into several sizes which carry different H-2 alloantigenic specificities. This separation is, in many respects, identical to the size separation of the HL-A alloantigen fragments.

The comparable molecular findings for the human HL-A alloantigens and the mouse H-2 alloantigens probably reflect a similar genetic mechanism, most likely that of multiple structural cistronic control.

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Antifungal Steroid Glycoside from Sea Cucumber

Abstract. *An antifungal steroid glycoside, holotoxin, has been isolated from the sea cucumber* Stichopus japonicus *(Selenka). In vitro, it exhibits high activity against various fungi, including vegetable pathogens, but has scarcely any activity against Gram-positive and Gram-negative bacteria and mycobacteria in vitro.*

As a result of a study on the chemical components of certain species of sea cucumber, such as *Stichopus japonicus* (Selenka), *St. chloronotus* (Brandt), *Holothuria pervicax* (Selenka), *H. monacaria* (Lesson), *H. leucospilota* (Brandt), *Cucumaria frondosa* var. *japonica* (Semper) which inhabit the sea surrounding Japan, a new antifungal substance has been discovered. It has a high activity against pathogenic fungi and has been named holotoxin.

After the viscera and body fluid were removed, 1 kg of *Stichopus japonicus*, consisting of body wall tissues, was sliced and dried. To about 100 g of the dried material, 500 ml of methanol was added; the mixture was heated under reflux on a water bath for 6 hours and filtered while hot. This procedure was repeated three times with 300 ml of methanol. The filtrates were combined, and the methanol was evaporated under reduced pressure. The residue was dried and redissolved in 300 ml of hot methanol. The methanol solution was filtered to remove insoluble substances, and the methanol was evaporated under reduced pressure. The residue was stirred with 100 ml of benzene, and the insoluble material was recovered by centrifugation. The residue then was treated with 50 ml of benzene, and the solids were recovered. The insoluble material was next stirred with the minimum quantity of water required to form a suspension, and the material insoluble in water, obtained by centrifugation of the suspension, was washed with a little water to give the crude holotoxin. The crude holotoxin was purified by recrystallization from ethanol several times (yield, 87 mg).

Table 1. Minimum inhibitory concentration of holotoxin. Controls without holotoxin were conducted in the same way as the experiments, and they showed no activity.

Organism	Inhibition effect (μg/ml)
Sabouraud agar, 28°C, 96 hours	
Trichophyton asteroides	6.25
T. rubrum	6.25–1.56
T. interdigitale	6.25–1.56
Sabouraud agar, 28°C, 39 and 63 hours	
Candida albicans	16.7
Torula utilis	2.78
Saccharomyces cerevisiae	2.78
Potato agar, 28°C, 39 and 63 hours	
Penicillium chrysogenum	16.7
Aspergillus niger	16.7
Fusarium lini	16.7
Gibberella saubinetii	16.7
Glomerella cingulata	16.7
Ophiobolus miyabeanus	16.7
Piricularia oryzae	2.78
Potato agar, 28°C, 111 hours	
Helminthosporium avenae	16.7

Holotoxin forms colorless needles (melting point 250°C, with decomposition) and shows the following elementary analysis. Found: C, 51.90 percent; H, 7.93 percent; it exhibits no absorption in the ultraviolet region; in the infrared spectrum it has bands at 1745 and 1640 cm^{-1}, indicative of a five-membered ring lactone and one double bond, respectively. It is estimated to be a steroid glycoside, because after acid hydrolysis the aglycon, which is soluble in chloroform, gave a positive Liebermann-Burchard color reaction, and sugars in aqueous solution were detected by a positive reaction to aniline-phosphoric acid reagent and through the reduction of silver oxide. The infrared spectrum of holotoxin is closely similar to that of holothurin, which is a steroid saponin isolated from the sea cucumber (*1*). Holothurin is a sulfate, whereas holotoxin is not. The glycosides including holothurin do not exhibit antifungal activity. To our knowledge, holotoxin is the first antifungal glycoside which has been isolated from the animal kingdom.

The antifungal activity of holotoxin was tested in vitro. Crystalline holotoxin was dissolved in a 17.5 percent aqueous solution of dimethylformamide to give a solution having a concentration of 2 mg/ml. The resulting solution was serially diluted with sterile water and then added to a series of agar culture plates, each of which had been inoculated with a different test organism. The minimum inhibitory concentration of holotoxin was determined (Table 1).

Holotoxin has high activity against various fungi in vitro, including pathogenic organisms of vegetable origin, but it has scarcely any activity against Gram-positive and Gram-negative bacteria and mycobacteria (*2*).

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2. The results of clinical tests with holotoxin against superficial dermatophytosis in Kyoto University, Kyushu University (Fukuoka), Juntendo University (Tokyo), and Kitano Hospital (Osaka) demonstrated that holotoxin produced some improvement of symptoms in 77 cases out of 87, and this is equivalent to 88.5 percent effectiveness. Almost no side effects were noted.

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