Bioresources from Echinoderms

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Abstract. More than 6,500 species have been recorded in the phylum Echinodermata. A variety of biologically active substances have been isolated from the echinoderm species: saponins, glycolipids, carotenoids, porphyrins, naphthoquinones, venoms and others. Several substances unique to the echinoderm have also been reported and some of them showed high potentiality as a new medicament. This chapter gives an overview of the history of the exploitation of echinoderm species in the Orient, presents studies on the biologically active substance obtained from them, and discusses questions related to the exploitation of the echinoderm and prospects of development of new medications.

1 Introduction

The sea occupies 71 % of the terrestrial globe. Its average depth is approximately 3,800 m. It is an entire complex of environmental conditions rather than a huge uniform hydrosphere. Marine faunas and floras resulted from adaptation to a variety of its environmental conditions. There is almost no light in the sea at depths below 150–200 m, as the seawater absorbs visible radiation. The dark environment, where visual communication is more disadvantageous than under terrestrial conditions, has forced organisms to evolve other means of communication, e.g., chemical communication. Marine organisms may contain substances that are not found in terrestrial organisms.

Marine organisms are anticipated as new resources of biologically active substances, e.g., as pharmacological reagents. The Echinodermata phylum

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consists of five major classes: Ophiuroidea, 1,900 species; Crinoidea, 600 species; Echinoidea, 1,000 species; Asteroidea, 1,500 species; and Holothuroidea, 500 species. Less pharmaceutical exploitation of marine organisms has taken place than that of terrestrial animals and plants, because we are not as familiar with marine life as with terrestrial life. Research and development of novel chemicals from unexplored echinoderms are current concerns, e.g., chemicals to fight tumors and microbes, depressors (hypotensives), pesticides and agrichemicals with low environmental impacts.

When we exploit echinoderm species as a new biological resource, we have to consider the economic and ecological standpoints. Historically and currently, the most fished animal among the echinoderms is the sea urchin. Some *Regularia* species have long been consumed and exploited as a gastronomic or an exotic food. In addition, the sea cucumber is fished and consumed in Japan and China. Therefore, market prices of new products from the edible sea urchin and sea cucumber must be higher than those of raw sea urchin or raw sea cucumber as a food. The price of sea urchin roes of the highest grade is 2,000–3,000 Japanese yen per 100 g. Judging only from the current situation of the sea urchin market, development of new products from sea urchins looks unprofitable, although most echinoderm species are non-edible and unexplored. The economic bottleneck described above will be solved by making use of non-edible species.

From an ecological viewpoint, we must not duplicate the error of overexploitation of sea urchins which caused a decrease in sea urchin resources in Japan and the Mediterranean Sea. However, the use of unexplored echinoderm species such as starfish may contribute to the management of fishery resources. Most asteroid species are carnivorous and final predators in the benthos population. In the 1960s, outbreaks of *Acanthaster planci* damaged the Great Barrier Reef; similarly in the 1970s, the coral reef in Okinawa, Japan, was also preyed upon seriously by *A. planci*. Blooms of asteroids in the Frigid and Temperate zones and the consequent impacts on shell fishery are also often reported. Exploitation of asteroids as a biological resource will contribute to the management of the benthic ecosystem. Although aquacultural farms of scallops, clams, and other shellfish remove starfish as a measure of damage control, the countermeasure is not intended to eradicate the starfish population. The landed starfish usually generates a new problem of industrial waste, and its disposal is an expense for aquacultural farms. When useful substances are prepared from collected starfish, two problems – how to decrease both predation by asteroids and the cost of waste disposal – are simultaneously solved.

Man has used a variety of organisms for medicines from time immemorial. Furthermore, marine organisms, in addition to terrestrial organisms, have also been employed as medicine in Japan and China. In this chapter, biologically active substances from echinoderms are described and their potential application is discussed.

2
Oriental Medicine and Historical Background

In Oriental medicine, traditional ideology has been inherited, expressing that the meal equals the medication. According to this ideology, every food is considered to be physiologically effective. For instance, in the Orient, foods abundant in collagen and chondroitin sulfate have long been recognized to contribute to maintaining smooth skin and preventing senescence and arteriosclerosis. The dried body wall of sea cucumber has been used as a corroborant and nutritious diet.

The sea cucumber is called "haishen" in Chinese, which literally means marine ginseng. Ginseng, *Panax ginseng*, is the most famous plant in Oriental medicine, best known for its multipotent pharmaceutical activities. It contains saponins which show depression effects on the central nervous system, and vasodilator effects. Ancient Japanese names "umiko" and "nameriko" for the sea cucumber were found as medications in an antique medical book, "Daidoruijuho" (808 A.D.). In the 17th century, a Chinese scholar, Xie Qi (1567–1624), reported that sea cucumbers were comparable to ginseng with respect to pharmaceutical activities in a book named "Wuzazhu". In Japan, dried and roasted sea cucumbers have been employed as a hemostatic for bleeding such as in hemophilia, a relaxant for convulsions and other remedies since the 11th century. It is recognized in Japan and China that sea cucumbers contain medicinal substances. The historical background described above seems to support pharmaceutical investigations into the Holothuroidea.

3
Saponins

It has long been known that some holothurian species contain toxins. In the southern Pacific and Tokara Islands (islands of southern Japan), a peculiar fishing method using sea cucumbers is employed. The autochthones catch paralyzed fish after they throw extracts or fragments of sea cucumbers into tidal pools. This toxic effect results from saponins. Cooper (1880) reported that some holothurians discharged white filamentous structures, called Cuvierian tubules. Contact with these tubules also caused skin irritation. Saponins concentrate in Cuvierian tubules. On the other hand, in holothurian species without these structures, saponins are distributed in the body wall. Dried sea cucumbers were used as a home remedy for skin diseases, and powdered starfish was employed as a pesticide in Japan. The pharmacological activity described is assumed to be due to saponins. In the animal kingdom, the occurrence of saponins is limited to only two echinoderm classes, namely holothurians and asteroids; in contrast, saponins are widely distributed in the plant kingdom. Saponins have been used as an expectorant, a cure for congestive heart failure and as a component of contraceptives and antirheumatic agents. The surfactant activity of saponins has been known and utilized for a long time in medicine. Studies on toxic and pharmaceutical activities of holothurians were initiated by Yamanouchi in the 1940s (Yamanouchi 1942, 1943a, b). Later, Nigrelli and Zahl (1952) named the toxic substance from Cuvierian tubules of *Actinpya agassizi* holothurin, and initiated a series of further investigations into the chemical and pharmacological properties of the substance. They demonstrated its lethal activities towards various organisms in e.g., Protozoa, Cnidaria, Nematoda, Mollusca, Annelida, and Amphibia. Antifungal activity of the holothurian saponin, holotoxin, was reported by Shimada (1969). He patented holotoxin as a cure for athlete's foot disease and commercialized it. Kitagawa et al. (1976a, b) showed that holotoxin is in fact composed of three molecules, holotoxin A, B and C. The inhibitory activities on fungal growth were much higher than those of various plant saponins (Table 1). Aglycons of holothurian saponins are terpenoids (Fig. 1), whereas those of asteroid saponins are sterols (Fig. 2). The molecular difference in saponins between the asteroid and holothurian seems to reflect phylogenic differences. Recently, novel cytotoxic triterpene glycosides have been isolated from sea cucumbers *Pentamera calcigera* (Avilov et al. 2000), *Staurocucumis liouvillei* (Maier et al. 2001), *Hemoiedema spectabilis* (Chludil et al. 2002) and *Mensamaria intercedens* (Zou et al. 2003). The glycosides from *P. calcigera*, *S. liouvillei* and *M. intercedens* showed antineoplastic activity against mammalian cancer cells. Terpene glycosides deserve to be investigated as anticancer pharmaceuticals. Two triterpene glycosides from *H. spectabilis* also showed antifungal activity against the phytopathogenic fungus. Antifungal activities of substances from sea cucumbers are thought to be due to their terpenoid structures.

Table 1. Antifungal activities of holotoxins. Minimum inhibitory concentration for growth of microorganisms (μg/ml)

Species	A	B	C
Trichophyton rubrum	0.78	0.78	6.25
Trichophyton mentagrphyte	1.56	1.56	12.5
Mictosporum gypseum	3.12	1.56	12.5
Candida albicans	6.25	6.25	25.0
Candida utilis	3.12	3.12	12.5
Tomla utilis	3.12	3.12	12.5
Aspergillus oryzae	6.25	12.52	5.0
Penicillium chrysogenum	3.12	6.25	12.5
Trishomonas vaginalis	3.12	1.56	3.12

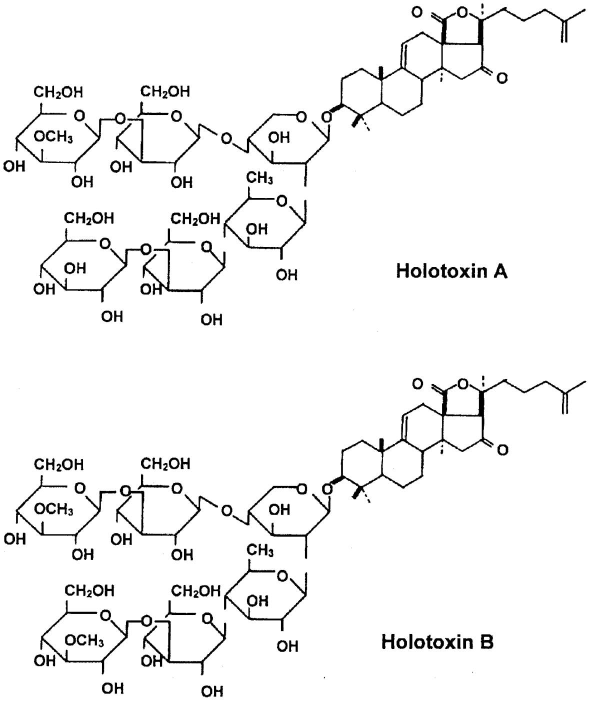


Fig. 1. Structural formulae of holotoxin A and B

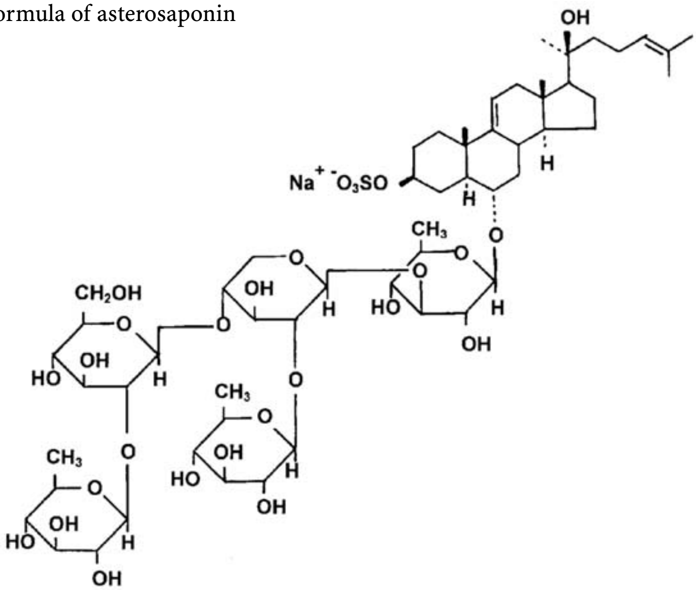


Fig. 2. Structural formula of asterosaponin

4 Glycolipids

Glycosphingolipids, which are important constituents of a variety of cell membranes of vertebrates, are also reported to be contained abundantly in echinoderms (Kubo et al. 1990; Higuchi et al. 1991, 1994). Glycosphingolipids have various physiological functions, e.g. as receptors of cytotoxins, cells, viruses, transmitters and hormones, in the regulation of cell proliferation and differentiation, cell recognition regulatory function of the cell membranes, signal transduction, etc. Complex carbohydrates have been revealed to play important roles in cancer and neuropathy. Some glycosphingolipids such as cerebrosides and gangliosides are believed to be useful in new medications.

Recently, the bioactive glycolipids of echinoderms have been intensively investigated, focusing on the development of new medicinal materials (Yamada 2002). Higuchi and coworkers examined neuritogenic activity of gangliosides isolated from the echinoderms *Stichopus japonicus*, *Holothuria leucospilota* (Yamada et al. 2001), *Asterias amurensis* (Higuchi et al. 1993) and *Holothuria pervicas* (Yamada et al. 2000), on cultured rat pheochromocytoma cells. They also demonstrated antitumoral activity of gangliosides from *Asteropecten latespinosus* (Higuchi et al. 1995). It was revealed that neuritogenic activity of echinoderm gangliosides is dependent on the amount of sialic acids in the carbohydrate moieties. Holothurians contain sphingoglycolipids with molecular structures different from those of mammalian sphingoglycolipids. A ganglioside, which shows a pharmaceutical activity higher than that of a mammalian ganglioside employed for the therapy of neuropathy, was found among holothurian gangliosides. The holothurian ganglioside appears capable of being employed in a new cure for neuropathy, since the chemical synthesis of sphingoglycolipids is less advantageous than isolation from echinoderms from an economic standpoint.

5 Pigments

Marine animals, including echinoderms, attract our attention because of their fantastic and bizarre colors. The coloration of echinoderms, in most cases, is due to chemical pigments. Carotenoids, melanins, porphyrins and naphthoquinones contribute to the pigmentation of the integument. In addition, the appetizing color of sea urchin roes is precisely that of carotenes. Carotenoids are known as antioxidants and widely used as a food supplement. As echinoderms are not capable of synthesizing carotenoids de novo, they obtain carotenoids from algae or animals that take them from algae. The occurrence of major carotenoids in echinoderms was documented by Fox and Hopkins (1966). The well-known antitumor promoter β-carotene and its derivatives are the most abundant carotenoids in echinoids and

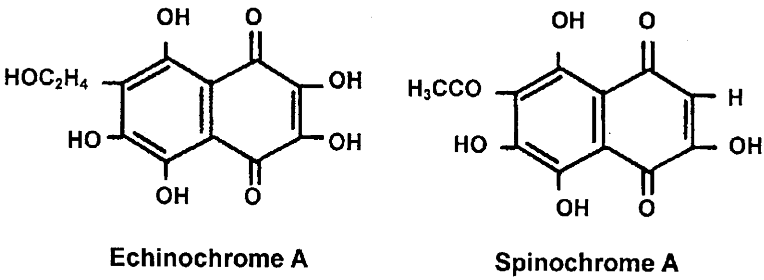


Fig. 3. Structures of echinochrome A and spinochrome A

asteroids. Preparations of major carotenoids from plants seem more economical than from echinoderms. Pigments other than major carotenoids, which are specific to echinoderm species, will be a focus of pharmaceutical development. Tsushima et al. (1995) carried out extensive studies on echinoderm carotenoids from the viewpoints of comparative biochemistry and pharmacology. They examined 51 carotenoids with different structures for the inhibitory effects on Epstein-Barr virus activation. A novel marine carotenoid from *Cucumaria japonica*, cucumariaxanthin C, showed such an effect (Tsushima et al. 1996). Quinone sulfates isolated from the crinoids *Tropiometra afra macrodisucus* and *Oxycomanthus japonicus* have shown antifeedant activity on fish (Takahashi et al. 2002). Spinochrome and echinochrome are assumed to show hypotensive activity (Kuzuya et al. 1973; Fig. 3). Pharmaceutical investigation into echinodermal quinones is required for the development of new anticancer reagents.

6
Venoms

Venoms occur in two echinoderm classes, namely echinoids and asteroids. Their distribution is limited to three echinoid families, Echinothuriidae, Diadematidae and Toxopneustidae. *Acanthaster planci* is the only species that has been reported to be venomous among starfish. A few studies on sea urchin venoms have been reported. Peditoxin, purified from the pedicellariae of *Toxopneustes pileolus*, is composed of a protein called pedin and a prosthetic group called pedoxin (Kuwabara 1994). Venomous activity results from pedoxin, which has a molecular mass of 206 Da. It causes sedation and anesthetic coma accompanied by muscular relaxation at sublethal doses. Another toxin, which was named contractin A, from the venom of *T. pileolus* pedicellaria, has been purified and characterized (Nakagawa et al. 1991). Contractin A, having an apparent molecular weight of 18,000 Da for a total of 138 amino-acid residues, caused contraction of the tracheal smooth muscle. Spine venom from *A. planci* has been extensively investigated by Japanese researchers. They demonstrated that *A. planci* venom has various toxic activities. The lethal factor was shown to be a potent hepatotoxic basic glycoprotein with a molecular weight of 20,000–25,000 Da (Shiomi et al. 1988, 1990). A new anticoagulant peptide with a native molecular mass of 7,500 Da from the spine venom of *A. planci*, plancinin, inhibits factor X activation in the human blood coagulation cascade (Koyama et al. 1998). A fraction of venom from the crown-of-thorns starfish causes smooth muscle contraction mediated by prostaglandins (Karasudani et al. 1996). In addition, the venom showed vasorelaxing and hypotensive effects, which were assumed to be due to the release of a platelet-activating factor or a factor-like substance (Yara et al. 1992). The fact that the molecular mass of pedoxin from *T. pileolus* is 206 Da may suggest that the development of its synthesis is possible. Further, pedoxin seems to show low antigenicity because of its low molecular mass when it is subcutaneously or intramuscularly injected. There are still many sea urchin species to be investigated, the venoms of which are not yet characterized.

7 Hemagglutinins

In echinoderms, hemagglutinins were reported in a starfish and sea urchins. Hemagglutinin occurs in the coelomic fluid of *Anthocidaris crassispina* (Giga et al. 1987) and *Hemicentrotus pulcherrimus* (Yamada and Aketa. 1982), and in eggs of *Anthocidaris crassispina* (Ozeki et al. 1991). Hemagglutinins of *A. crassispina* have been extensively investigated and their complete amino-acid sequence has been reported. Also in holothurians, hemagglutinin was reported (Hatakeyama et al. 1994; Matsui et al. 1994).

In general, hemagglutinins from echinoderms do not seem advantageous for clinical use, as most of them have not shown any blood group specificity.

8 Prospects

Organisms are capable of synthesizing many kinds of biologically active substances with diverse molecular structures. Various antibiotics have been discovered and incorporated in the treatment of infection in the last century. Development of biologically active chemicals from the half million species of marine organisms is one interest. In order to efficiently fulfill biological and pharmaceutical surveys of substances, a globally standardized system for biological screening must be established. Biologically active substances are generally divided into two categories: those that are antigenic and those that are non-antigenic. When an antigenic substance is subcutaneously or intramuscularly administered as a medication, there is a conflict with the host immune system. It is not to say that oral administration of peptides and proteins is impossible. This is why antitumoral proteins found in the sea urchins *Strongylocentrotus purpuratus*, *S. intermedius* and *S. nudus* (Pettit et al. 1979; Sasaki and Endo 1987) are not yet employed in therapy. However, analyses of the action mechanism and the notion of active sites in biologically active peptides may contribute to the development of novel pharmaceuticals without the side effect of antigenicity.

A large variety of toxic substances have been used for a long time as drugs. In addition, a number of useful biologically active substances such as anodynes have been synthesized based on the molecular structure of toxins. The fact that saponins and terpenoids occur only in Echinodermata in the animal kingdom suggests that echinoderms are capable of synthesizing unknown substances. Saponins from asteroids and holothurians display a variety of pharmaceutical activities. The utilization of synthetic activities specific to asteroids and holothurians is one way of producing new medications. Novel biologically active substances may act as potential leading compounds even though their isolation for such use is not commercially practical.

Finally, the blooming of starfish and sea cucumber, *Cucumaria echinata*, is a serious obstacle to fishery and the only countermeasure against it is their extirpation. The 2000 annual catch of starfish in Hokkaido, Japan, was reported to be 16,000 t. The disposal of extirpated animals is a serious problem for the fishery sectors. Exploitation of chemicals from these animals would be beneficial regarding both disposal of industrial waste and production of useful substances.

9 Appendix: Antique Illustrations of Echinoderms in Japan

We can go back to an encyclopedia, *Wakansansaizue*, edited by the physician Ryoan Terashima in 1712, to the scholarly description and illustration of the echinoderm. A sea urchin, a supposed starfish, a sand dollar and a sea cucumber were described and illustrated (Figs. 4–6). The encyclopedia is composed of 105 volumes. It was edited on the basis of an antique idea that human diseases were generated by three components: the heavens, earth (including animals and plants) and humans. *Wakansansaizue* literally means Japanese–Chinese encyclopedia on the three components.

In 1762, Yoritaka Matsudaira completed a series of picture books on natural history entitled *Shurinshukan* in order to enhance the local activity of fishery and related industries. Four of them, called *Shurinzu* and dealing with fishes, are highly valued for the quality of their drawings. Even echinoderms, sea cucumbers, starfish and brittle stars are illustrated in the third issue of *Shurinzu* (Figs. 7 and 8).

Senchufu (1811) by Tanshu Kurimoto (1756–1834) describes 27 species of echinoderms. This number seems incorrect because some species appeared

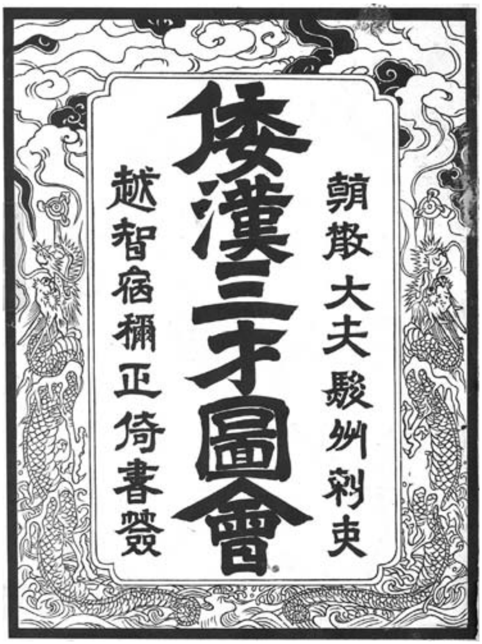


Fig. 4. Cover of *Wakansansaizue* (from the reprint published in 1902 by Chugai Shuppan, Tokyo)

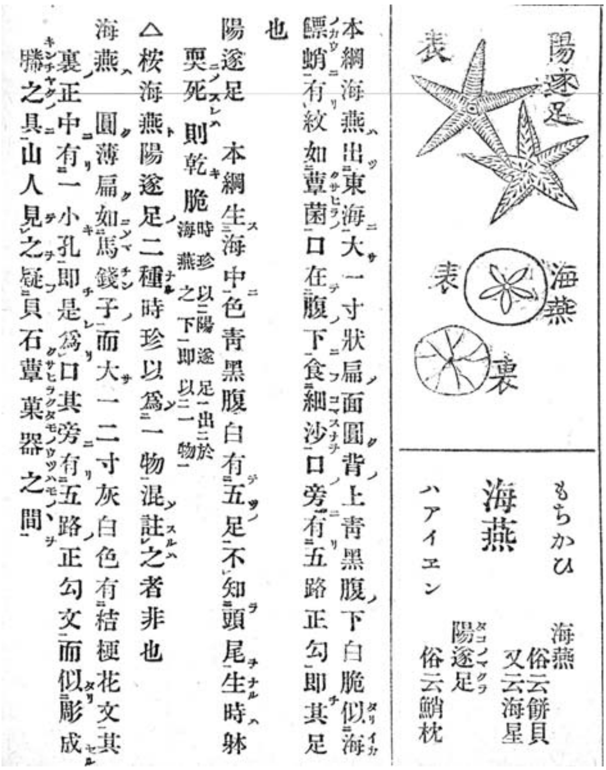
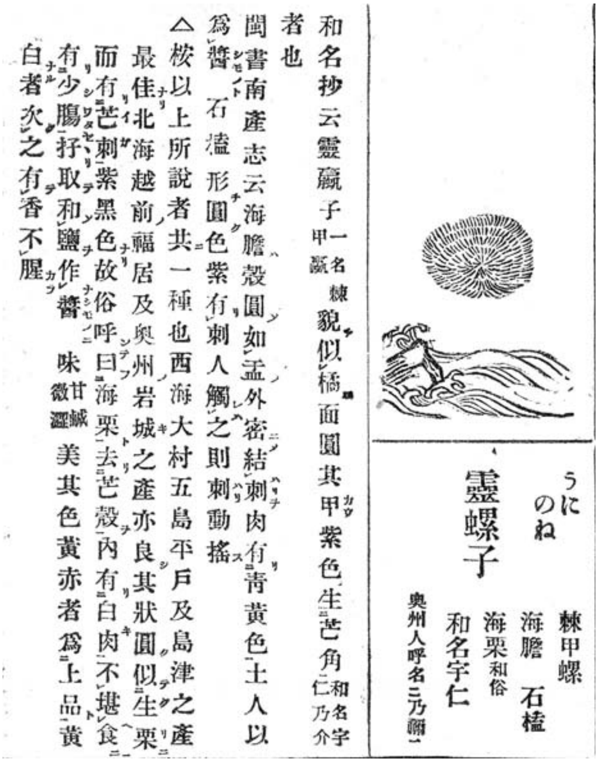


Fig. 5. Illustration and description of starfish and sand dollar in *Wakansansaizue* (from the reprint published in 1902 by Chugai Shuppan, Tokyo). The author classified these two animals into different animal groups, although a famous Chinese scholar, Li Shizhen (1518–1594), described them as belonging to the same category in his pharmaceutical book *Bencaogangmu*. A starfish, named Takonomakura, is a blue-grey animal with five podia. A sand dollar, called Mochikai, has a thin circular body, on which a penta-radiated pattern is seen. A starfish is assumed *Asteropecten* sp. judging from the explanation given. An animal called Takonomakura is today *Clypeaster japonicus*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靈螺子
棘甲螺
海膽 石橲
海栗 和俗
和名宇仁
奥州人呼名乃爾

和名抄云靈蠃子一名甲蠃棘貌似橘面圓其甲紫色生芒角和名宇仁乃介
者也
閩書南產志云海膽殼圓如盂外密結刺肉有青黄色土人以
爲醬石橲形圓色紫有刺人觸之則刺動搖
△按以上所說者共一種也西海大村五島平戶及島津之產
最佳北海越前福居及奥州岩城之產亦良其狀圓似生栗
而有芒刺紫黑色故俗呼曰海栗去芒殼內有白肉不堪食
有少腸抒取和鹽作醬 味甘微鹹澀 美其色黄赤者爲上品黄
白者次之有香不腥

Fig. 6. Description of sea urchin in *Wakansan-saizue* (from the reprint published in 1902 by Chugai Shuppan, Tokyo). The body is round and similar to a bur. The animal moves its spines in response to contact stimuli. Localities of sea urchin production and quality of sea urchin roes were explained. The description of the animal in the text suggests that the species is *Anthocidaris crassispina*

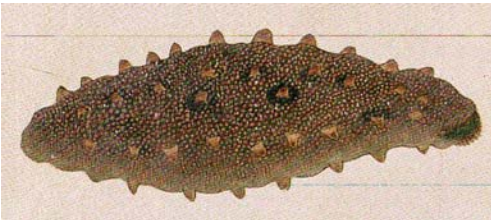

Fig. 7. Sea cucumber in *Shurinzu* drawn by Bunryu Miki (1762) (from a publication of the Kagawa History Museum, 2003). The animal appears to be *Apostichopus japonicus*. Original drawing is in color

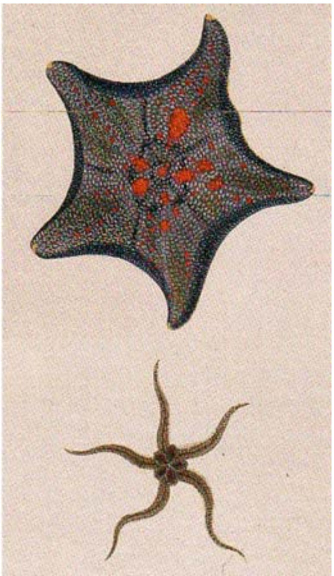

Fig. 8. Starfish and brittle star in *Shurinzu* (from a publication of the Kagawa History Museum, 2003). The starfish is recognized as *Asterina pectinifera*. Original in color

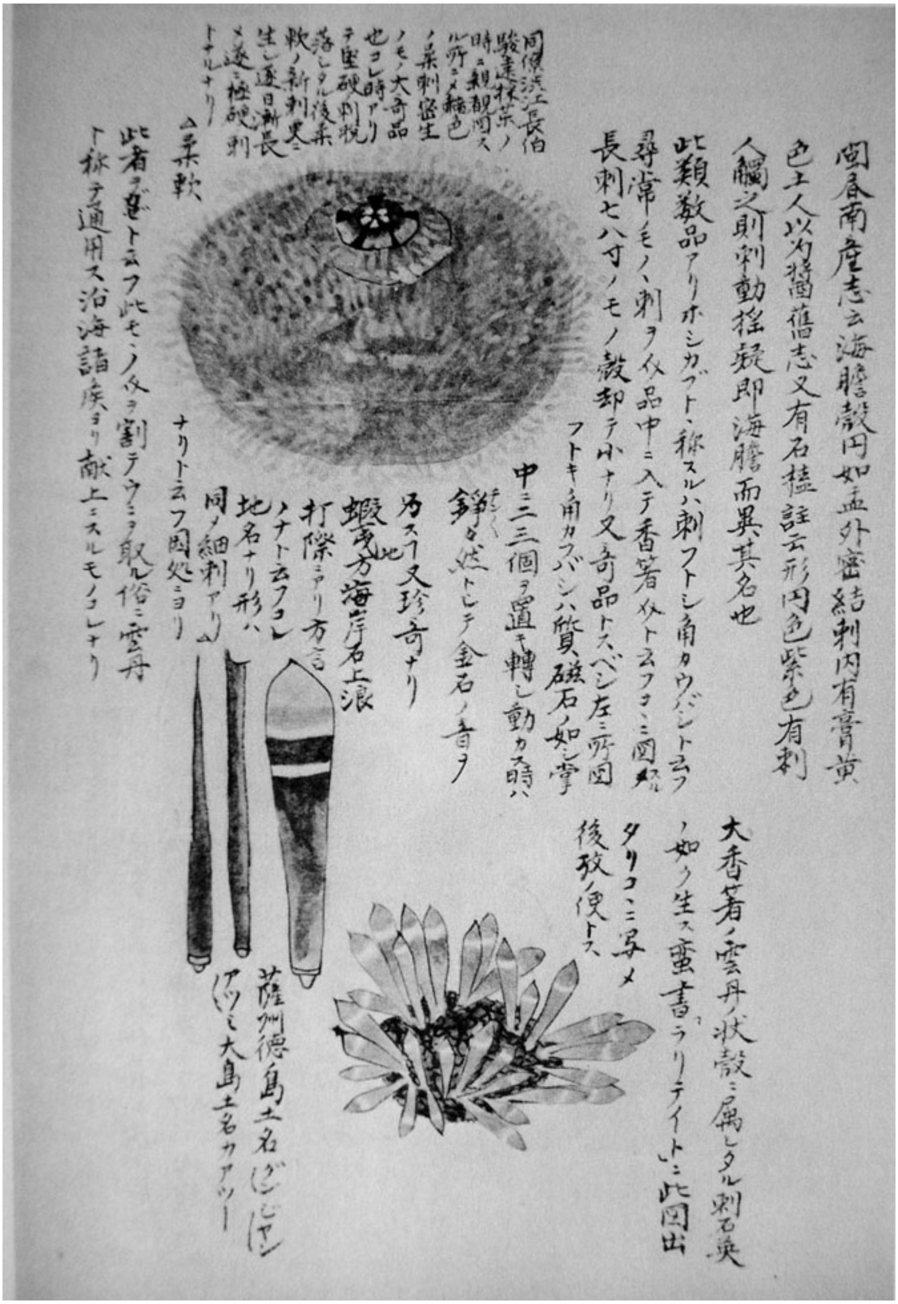

Fig. 9. Description of *Heterocentrotus mamillatus* in *Senchufu* (1811) (from the reprint published by Kowa Shuppan, 1982). It was described that this species occurred in southern Japan

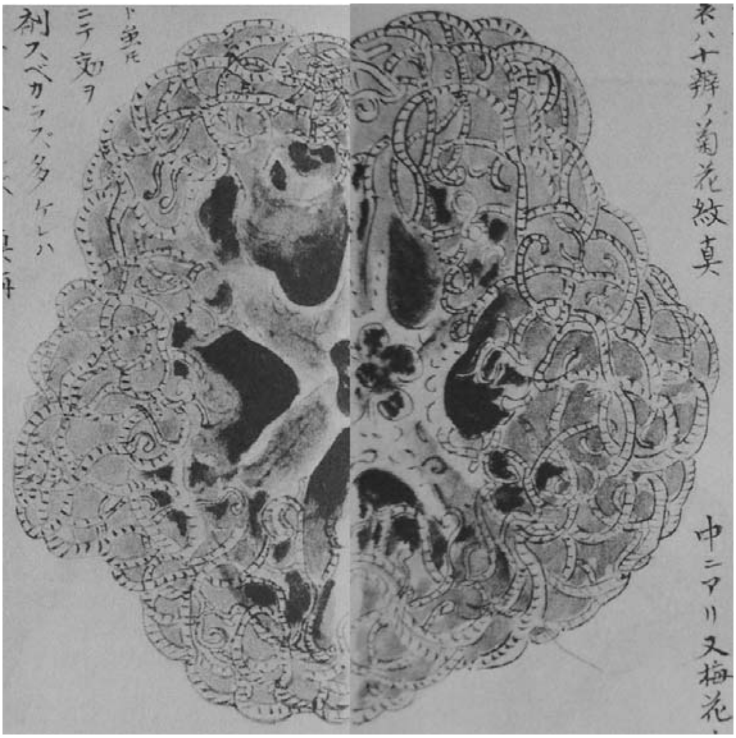

Fig. 10. Illustration of *Gorgonocephalus eucnemus* from *Senchufu* (1811) (from the reprint published by Kowa Shuppan, 1982). This animal was cited as a peculiar species of starfish. It was written that powdered *Gorgonocephalus* was used as a medication for bruising. The animal was reported to have fundamentally five large tentacles separating into a large number of small tentacles. A penta-radiated pattern like a cherry blossom was described in the center of the body. The animal is illustrated on two facing pages

two or more times in the different sections and the identification of species was unclear. *Stichopus japonicus*, *Asteropecten*, *Diadema setosum*, *Asterina pectinifera*, *Strongylocentrotus intermedius*, *Heterocentrotus mamillatus*, *Astriclypeus manni*, *Asterias amurensis*, *Gorgonocephalus eucnemus*, etc. were illustrated (Figs. 9 and 10). The original book of *Senchufu* was lost due to fire and only some reproductions remain.

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