Kornprobst 2005; Kalinin et al. 2005). Possessing such a molecular diversity should be a selective advantage for the animal, different molecular structures seemingly conferring different properties to the saponins. According to Kalinin (2000), the presence of a sulfate group enhances the hydrophilicity of the saponin while the length and composition of the carbohydrate chain is important for its membranolytic action. This could explain at least partly the variation of saponin composition between the body wall and the Cuvierian tubules in a single species. To make the picture even more complex, it has been shown recently that, in the Cuvierian tubules of *H. forskali*, a prolonged stress induces the modification of some congeners into others by addition of a disaccharide (Van Dyck et al. in press). All the data taken together therefore

indicate a complex chemical defence mechanism with, for a single species, different sets of saponins originating from different body compartments and presenting different responses to stress. This presumably finely tunes the saponin properties according to their ecological role(s).

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