

Recent Development in Antineoplastic
Biopolymers
from Marine Organisms*¹

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Intrudocotion

Marine organisms comprise over a half million species and inhabit very unusual circumstances as compared with terrestrial organisms. Some organisms, such as marine algae, marine sponges, tunicates, nudibranchs, and bryozoans, lack any physical defence means in thier structures to avoid the menace of predators and the infection of pathogenic microorganisms. In order to keep alive and adapt in these severe environment, marine organisms seem to develop suitable bio-defence systems with characteristic metabolisms which are significantly different from those known in terrestrial organisms.

In this relation, the chemical metabolites of marine organisms have been paid much attention in recent years and an increasing number of marine natural products have been discovered. Those substances are not only diverse in their chemical structures, but also shown to exhibit varieties of biological activities, e.g. growth inhibitions against pathogenic microorganisms, physiological functions (pheromone, allelochemic and synomene), pharmacological actions, cytotoxicities and anti-cancer activities, and toxicities.

In this mean, the effort to discover new anti-cancer substances from marine organisms has been also undertaken. The resulting several interest biopolymers, for example the antineoplastic glycoproteins such as sphyrnastains from the hummerhead shark (Sphyrna lemini), stronglostains from the sea urchin (Stronglocontrolus drobachiensis), aplysianins from the sea hare (Aplysia kurodai) and dolabellanins from another sea hare (Dolabella auricularia) have been isolated recently. On the other hand, many novel antineoplastic compounds having usual molecular weight (e.g. macrolides, peptides, alkaloids, terpenoids and others) have been isolated from many marine organisms.

In this article, the important chemically clarified biopolymers, having strongly cytostatic and potent antineoplastic activities, from marine organisms were reviewed.

1. Example of The Biopolymer Screening

Recently, Sasaki and Endo¹⁾ have discovered the important antineoplastic polymer fractions from the water extracts of 25 marine organisms, in which the popular marine animals as Japanese foods, e.g. Awabi (Haliotis discus hannai), Sazae (Butillus cornutus), Hotategai (Aequipecten irradians), etc. are including.

The molecular weights and antineoplastic (*in vivo*) activities against sarcoma-180 of fractionated polymers were summarized in Table 1. Each polymer fraction was yielded by usual fractionation with Sephadex series.

The *in vivo* activity against Sarcoma-180 (mice) for polymer fractions was obtained by the systematic injection (i.p. 400mg/Kg/dose) to ICR mice (female). From the data in Table 1, it was found that many marine organisms produced the original antineoplastic polymers. In them, the polymers from Haliotis diversicolor aquatilis (Tokobushi), Butillus cornutus (Sazae), Placopecten magellanicus (Hotategai), Mercenaria mercenaria (Honbinosugai), Crassostrea virginica (Americagaki) and Ecteinascidia turbinata (Guntaiboya) showed the remarkable antineoplastic activities over 80% effect, respectively.

However, unfortunately, the extended purification and identification of these biopolymer fractions are not completed at this time.

Table 1. Antitumor Activity of the Polymers isolated from the Water Extracts of Marine Organisms ¹⁾

Japanese Name	Scientific Name	Molecular Weight of Polymers	Antitumor Activity (%) ^{a)}	Rate of Perfect Recovery
Ezoawabi	<i>Haliotis discus hannai</i>	MW > 10	61.6	0/6
		MW < 10	7.5	0/6
		MW > 50	87.6	0/6
		50 > MW > 10	57.3	0/6
Tokobushi	<i>H. diversicolor aquatilis</i>	300 > MW > 100	83.5	3/6
Sazae	<i>Butillus cornutus</i>	300 > MW > 100	96.7	1/6
Hotategai	<i>Placopecten magellanicus</i>	MW > 300	47.6	1/6
		300 > MW > 100	81.6	1/6
Hotategai	<i>Aequipecten irradians</i>	MW > 300	56.3	2/6
		300 > MW > 100	76.7	3/6

Table 1 (continued)

Japanese Name	Scientific Name	Molecular Weight of Polymers	Antitumor Activity (%) ^{a)}	Rate of Perfect Recovery
Honbinosugai (Quahog)	<i>Mercenaria mercenaria</i>	MW > 300	61.7	0/6
		300 > MW > 100	20.4	0/6
Honbinosugai (Little necked clam)	<i>M. mercenaria</i>	MW > 300	86.0	1/6
		300 > MW > 100	27.1	0/6
Honbinosuagi (Cherry stone clam)	<i>M. mercenaria</i>	MW > 300	50.5	0/6
		300 > MW > 100	75.7	0/6
Ohonogai	<i>Mya arenaria</i>	MW > 300	23.4	0/6
		300 > MW > 100	77.6	0/6
Igai	<i>Mytilus edulis</i>	MW > 300	12.1	0/6
		300 > MW > 100	4.7	0/6
Americagaki	<i>Crassostrea virginica</i>	MW > 300	29.0	0/6
		300 > MW > 100	82.2	0/6
Begaika	<i>Loligo paelici</i>	MW > 300	35.5	0/6
		300 > MW > 300	50.5	0/6
Ezobafununi	<i>Strongylocentrotus intermedius</i>	MW > 10	43.8	0/6
		MW < 10	34.9	0/6
Kitamura - sakiuni	<i>S. nudus</i>	MW > 10	74.0	0/6
		MW > 300	56.3	0/6
		300 > MW > 50	76.7	0/6
		50 > MW > 10	20.4	0/6
Literhoya	<i>Halocynthia hilgendorfi f. ritteri</i>	MW > 10	74.8	0/6
		10 > MW > 0.5	56.3	0/6
		50% EtOH extract	54.4	0/6
Shiroboya	<i>Styela plicata</i>	MW > 10	61.2	0/6
		10 > MW > 0.5	17.5	0/6
Yureiboya	<i>Ciona intestinalis</i>	50% EtOH extract	45.6	0/6
Guntaiboya	<i>Ecteinascidia turbinata</i>	MW > 10	92.2	3/6
Akafuji-tsubo	<i>Megabalanus rosa</i>	body fluid	41.1	0/6
		MW > 50	63.0	0/6
		50 > MW > 10	65.1	0/6
Erako kani	<i>Pseudopotamilla accelata</i>	MW > 10	28.1	0/6
Madaragani	<i>Geryon quinquedens</i>	MW > 300	47.7	0/6
		300 > MW > 100	47.7	0/6
Madaragani	<i>Canceræla irroratus</i>	MW > 300	56.1	0/6
		300 > MW > 100	52.3	0/6

a) Antitumor in vivo Activity against Sarcoma-180 (abdominal dropsy cell) was estimated by the followed formula.

$$\text{Activity (\%)} = (A - B) / A \times 100$$

A: Avarage of tumor weight on control

B: Avarage of tumor weihgt on treatment

In the experiment, ICR mice (female, ca. 23g weight) was used.

2. Sphyrnastatins and Strongylostatins

In 1977, Pettit and Ode reported that two antineoplastic glycoproteins, designated sphyrnastatin 1 and sphyrnastatin 2, were isolated from the water extract of blood and body fluids of the hammerhead shark *Sphyrna lemini* by chromatography on macroreticular resin followed by gel permeation chromatography.

Sphyrnastatins 1 and 2 were glycoproteins of 22% and 26% carbohydrate, respectively, with apparent molecular weights of at least 40×10^6 . Amino acid analyses indicated a minimum protein segment of 274 amino acid units for sphyrnastatin 1 and 380

amino acid units for sphyrnastatin 2. Upon biological evaluation, both compounds produced a 30-40% (at 11-13 mg/kg dose) life extension in mice inoculated with P-388 murine lymphocytic leukemia. ²⁾

In 1979, Pettit and co-workers reported that a new glycoprotein antineoplastic agent designated strongylostatin 1 was isolated from the water extract of the well-known green sea urchin *Strongylocentrotus drobachiensis* (Muller) of the Strongylocentrotidae family. A series of gel permeation chromatography was used for separation. The apparent molecular weight of this compound appeared to be over 40×10^6 . The results of amino acid analyses suggested a minimum protein segment of 546 amino acid units. Strongylostatin 1 (approximately 1.6% yield based on the aqueous extract) was found to produce 35-53% life extension in the P388 murine lymphocytic leukemia system. ³⁾

In 1981, Pettit and co-workers described that the same green sea urchin was found to contain a second protein anticancer constituent designated strongylostatin 2. It was found to be a glycoprotein with a unit/subunit molecular weight of at least 65,000. An amino acid analysis showed Gly to be most prominent, and the carbohydrate portion was estimated to represent 22% of the glycoprotein. Treatment of the murine P388 lymphocytic leukemia with strongylostatin 2 resulted in 39-42% life extension at a dose of 4.5mg/kg. ⁴⁾

3. Aplysianins

Recently, Kamiya and co-workers reported that three antitumor factors, aplysianins A, E and P, inducing tumor lysis were isolated from the sea hare *Aplysia kurodai*. Aplysianin E was purified to apparent homogeneity from the supernatant of a homogenate of eggs of *Aplysia kurodai*. Purified aplysianin E was a 250kDa glycoprotein containing

three different subunits. This factor was half-maximally active at 2-114 ng protein/ml and lysed all the tumor cells tested but did not lyse normal white or red blood cells. Aplysianin E was labile on treatments with heat, low pH, urea, guanidine, sodium lauryl sulfate, and periodate, but not with proteases or organic solvents. Aplysianin E completely inhibited the syntheses of DNA, RNA, and protein by tumor cells within 2 h and caused their complete cytolysis within 15 h. Tumor lysis by aplysianin E was inhibited by N-acetylneuraminic acid, suggesting that recognition of the sugar moiety is a key step in cytolysis induced by aplysianin E. Aplysianin E also prolonged the survival of mice bearing syngeneic MM46 ascites or solid tumors.^{5) 6)}

The cytolytic activity against various target cells was examined as shown in Table 2. All the murine and human tumor cells tested were lysed by purified aplysianin E in the concentration range of 2-114 ng protein/ml. In contrast, normal spleen cells and erythrocytes were resistant to this cytolytic factor. These results indicate that tumor cells are relatively susceptible to aplysianin E.

6) 8)

Table 2 Target cell specificity of aplysianin E

Target cells		ED ₅₀ ^a (ng protein/ml)
Murine	MM46 (mammary carcinoma)	2
	MM48 (mammary carcinoma)	35
	L1210 (leukemia)	2
	EL-4 (lymphoma)	30
	L929 (fibroblast)	114
Human	Raji (leukemia)	9
	Molt-3 (leukemia)	13
	K-562 (leukemia)	30
	PC-6 (lung adenocarcinoma)	30
	PC-7 (lung adenocarcinoma)	30
	PC-9 (lung adenocarcinoma)	60
Murine	Spleen cells	>1600
Sheep	Erythrocytes	>1600

^a Target cells were incubated with aplysianin E for 18 h. ED₅₀, concentration for 50% lysis of target cells.

Table 3 shows that aplysianin E as well as mitomycin C had a protective effect against the MM 46 tumor. Some mice that survived were resistant to challenge with a lethal dose of fresh tumor cells.

Table 3 Neutralizing effects of aplysianin E on MM46 tumor cells 5) 6)

Treatment	Dose/ mouse	Neutralization ^a		Challenge ^b	
		Survival ratio	Survival days	Survival ratio	Survival days
None		0/5	16.2 ± 0.8 ^c	0/5	18.8 ± 1.6
Mitomycin C	10 µg	5/5	>45	0/5	15.2 ± 2.5
Aplysianin E	0.1 unit ^d	1/5	17.0 ± 0.0	1/1	55
Aplysianin E	1 unit	4/5	23	2/4	18.0 ± 1.4
Aplysianin E	10 units	4/5	25	3/4	19

^a Murine MM46 tumor cells were pretreated with mitomycin C or aplysianin E *in vitro* at 37°C for 60 min. Then the cells (5×10^4 cells/mouse) were inoculated i.p. into C3H/He mice. Data are representative of two similar experiments.

^b Fresh MM46 tumor cells (5×10^4 cells/mouse) were inoculated i.p. into surviving mice 50 days after neutralization.

^c Mean ± SD.

^d One unit equals 8.2 µg of protein.

Aplysianin A was isolated from the albumen gland of *Aplysia kurodai*. The aplysianin A obtained showed similar physiological activity to that of aplysianin E, an active factor in eggs. It showed a 50% inhibition of *B. subtilis* growth at a concentration of 4µg protein/ml. The concentration required for 50% lysis of murine MM46 tumor cells was as low as 14ng protein/ml. Physicochemical properties were, on the other hand, quite different from those of aplysianin E. Aplysianin A, which has a molecular weight of approximately 320,000 and contains four M_r 85,000 subunits, is larger than aplysianin E. However, these two factors have a common antigen and a similar amino acid composition, suggesting that aplysianin A is a precursor of aplysianin E. ⁷⁾

Aplysianin P was purified from the purple fluid of *Aplysia kurodai*. Purified aplysianin P was a single polypeptide of m.w. 60,000. This factor was half-maximally active at 3-25 ng protein/ml and lysed all the tumor cells tested but did not lyse normal WBC or RBC. Aplysianin P was labile on treatments with heat, low pH, urea, and periodate, but not with Pronase. The factor completely inhibited the syntheses of DNA, RNA, and protein by tumor cells within 2 h and caused their complete cytolysis within 18 h. Tumor lysis by aplysianin P was also inhibited by N-acetylneuraminic acid, suggesting that recognition of the sugar moiety is a key step in the cytolysis induced by aplysianin P. The factor also prolonged the survival of mice bearing syngeneic MM46 ascites. It did not resemble previously isolated antineoplastic glycoproteins from the eggs (aplysianin E) or albumen gland (aplysianin A) of *A. kurodai* in terms of molecular size, antigenicity, or amino acid composition. ^{8) 9)}

The cytolytic activity against various target cells was examined, as shown in Table 4 all the murine and human tumor cells tested were lysed by purified aplysianin P in the concentration range of 3-25 ng protein/ml. In contrast, normal spleen cells and erythrocytes were resistant to this cytolytic factor. These results indicate that tumor cells are relatively susceptible to aplysianin P.

Table 4 Target cell specificity of aplysianin P 819)

Target cells	ED ₅₀ ^a (ng/protein/ml)
Murine	
MM46 (mammary carcinoma)	10
MH134 (hepatoma)	8
L1210 (leukemia)	3
P388 (leukemia)	5
YAC-1 (lymphoma)	11
EL-4 (lymphoma)	4
Human	
K-562 (leukemia)	25
Raji (leukemia)	3
Molt-3 (leukemia)	5
PC-6 (lung adenocarcinoma)	20
PC-7 (lung adenocarcinoma)	25
PC-9 (lung adenocarcinoma)	25
Murine	
Spleen cells	>40,000
Blastogenic spleen cells	>40,000
Sheep	
Erythrocytes	>40,000

^a Target cells were incubated with aplysianin P for 18 h. ED₅₀, concentration for 50% lysis of target cells.

4. Dolabellanins

The Indian and the Pacific Ocean sea hare, Dolabella auricularia, is a large shell-less opisthobranch mollusk which is found intertidally feeding on brown algae. Dolabella auricularia contain cytotoxic and/or antineoplastic substances such as dolastatins, as shown in the section of peptides. These substances are low molecular weight compounds. Recently, Kamiya and co-workers found high molecular weight compounds with antineoplastic and antibacterial activities in the body fluid, albumen gland and purple fluid of Dolabella auricularia. These active substances of Dolabella auricularia were also isolated as glycoproteins, which differed from aplysianins in Aplysia kurodai.^{18) - 13)}

An antineoplastic factor, dolabelanin C, including tumor lysis activity was purified to apparent homogeneity from the body fluid of the sea hare Dolabella auricularia. Purified dolabellanin C is a glycoprotein of 215

kDa containing 3 subunits of 70 kDa. The amino acid sequence of the amino terminal region was also determined. This factor was active even at 0.38ng protein/ml, but did not lyse normal white or red blood cells.¹⁰⁾

Dolabellin A was purified to apparent homogeneity from the albumen gland of the sea hare *Dolabella auricularia*. Dolabellin A was a glycoprotein of 250 kDa containing 4 subunits. The amino acid composition and the N-terminal amino acid sequence of the factor were determined. By dolabellin A, all the murine and human tumor cells tested were lysed at the concentration range of 1-18 ng protein/ml (Table 5). The *in vivo* antitumor activity of dolabellin A was tested by incubating mixtures of tumor cells and dolabellin A *in vitro* and then injecting them intraperitoneally into mice. (Table 6). Mitomycin C was used as a positive control. Dolabellin A had protective effect against the MM46 tumor ($P > 0.001$) and also some animals survived after injection of 4 ng/mouse daily for 6 days (see Table 6).^{11) 12)}

Table 5 Target Cell Specificity of Dolabellin A 11)

	Target cells	ED ₅₀ ^{a)} (ng protein/ml)
Murine	MM46 (mammary carcinoma)	18
	MM48 (mammary carcinoma)	6
	MH134 (hepatoma)	2
	Macrophages	525
	Lymphocytes	1×10^5
	Red blood cells	$> 2 \times 10^5$
Human	A549 (lung carcinoma)	1.2
	LS174T (colon carcinoma)	3.4
	T24 (bladder carcinoma)	1.2
	WiDr (colon carcinoma)	0.9
	TIG-1 (fetal lung)	62
	IMR-90 (fetal lung)	68
	WI-38 (lung diploid)	120
	Red blood cells	$> 2 \times 10^5$

a) Target cells were incubated with dolabellin A for 18 h. ED₅₀ concentration for 50% lysis of target cells.

Table 6 Effect of Dolabellamin A on Survival of Mice Bearing Ascitic MM46 Tumor Cells

12)

Treatment	Dose/ mouse	Survival ratio	Survival days
Expt. I (Neutralization)^{a)}			
Saline		0/5	16.8 ± 0.8
Mitomycin C	10 µg	5/5	> 50
Dolabellamin A	0.2 µg	0/5	17.8 ± 0.8
Dolabellamin A	2 µg	0/5	19.2 ± 1.6 <i>p</i> < 0.05
Dolabellamin A	20 µg	3/5	22.5 ± 2.1 <i>p</i> < 0.001
Expt. II (i.p.injection)^{b)}			
Saline	0.2 ml × 6	0/6	16.3 ± 1.0
Mitomycin C	10 µg × 6	5/5	> 50
Dolabellamin A	0.8 µg × 6	0/6	17.3 ± 1.4
Dolabellamin A	4 µg × 6	3/6	20.3 ± 4.2 <i>p</i> < 0.001

^{a)} Murine MM46 tumor cells were treated with mitomycin C or dolabellamin A *in vitro* at 37°C for 30 min. Then the cells (5×10^4 cells/mouse) were injected i.p. into C3H/He mice. ^{b)} C3H/He mice received ascitic MM46 cells i.p. on day 0 and were treated i.p. with saline, mitomycin C or dolabellamin A daily for 6 d from day 0.

Third cytolytic factor, dolabellamin P, was purified to apparent homogeneity from the purple fluid of *Dolabella auricularia*. Purified dolabellamin P is a single polypeptide of 60 kDa. Examination of the amino acid composition of dolabellamin P revealed that it contained a relatively large amount of Asx and Glx. The sequence of the amino terminal region of dolabellamin P was shown:

Val-()-Arg-Glu-Asp-Gln-Lys-Trp-Lys-Leu-Ser-Phe-Asp-Lys-Glu-----

This factor nonspecifically lysed all the cell tested at 50-200ng protein/ml. Dolabellamin P caused completed cytolysis within 2h.¹³⁾, as shown in Table 7.

Table 7 Target Cell Specificity of Dolabellamin P ¹³⁾

	Target cells	ED ₅₀ ^{a)} (ng protein/ml)
Murine	MM46 (mammary adenocarcinoma)	100
	MM48 (mammary adenocarcinoma)	80
	MHI34 (lung adenocarcinoma)	60
Human	A 549 (lung adenocarcinoma)	100
	T 24 (urinary bladder carcinoma)	50
	SK LEU-1 (lung adenocarcinoma)	200
Murine	Spleen cells	67
	Thymus cells	150

^{a)} Target cells were incubated with dolabellamin P for 18 h. ED₅₀: concentration giving 50% lysis of target cells.

5. Halitoxin

Baslow et al. reported that the aqueous extracts of the sponge *Haliclona viridis* was toxic for mice.¹⁴⁾ Ten years later (1978), from several species of the same genus *Haliclona*, a cytotoxic metabolite called halitoxin (1) was isolated by Schmitz and co-workers. The structure proposed for halitoxin was a polymer consisting of various combination of different pyridinium units of two general structural types, as shown in Chart.1²⁾ Halitoxin was hemolytic and toxic to fish and mice. The cytotoxicity of fractionated halitoxin R (m.w. 500-1000) was reported as ED₅₀ 2.8 μg/ml in KB cell culture.¹⁵⁾

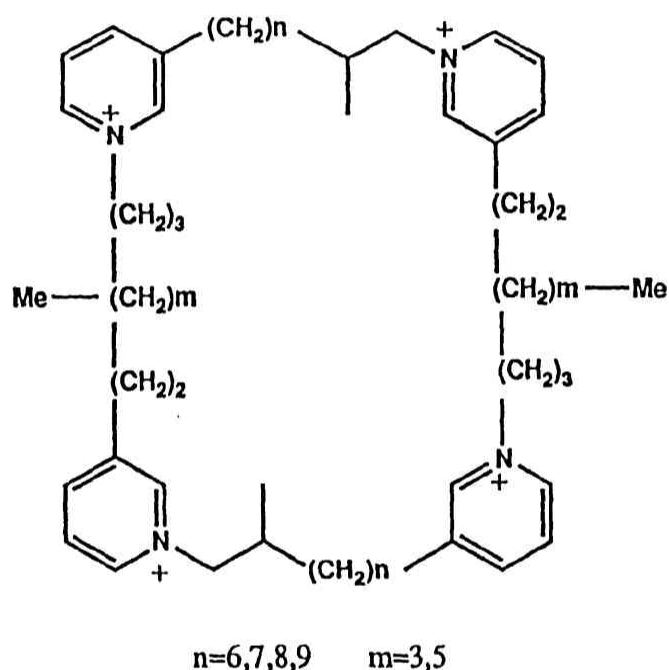


Chart 1

6. Polypeptides

Mucus secreted from the integument of the marine heteronemertine *Cerebratulus lacteus* (Leidy) contains two major types of polypeptide neurotoxin. The A toxins (11,000 daltons) which are lethal to a variety of animal species including mammals. The B toxins (6,000 daltons) which appear to be selectively toxic for crustaceans. Both types of toxin were readily obtained from live worms by stimulation and collection of mucus with 1% acetic acid followed by batch adsorption of the basic polypeptide fraction on CM-cellulose.¹⁶⁾

A toxins were homogeneous by gel electrophoreses and amino acid composition analyses, and by NH₂-terminal and COOH-terminal partial sequence analyses.

As shown in Chart 2, each of three A toxins(2) consists of a single basic polypeptide chain of 93 to 99 residues, cross-linked by three or four disulfide bonds, lacking reducing sugar and cyteinyl residues. The three A toxins rapidly lysed human red cells and Ehrlich ascites tumor cells at 1 to 10 $\mu\text{g/ml}$ concentration. ¹⁷⁾

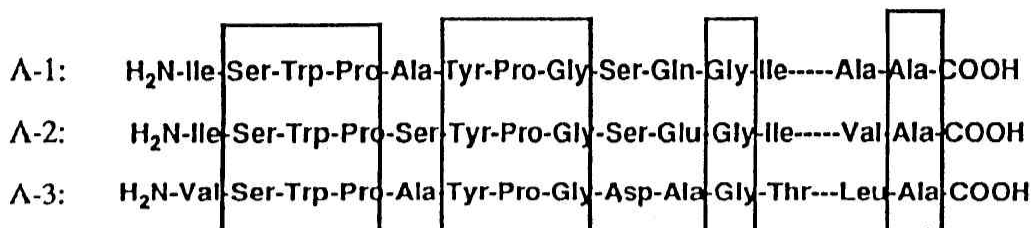


Chart 2

7. Antineoplastic Polymers from Marine Bacteria

An active polysaccharide, named marinactan was obtained by Umezawa and co-workers by a screening of antitumor activity against sarcoma 180 solid tumor in mice from the marine bacterium *Flavobacterium uliginosum*. Marinactan is a novel heteroglycan consisting of glucose, mannose and fructose in a ratio of approximately 7:2:1. Marinactan, 10-50mg/kg per day for 10 days i.p., produced 70-90% inhibition of the growth of solid sarcoma 180. Complete regression of the tumor was observed in some treated mice. Its administrations before and after tumor transplantation showed almost the same inhibitory effect. Marinactan prolonged markedly the survival period of mice bearing ascites sarcoma 180. ¹⁸⁾

Antitumor activity of the marine bacterium, *Vibrio anguillarum* P-B-1 against Ehrlich carcinoma cells in ddY mice, was investigated by Shimizu and co-workers. The aqueous layer obtained by the hot phenol-water procedure exhibited more antitumor activity than did the middle layer or the phenol layer. This finding indicated that lipopolysaccharide (LPS) derived from *V. anguillarum* exhibited significant antitumor activity. In fact, mice injected with LPS obtained by ultracentrifugation and treatment with RNase had a longer mean survival period than the control mice. *V. anguillarum* LPS also inhibited the growth of syngeneic fibrosarcoma induced by 3-methylcholanthrene in C57BL/6 mice. ¹⁹⁾

By Creech and Breuninger, the antitumor properties of polysaccharide fractions, obtained by high speed centrifugation of aqueous extracts of the cells of *Serratia*

marcescens followed by phenol extraction and trypsin digestion, were investigated. The polysaccharide fractions were highly effective in causing complete regression of well established solid sarcoma 37 in Swiss mice. The products derived primarily from the cell wall were more potent than the polysaccharides of extracellular and cytoplasmic origins.²⁰⁾

8. Miscellaneous

*Boiled Scallop Extract.*²¹⁾ Sasaki and co-workers reported that a fraction from boiled scallop extract modified by a sonication procedure exhibited marked antitumor activity when it was injected intradermally into either ICR or C3H/He mice that had been given SC implants of sarcoma 180 and Ehrlich carcinoma. However, this fraction was almost ineffective against L1210 cells. In a smaller dose (100 mg/kg) in *in vivo* sarcoma 180 test, this fraction was still effective. It had an inhibition ratio of 74.2% and caused complete tumor regression in 2 of 5 mice. The inhibition ratio observed with Ehrlich carcinoma was 73.5%. From the result of a further fractionation and purification, this fraction was found to contain two active principles -one was probably a heat-stable protein (mol. wt., ~21,000 daltons) and the other, a heat-unstable peptidoglycan (mol. wt., ~ 100,000 daltons).

*Mercenaria extract.*²²⁾ In 1964, Schmeer reported that a polymeric fraction, which was obtained from the water extract of *Mercenaria mercenaria* by partial purification with sephadex G-25, inhibited both sarcoma 180 and Krebs 2 carcinoma in Swiss albino mice. Unfortunately, further investigation has not been done.

*Marine Animal Lectin.*²³⁾ By Kamiya and co-workers, several lectins from marine animals, such as *Balanus roseus* hemagglutinin, *B. balanoides* hemagglutinin, *Tetraclita squamosa japonica* hemagglutinin and *Aplysia kurodai* agglutinin, were tested for induction of tumor lysis mediated by macrophages. Among them, *B. roseus* and *B. balanoides* lectins agglutinated several murine tumor cells and induced binding of macrophages to tumor cells. Binding of these cells was inhibited by galacturonic acid, suggesting that carbohydrate moieties on the cell membrane of the two types of cells are recognized by these lectins. *B.roseus* lectin was also effective *in vivo*.

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