

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

Guillaume Caulier,^{1*} Séverine Van Dyck,¹ Pascal Gerbaux,² Igor Eeckhaut¹ and Patrick Flammang¹

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 bond 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

1. University of Mons, Marine Biology Laboratory, 7000 Mons, Belgium.

2. University of Mons, Organic Chemistry Laboratory, Interfaculty Center for Mass Spectrometry (CISMa), 7000 Mons, Belgium.

* Corresponding author: guillaume.caulier@umons.ac.be

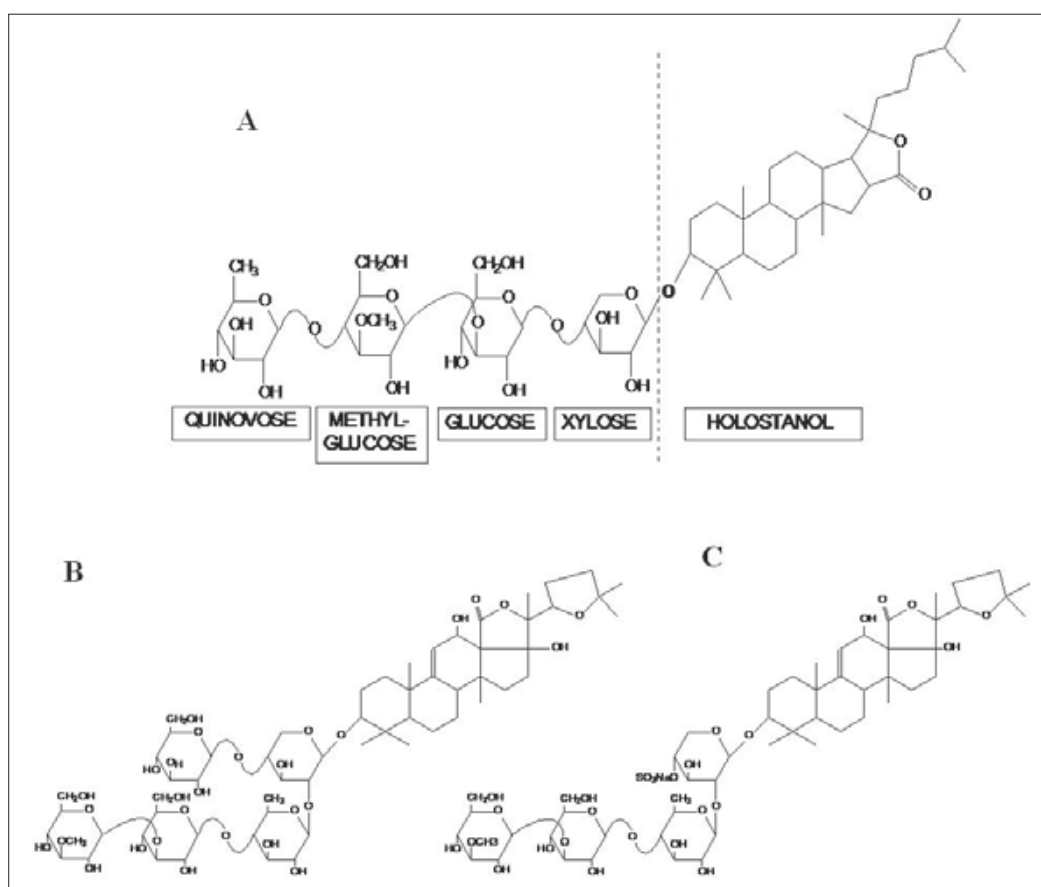


Figure 1. Molecular structure of (A) a hypothetical saponin composed of an aglycone of holostanol (according to the Comparative Toxicogenomic Database) and a linear glycosidic chain constituted by the four most frequent monosaccharides found in holothurian saponins; (B) holothurinoside A, a non-sulfated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

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).

Acknowledgements

SVD and GC benefited from a FRIA doctoral grant (Belgium). PF and PG are respectively Senior Research Associate and Research Associate of the Fund for Scientific Research of Belgium (F.R.S.-FNRS). PG thanks the F.R.S.-FNRS for financial support in the acquisition of the Waters Q-ToF Premier Mass Spectrometer and for continuing support. Work supported by a FRFC Grant n° 2.4535.10. This study is a contribution of the "Centre Interuniversitaire de Biologie Marine" (CIBIM).

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. Leucospilataside C, a new sulphated triterpene glycoside from sea cucumber *Holothuria leucospilota*. *Chinese Chemical letters* 19:1462–1464.
- 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., Afiyatullof 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. *Phytochemistry 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.
- Rodriguez 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., Kalinovskiy A.I., Zaharenko A.M., Smirnov A.V., Mollo E., and Cimino G. 2005. Holothurins B₂, B₃ and B₄, 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-hydroxy lanostan-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 Biological Laboratory* 4:183–203
- Yasumoto T., Nakamura K. and Hashimoto Y. 1967. A new saponin holothurin isolated from the sea cucumber *Holothuria vagabunda*. *Agricultural Biology 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.