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COMMUNICATIONS

Antineoplastic Agents XLV: Sea Cucumber Cytotoxic Saponins

Keyphrases □ Antineoplastic agents, potential—three saponins isolated from sea cucumbers, structures partially determined, activity evaluated □ Cytotoxic activity—evaluated in three saponins isolated from sea cucumbers □ Sea cucumbers—three saponins isolated and identified, screened for antineoplastic and cytotoxic activity □ Saponins—isolated from sea cucumbers, structures partially determined, antineoplastic and cytotoxic activity evaluated

To the Editor:

In Asia, various sea cucumbers (Echinodermata phylum, Holothurioidea class) are readily available under the name Trepang. One of these, *Stichopus japonicus* Selenka, is used for various medicinal purposes[1] (1) and human food. In 1929, Yamanouchi began to explore sea cucumber toxins; in 1942, he reported the isolation of a crystalline saponin mixture from *Holothuria vagabunda* Selenka (2). Subsequently, such saponin mixtures from sea cucumbers have been shown to possess various physiological activities, *e.g.*, neurotoxic (3), hemolytic (4), and the ability to inhibit growth of Crocker mouse sarcoma 180 and Krebs-2 ascites tumors in Swiss mice (5).

The actual composition of these saponin mixtures continues to be a challenging area for chemical study. So far, evidence has been presented only for the structure of the antifungal agent holotoxin A (I) from *S. japonicus* Selenka (6). However, the lanostane derivatives obtained by acid hydrolysis of the saponin mixtures received detailed investigation (7), particularly by Tan *et al.* (8) who recently provided definitive structural evidence for holotoxinogenin (II*a*) (6) and its 25-methyl ether derivative (II*b*).

About 10 years ago, in collaboration with the National Cancer Institute, we began the first systematic and worldwide survey of marine animals as potential sources of new antineoplastic agents (9). Many marine animal species have been located which yield extracts with a confirmed level of activity in the National Cancer Institute's P-388 murine lymphocytic leukemia, 9KB cell culture, and/or the P-388 and L-1210 cell culture evaluation systems (10). Of these potentially important marine animal species, six were Holothurioidea. Three were selected for detailed study: two from the family Stichopodidae, namely, *Stichopus chloronotus* Brandt (from Australia) and *Thelenota ananas* Jaeger (from Taiwan and the Marshall Islands), and one from the family Holothuriidae, *Actinopyga mauritiana* (from Hawaii).

We now wish to report that separation of the cytotoxic and antineoplastic constituents, guided by bioassay, led in each case to a complex mixture of lanostane-type saponins characteristic of sea cucumber Cuvierian organ toxins. The major cytotoxic component of each saponin mixture was isolated and designated stichostatin 1, thelenostatin 1, and actinostatin 1, respectively. A description of the isolation, purification, and partial characterization of stichostatin 1 (P-388, ED_{50} = 2.9) provides a summary of the techniques found most useful in this first detailed study of sea cucumber cytotoxic constituents.

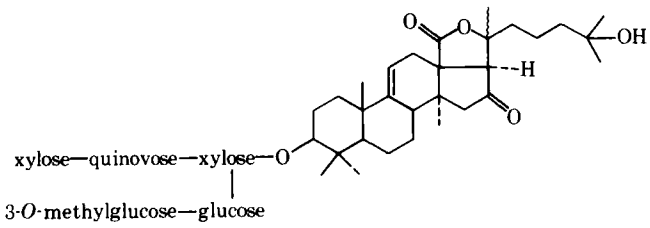
A 2-propanol extract (59 g) obtained from *S. chloronotus* Brandt was partitioned between benzene and water. The water phase was separated and extracted with 1-butanol, and the extracted material was partitioned between ether and water. Freeze drying of the water phase afforded 1 g of the saponins, which were carefully separated on a specially prepared (11) prepacked silica gel column [elution with chloroform–methanol–water (20:5:0.5)].

Fractions 71–106 (6-ml volumes) contained stichostatin 1 (0.19 g). Recrystallization from methanol–chloroform gave needles (0.13 g) melting at 279–280°. Elemental analyses and molecular weight determinations by osmometry methods suggested a molecular weight of 1200 and an empirical formula of $C_{54-56}H_{93-98}O_{27-28}$. A series of mass (molecular ions were not observed), PMR, IR, and UV spectral studies, combined with preliminary chemical evidence, suggests that stichostatin 1 contains a lanostane nucleus related to that of Structure II*c* (8) and a glycoside system related to that of holotoxin A (I).

The structurally related cytotoxic saponins thelenostatin 1 (needles, mp 213–217°, from chloroform–methanol–water) and actinostatin 1 (needles, mp 218–220°, from chloroform–methanol–water) were obtained and partially characterized by similar methods from *T. ananas* Jaeger and *A. mauritiana*, respectively. In addition, both thelenostatin 1 (P-388, ED_{50} = 1.5) and actinostatin 1 (KB, ED_{50} = 2.6 and L-1210, ED_{50} = 2.1) were found to be half-esters of sulfuric acid similar to holothurin A, which is believed to contain the lanostane system illustrated by Structure III (12).

A definitive structural elucidation of these three new cytotoxic saponins, combined with further cytotoxic and antineoplastic evaluations, will be undertaken. Pres-

[1] Trepang is prepared by boiling the sea cucumber to cause evisceration and partial deactivation of the toxins.



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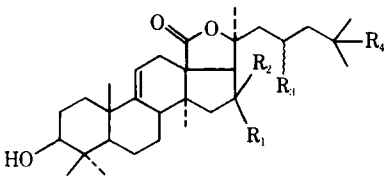
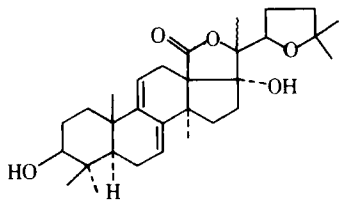


IIa: $R_1 = R_2 = O, R_3 = H, R_4 = OH$
IIb: $R_1 = R_2 = O, R_3 = H, R_4 = OCH_3$
IIc: $R_1 = OH, R_2 = H, R_3 = OCOCH_3, R_4 = H$



III

ently available evidence strongly indicates that such sea cucumber saponins have potentially useful and interesting biological properties. The inhibition of cell growth by the three tetracyclic triterpene saponins described in this report may be due to the ability of certain sea cucumber saponins to inhibit protein synthesis (rat bone marrow tissue culture) and RNA synthesis (yeast cell culture) (13).

(1) G. B. Elyakov, T. A. Kuznetsova, and V. E. Vaskovsky, *Khim. Prir. Soedin*, **4**, 253(1968); through *Chem. Abstr.*, **70**, 45173(1969).

(2) T. Yasumoto, K. Nakamura, and Y. Hashimoto, *Agr. Biol. Chem.*, **31**, 7(1967). B. W. Halstead, "Poisonous and Venomous Marine Animals of the World," vol. 1, U.S. Government Printing Office, Washington, D.C., 1965, p. 574.

(3) S. L. Friess, F. G. Standaert, E. R. Whitcom, R. F. Nigrelli, J. D. Chanley, and H. Sobotka, *J. Pharmacol. Exp. Ther.*, **126**, 323(1959).

(4) J. Lasley and R. F. Nigrelli, *Zoologica*, **56**, 1(1971).

(5) R. F. Nigrelli, *ibid.*, **37**, 89(1952). T. D. Sullivan, K. T. Ladue, and R. F. Nigrelli, *ibid.*, **40**, 49(1955).

(6) I. Kitagawa, T. Sugawara, and I. Yosioka, *Tetrahedron Lett.*, **1975**, 963; I. Kitagawa, T. Sugawara, and I. Yosioka, *Chem. Pharm. Bull.*, **24**, 275(1976).

(7) J. D. Chanley and C. Rossi, *Tetrahedron*, **25**, 1897(1969). B. Tursch, R. Cloetens, and C. Djerassi, *Tetrahedron Lett.*, **1970**, 467. P. Roller, C. Djerassi, R. Cloetens, and B. Tursch, *J. Am. Chem. Soc.*, **91**, 4918(1969). G. Habermehl and G. Volkwein, *Justus Liebigs Ann. Chem.*, **731**, 53(1970). P. Roller, B. Tursch, and C. Djerassi, *J. Org. Chem.*, **35**, 2585(1970). I. Rothberg, B. M. Tursch, and C. Djerassi, *ibid.*, **38**, 209(1973). J. D. Chanley, R. Ledeen, J. Wax, R. F. Nigrelli, and H. Sobotka, *J. Am. Chem. Soc.*, **81**, 5180(1959).

(8) W. L. Tan, C. Djerassi, J. Fayos, and J. Clardy, *J. Org. Chem.*, **40**, 466(1975). G. B. Elyakov, V. A. Stonik, E. V. Levina, V. P. Slanke, T. A. Kuznetsova, and V. S. Levin, *Comp. Biochem. Physiol.*, **44B**, 325(1973).

(9) G. R. Pettit, J. F. Day, J. L. Hartwell, and H. B. Wood, *Nature*, **227**, 962(1970) and Abstracts (No. 190) of the 30th Annual Northwest Regional Meeting of the American Chemical Society, University of Hawaii, Honolulu, Hawaii, June 12–13, 1975.

(10) R. I. Gueran, N. H. Greenberg, M. M. Macdonald, A. M. Schumacher, and B. J. Abbott, *Cancer Chemother. Rep., Part 3*, **3**, No. 2 (Sept. 1972).

(11) D. L. Herald, R. H. Ode, and G. R. Pettit, *J. Chromatogr. Sci.*, **14**, 356(1976).

(12) S. L. Friess, R. C. Durant, W. L. Fink, and J. D. Chanley, *Toxicol. Appl. Pharmacol.*, **22**, 115(1972).

(13) M. M. Anisimov, N. G. Prokof'eva, T. A. Juznetsova, and N. V. Peretolchin, *Izv. Akad. Nauk SSSR, Ser. Biol.*, **1**, 137(1971); through *Chem. Abstr.*, **74**, 73825(1971). S. I. Baranova, A. L. Kul'ga, M. M. Anisimov, V. A. Stonik, E. V. Levina, V. S. Levin, and G. B. Elyakov, *ibid.*, **2**, 284(1973); through *Chem. Abstr.*, **78**, 143997(1973).

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For Part XLIV of this series, see G. R. Pettit, *China Q.*, in press.

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Starch Paste Granulations: Factors Causing Binder Dilution Effects on Granulations and Tablets

Keyphrases □ Starch paste granulations—effect of mixing time, speed, and binder dilution on physical properties of tablets □ Granulations, starch paste—effect of mixing time, speed, and binder dilution on physical properties of tablets □ Dosage forms—tablets, effect of mixing time, speed, and binder dilution on physical properties of tablets

To the Editor:

In a recent communication (1), the dilution factor of starch paste binder and its effects on granulations and tablets were reported. The conventional wet granulation process was used to prepare granulations in a small

Table I—Starch Paste Dilutions

	Formulation A	Formulation B	Formulation C
Lactose, g	860	860	860
Starch (in dry mix), g	47	47	47
Starch (in paste), g	26	26	26
Water (for paste), ml	100	130	160
Water (used to *qs*), ml	100	70	40