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 $D^{9(11)}$ 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 Rodrigez et al. (1991). Also, authors should homogenize the

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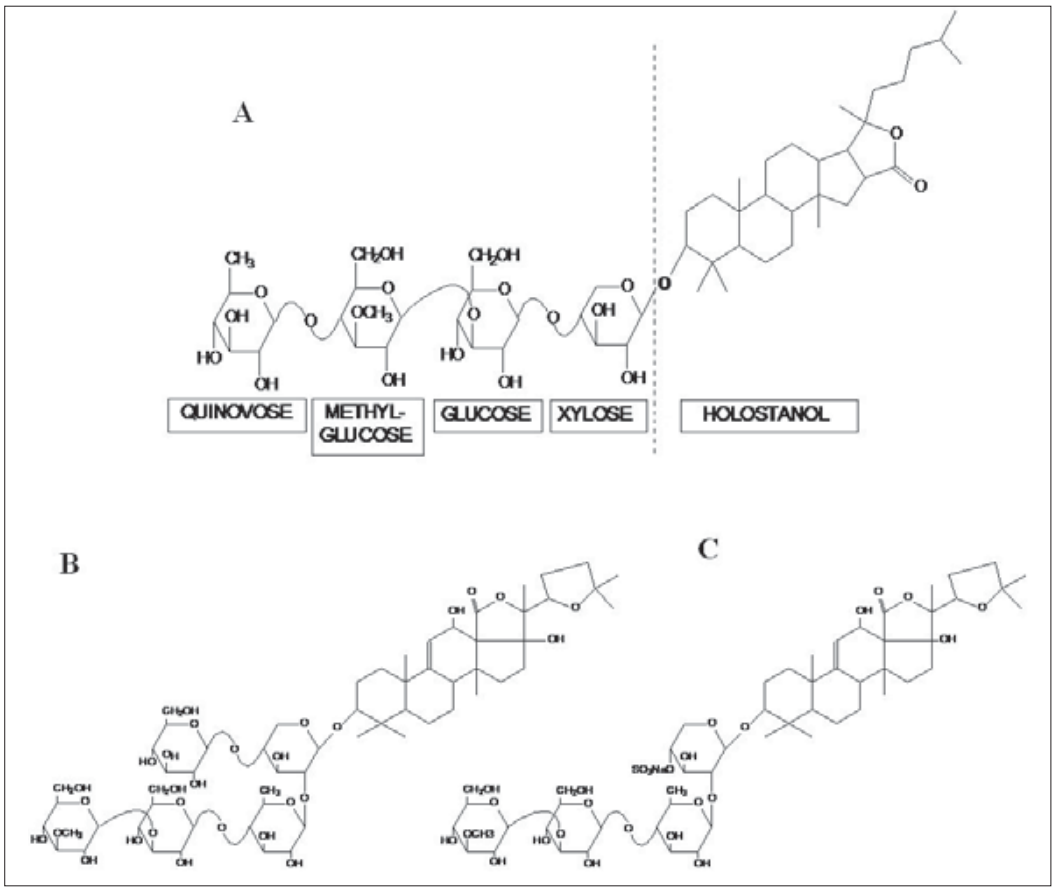

Figure 1. Molecular structure of (A) a hypothetic saponin composed of an aglycone of holostanol (according to the Comparative Toxicogenomic Database) and a linear glycosidic chain constituted by the four most frequent monosaccharides found in holothurian saponins; (B) holothurinoside A, a non-sulfated 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
Actinopyga agassizi	24-dehydroholothurin A_2		x	Kitagawa et al. (1982)
	Holothurin A		x	Kitagawa et al. (1982)
	Holothurin A_2			Kitagawa et al. (1980)
	Holothurin B			Elyakov et al. (1975)
	Holothurin B_1			Kitagawa et al. (1980)
Actinopyga echinites	Fuscocineroside B/C*	x	x	Chapter 2
	Holothurin A	x	x	Elyakov et al. (1973)
	Holothurin A_2	x		Kitagawa et al. (1980)
	Holothurin B			Elyakov et al. (1973)
	Holothurin B/B_4*			Chapter 2
	Holothurin B_1	x		Kitagawa et al. (1980)
	Holothurin B_2	x		Chapter 2
	Holothurin B_3	x		Chapter 2
Actinopyga flammea	Holothurin A			Bhatnagar et al. (1985)
	Holothurin B			Bhatnagar et al. (1985)
Actinopyga lecanora	Holothurin A			Elyakov et al. (1973)
	Holothurin B			Elyakov et al. (1973)
Actinopyga mauritana	24-dehydroholothurin A_2	x		Kobayashi et al. (1991)
	24-dehydroholothurin B_1	x		Kobayashi et al. (1991)
	Holothurin A			Elyakov et al. (1973)
	Holothurin A_2	x		Kobayashi et al. (1991)
	Holothurin B	x		Elyakov et al. (1973)
	Holothurin B_1	x		Kobayashi et al. (1991)
Actinopyga miliaris	Holothurin A			Elyakov et al. (1973)
	Holothurin B			Elyakov et al. (1973)
Bohadschia argus	Arguside A			Liu et al. (2007)
	Arguside B			Liu et al. (2008a)
	Arguside C		x	Liu et al. (2008a)
	Arguside D	x	x	Liu et al. (2008b)
	Arguside E (Desholothurin A_1)**			Liu et al. (2008b)
Bohadschia bivittata	Bivittoside A		x	Ohta and Hikino (1981)
	Bivittoside B		x	Ohta and Hikino (1981)
	Bivittoside C		x	Ohta and Hikino (1981)
	Bivittoside D		x	Ohta and Hikino (1981)
Bohadschia marmorata	17 -hydroxy impatienside A			Yuan et al. (2009)
	25-acetoxy bivittoside D			Yuan et al. (2009)
	Bivttoside 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)
Bohadschia subrubra	Arguside C		x	Chapter 2
	Bivittoside B		x	Chapter 2
	Bivittoside C	x	x	Chapter 2
	Bivittoside D		x	Chapter 2
	Impatienside A (Marmoratoside A)**	x		Chapter 2
Bohadschia subrubra (Continued)	Holothurinoside F	x		Chapter 2
	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		Chapter 2
Bohadschia tenuissima	Bivittoside C	x		Radhika et al. (2002)
	Bivittoside D	x		Radhika et al. (2002)
Bohadschia vitiensis	Bivittoside C			Radhika et al. (2002)
	Bivittoside D			Radhika et al. (2002)
Holothuria arenicola	Holothurin A			Elyakov et al. (1973)
	Holothurin B			Elyakov et al. (1975)
Holothuria atra	Holothurin A			Kobayashi et al. (1991)
	Holothurin A_2			Kobayashi et al. (1991)
	Holothurin B	x		Kobayashi et al. (1991)
	Holothurin B/B_4*			Chapter 2
	Holothurin B_1	x		Kobayashi et al. (1991)
	Holothurin B_2	x		Chapter 2
	Holothurin B_3	x		Chapter 2
Holothuria axiloga	Axilogoside A			Yuan et al. (2008)
(Microthele)	Holothurin A			Kobayashi et al. (1991)
	Holothurin A_2			Kobayashi et al. (1991)
	Holothurin B			Yuan et al. (2008)
Holothuria cinerascens	Holothurin A			Elyakov et al. (1973)
Holothuria coluber	Holothurin A			Elyakov et al. (1973)
	Holothurin B			Elyakov et al. (1973)
Holothuria cubana	Holothurin A			Elyakov et al. (1975)
	Holothurin B			Elyakov et al. (1975)
Holothuria difficilis	Holothurin A			Elyakov et al. (1973)
	Holothurin B			Elyakov et al. (1973)
Holothruia edulis	Holothurin A	x		Kobayashi et al. (1991)
	Holothurin A_2			Stonik (1986)
	Holothurin B			Stonik (1986)
Holothuria floridana	Holothurin A_1			Stonik (1986)
	Holothurin A_2			Stonik (1986)
	Holothurin B_1			Elyakov et al. (1982)
Holothuria forskali	Desholothurin A (Nobiliside 2a)**	x	x	Rodrigez et al. (1991)
	Desholothurin A_1 (Arguside E)**		x	Chapter 1
	Holothurinoside A	x	x	Rodrigez et al. (1991)
	Holothurinoside A_1		x	Chapter 1
	Holothurinoside B	x	x	Rodrigez et al. (1991)
	Holothurinoside C	x	x	Rodrigez et al. (1991)
	Holothurinoside C_1		x	Chapter 1
	Holothurinoside D	x	x	Rodrigez et al. (1991)

Table 1 (continued)

Species	Saponins	BW	CT	References
Holothuria forskali	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
Holothuria fuscocinerea	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)
Holothuria gracilis	Holothurin A			Elyakov et al. (1973)
	Holothurin B			Elyakov et al. (1973)
Holothuria grisea	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)
Holothuria hilla	Hillaside C			Wu et al. (2006c)
	Holothurin A			Elyakov et al. (1973)
	Holothurin B			Elyakov et al. (1973)
Holothuria impatiens	Bivittoside D			Sun et al. (2007)
	Holothurin A			Elyakov et al. (1973)
	Impatienside A (Marmoratoside A)**			Sun et al. (2007)
Holothuria leucospilota	Bivittoside C		x	Chapter 2
	Bivittoside 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_2	x		Chapter 2
	Holothurin B_3	x		Chapter 2
	Holothurinoside E_1		x	Chapter 2
	Leucospilotaside A			Han et al. (2007)
	Leucospilotaside C			Han et al. (2008)
Holothuria lubrica	Holothurin A			Yasumoto et al. (1967)
	Holothurin B			Yasumoto et al. (1967)
Holothuria mexicana	Holothurin A			Elyakov et al. (1975)
	Holothurin B			Elyakov et al. (1975)
Holothuria moebi	Holothurin A			Matsuno and Iba (1966)
Holothuria monacaria	Holothurin A			Matsuno and Iba (1966)
Holothuria nobilis	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)
Holothuria pervicax	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)
Holothuria polii	Holothurin A	x		Silchenko et al. (2005)
	Holothurin B	x		Silchenko et al. (2005)
	Holothurin B_2	x		Silchenko et al. (2005)
	Holothurin B_3	x		Silchenko et al. (2005)
	Holothurin B_4	x		Silchenko et al. (2005)
Holothuria pulla	Holothurin A			Elyakov et al. (1973)
	Holothurin B			Elyakov et al. (1973)
Holothuria scabra	24-dehydroholothuin A_2	x		Kobayashi et al. (1991)
	Holothurin A	x		Dang et al. (2007)
	Holothurin A_2	x		Dang et al. (2007)
	Holothurin A_3			Dang et al. (2007)
	Holothurin A_4			Dang et al. (2007)
	Holothurin B			Elyakov et al. (1973)
Holothuria squamifera	Holothurin A			Stonik (1986)
Holothuria surinamensis	Holothurin A			Elyakov et al. (1975)
	Holothurin B			Elyakov et al. (1975)
Holothuria tubulosa	Holothurin A			Silchenko et al. (2005)
	Holothurin B			Silchenko et al. (2005)
Pearsonothuria graeffei	Bivittoside 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_2	x	x	Stonik (1986)
	Holothurin B	x	x	Elyakov et al. (1973)
	Holothurin B/B_4*			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 Cuvieran 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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References

Becker P. and Flammang P. in press. Unravelling the sticky threads of sea cucumbers. A comparative study on Cuvierian tubule morphology and histochemistry. In: von Byern J. and Grunwald I. (eds). Biological adhesive systems — From nature to technical and medical application. Springer Press.

Bhatnagar S., Dudoet B., Ahond A., Poupat C., Thoison O., Clastres A., Laurent D. and Potier P. 1985. Invertébrés marins du lagon néocalédonien. IV: Saponines et sapogénines d'une holothurie, *Actinopyga flammea*. Bulletin de la société chimique de France 1:124–129.

Bingham B.L. and Braithwaite L.F. 1986. Defense adaptations of the dendrochirote holothurian *Psolus chitonoides* Clark. Journal of Experimental Marine Biology and Ecology 98:311–322.

Dang N.H., Thanh N.V., Kiem P.V., Huong L.M., Minh C.V., and Kim Y.H. 2007. Two new triterpene glycosides from the Vietnamese sea cucumber *Holothuria scabra*. Archives of Pharmacal Research 30:1387–1391.

Elyakov G.B., Stonik V.A., Levina E.V., Slanke V.P., Kuznetsova T.A. and Levin V.S. 1973. Glycosides of marine invertebrates-I. A comparative study of the glycosides fraction of Pacific sea cucumbers.Comparative Biochemistry and Physiology 44:325–336.

Elyakov G.B., Kuznetsova T.A., Stonik V.A., Levin V.S. and Albores R. 1975. Glycosides of marine invertebrates-IV. A comparative study of the glycosides from Cuban sublittoral holothurians. Comparative Biochemistry and Physiology 52:413–417.

Hamel J.F. and Mercier A. 2000. Cuvierian tubules in tropical holothurian: usefulness and efficiency as a defence mechanism. Marine and Freshwater Behaviour and Physiology 33:115–139.

Han H., Yi Y.H., Li L., Wang X.H., Liu B.S., Sun P. and Pan M.X. 2007. A new triterpene glycoside from sea cucumber *Holothuria leucospilota*. Chinese Chemical letters 18:161–164.

Han H., Yi Y.H., Liu B.L., Wang X.H. and Pan M.X. 2008. Leucospilotaside C, a new sulphated triterpene glycoside from sea cucumber *Holothuria leucospilota*. Chinese Chemical letters19:1462–1464.

Kalinin V.I. 2000. System-theoretical (holistic) approach to the modelling of structural-functional relationships of biomolecules and their evolution: An example of triterpene glycosides from sea cucumbers (Echinodermata, Holothuroidea). Journal of Theoretical Biology 206:151–168.

Kalinin V.I., Anisimov M.M., Prokofieva N.G., Avilov S.A., Afiyatullov SH.SH., and Stonik V.A. 1996a. Biological activities and biological role of triterpene glycosides from holothuroids (Echinodermata). p. 139–181. In: Jangoux M. and Lawrence L.M. (eds). Echinoderm studies. Balkema, Rotterdam.

Kalinin V.I., Prokofieva N.G., Likhatskaya G.N., Schentsova E.B., Agafonova I.G., Avilov S.A. and Drozdova O.A. 1996b. Hemolytic activities of triterpene glycosides from the holothurian order dendrochirotida: Some trends in the evolution of this group of toxins.Toxicon 34:475–483.

Kalinin V.I., Silchenko A.S., Avilov S.A., Stonik V.A. and Smirnov A.V. 2005. Sea cucumbers triterpene glycosides, the recent progress in structural elucidation and chemotaxonomy. Pytochemistry Reviews 4:221–236.

Kerr R.G. and Chen Z. 1995. In vivo and in vitro biosynthesis of saponins in sea cucumbers. Journal of Natural Products 58:172–176.

Kitagawa I., Nishino T., Matsuno T., Akutsu H. and Kyogoku Y. 1978. Structure of holothurine B. A pharmacologically active triterpene-oligoglycoside from the sea cucumber *Holothuria leucospilota* Brandt. Tetrahedron Letters 11:985–988.

Kitagawa I., Nishino T. and Kyogoku Y. 1979. Structure of holothurine A. A pharmacologically active triterpene-oligoglycoside from the sea cucumber *Holothuria leucospilota* Brandt. Tetrahedron Letters 16:1419–1422.

Kitagawa I., Inamoto T., Fuchida M., Okada S., Kobayashi M., Nishino T. and Kyogoku Y. 1980. Structures of echinoside A and B, two antifungal oligoglycosides from the sea cucumber *Actinopyga echinites* (Jaeger). Chemical and Pharmacological Bulletin 28:1651–1653.

Kitagawa I., Kobayashi M. and Kyogoku Y. 1982. Marine natural products. IX. Structural elucidation of triterpenoidal oligoglycosides from the Bahamean sea cucumber *Actinopyga agassizi* Selenka. Chemical and Pharmacological Bulletin 30:2045–2050.

Kitagawa I., Kobayashi M., Son B.W., Suzuki S. and Kyogoku Y. 1989. Marine natural products. XIX. Pervicosides A, B and C, lanostane-type triterpene-oligoglycoside sulfates from the sea cucumber *Holothuria pervicax*. Chemical and Pharmacological Bulletin 37:1230–1234.

Kobayashi M., Hori M., Kan K., Yasuzawa T., Matsui M., Suzuki S. and Kitagawa I. 1991. Marine Natural Product. XXVII. Distribution of lanostane-type triterpene oligoglycosides in ten kinds of Okinawan sea cucumbers. Chemical and Pharmacological Bulletin 39:2282–2287.

Kornprobst J.-M. 2005. Substances naturelles d'origine marine : chimiodiversité, pharmacodiversité, biotechnologies. Éditions Tec & Docs. 1834 p.

Li R., Zhou Y., Wu Z. and Ding L. 2006. ESI-QqTOF-MS/MS and APCI-IT-MS/MS analysis of steroid saponins from the rhizomes of *Dioscorea panthaica*. Journal of Mass Spectrometry 41:1–22.

Liu B.-S., Yi Y.-H., Li L., Zhang S.-L., Han H., Weng Y.-Y. and Pan M.-X. 2007. Arguside A: a new cytotoxic triterpene glycoside from the sea cucumber *Bohadschia argus* Jaeger. Chemistry and Biodiversity 4:2845–2851.

Liu B.-S., Yi Y.-H., Li L., Sun P., Yuan W.-H., Sun G.-Q., Han H. and Xue M. 2008a. Arguside B and C, two new cytotoxic triterpene glycosides from the sea cucumber *Bohadschia argus* Jaeger. Chemistry and Biodiversity 5:1288–1297.

Liu B.-S., Yi Y.-H., Li L., Sun P., Han H., Sun G.-Q., Wang X.H. and Wang Z.-L. 2008b. Arguside D and E, two new cytotoxic triterpene glycosides from the sea cucumber *Bohadschia argus* Jaeger. Chemistry and Biodiversity 5:1425–1433.

Mackie A.M. and Turner A.B. 1970. Partial characterization of biologically active steroid glycoside isolated from the starfish *Marthasterias glacialis*. Biochemical Journal 117:543–550.

Matsuno T. and Iba J. 1966. Studies on the saponins of the sea cucumber. Yakugaku Zasshi 86:637–638.

Matsuno T. and Ishida T. 1969. Distribution and seasonal variation of toxic principles of sea cucumber (*Holothuria leucospilota* Brandt). Experientia 25:1261.

Nigrelli R.F. 1952. The effect of holothurin on fish, and mice with sarcoma 180. Zoologica 37:89–90.

Ohta T. and Hikino H. 1981. Structures of four new triterpenoidal oligosides, bivittoside A, B, C and D, from the sea cucumber *Bohadschia bivittata* Mitsukuri. Chemical Pharmacological Bulletin 29:282–285.

Prokofieva N.G., Chaikina E.L., Kicha A.A. and Ivanchina N.V. 2003. Biological activities of steroid glycosides from starfish. Comparative Biochemistry and Physiology 134:695–701.

Radhika P., Anjaneyulu V., Rao P.V.S., Makarieva T.N. and Kalinovosky A.I. 2002. Chemical examination of the echinoderms of Indian Ocean: The triterpene glycosides of the sea cucumbers: *Holothuria nobilis*, *Bohadschia aff. Tenuissima* and *Actinopyga mauritana* from Lakshadweep, Andaman and Nicobar Islands. Indian Journal of Chemistry 41:1276–1282.

Rodrigez J., Castro R. and Riguera R. 1991. Holothurinosides: new antitumour non sulphated triterpenoid glycosides from the sea cucumber *Holothuria forskali*. Tetrahedron 47:4753–4762.

Silchenko A.S., Stonik V.A., Avilov S.A., Kalinin V.I., Kalinovsky A.I., Zaharenko A.M., Smirnov A.V., Mollo E., and Cimino G. 2005. Holothurins B_2, B_3 and B_4, new triterpene glycosides from Mediterranean sea cucumbers of the genus *Holothuria*. Journal of Natural Products 68:564–567.

Sun G.-Q., Li L., Yi Y.-H., Yuan W.-H., Liu B.-S., Weng Y.Y., Zhang S.-L., Sun P. and Wang Z.-L. 2008. Two new cytotoxic nonsulfated pentasaccharide holostane (=20-hydroxylanostan-18-oic acid -lactone) glycosides from the sea cucumber *Holothuria grisea*. Helvetica Chimica Acta 91:1453–1460.

Thompson J.E., Walker R.P. and Faulkner D.J. 1985. Screening and bioassays for biologically-active substances from forty marine sponge species from San Diego, California, USA. Marine Biology 88:11–21.

Van Dyck S., Gerbaux P. and Flammang P. 2009. Elucidation of molecular diversity and body distribution of saponins in the sea cucumber *Holothuria forskali* (Echinodermata) by mass spectrometry. Comparative Biochemistry and Physiology B- Biochemistry and Molecular Biology 152(2):124–134.

Van Dyck S., Gerbaux P. and Flammang P. 2010. Qualitative and quantitative saponin contents in five sea cucumbers from the Indian Ocean. Marine Drugs 8(1):173–189.

Van Dyck S., Flammang P., Meriaux C., Bonnel D., Salzet M. Fournier I. and Wisztorski M. in press. Localization of secondary metabolites in marine invertebrates: Contribution of MALDI MSI for the study of saponins in Cuvierian tubules of *H. forskali*. PLoS ONE.

Wu, J., Yi, Y.-H., Zou, Z.-R., Wu, H.-M., and Tang, H.-F. 2006a. Two new triterpene glycosides from sea cucumber Holothuria nobilis. Chinese Traditional Herbal Drugs. 37: 497-500.

Wu, J., Yi, Y.-H., Tang, H.-F., Wu, H.-M., Zou, Z.-R., and Lin, H.-W. 2006b. Nobilisides A–C, three new triterpene glycosides from the sea cucumber Holothuria nobilis. Planta Medica 71:932–935.

Wu J., Yi Y.-H., Tang H.-F., Zou Z.-R. and Wu H.-M. 2006c. Structure and cytotoxicity of a new lanostan-type triterpene glycoside from the sea cucumber *Holothuria hilla*. Chemical Biodiversity 3:1240–1254.

Yamanouchi T. 1955. On the poisonous substance contained in holothurians. Publications of the Seto Marine Biologica Laboratory 4:183–203

Yasumoto T., Nakamura K. and Hashimoto Y. 1967. A new saponin holothurin isolated from the sea cucumber *Holothuria vagabunda*. Agricultural Biolology and Chemistry 31:7.

Yuan W.-H., Yi Y.-H., Xue M., Zhang H-.W. and La M.-P. 2008. Two antifungal active triterpene glycosides from sea cucumber *Holothuria* (*Microthele*) *axiloga*. Chinese Journal of Natural Medicines 6:105–108.

Zhang S.-Y., Yi Y.-H. and Tang H.-F. 2006. Bioactive triterpene glycosides from the sea cucumber *Holothuria fuscocinerea*. Journal of Natural Products 69:1492–1495.