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Novel cytotoxins and fungicides from blue-green algae and marine animals possessing algal symbionts

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Abstract - A status report of a program initiated in early 1981 to evaluate extracts of cultured blue-green algae for pharmacological activity and to isolate and identify active agents is presented. Novel macrolides and nucleosides, for example, have been shown to be responsible for the cytotoxic and fungicidal activities of many blue-green algae belonging to the Scytonemataceae. In the last two years this program has been expanded to include studies on marine animals that harbor symbiotic algae. Macrolides, for example, have been found to account for the potent cytotoxicity of an Indonesian sponge *Hyattella* sp. and a Guamanian tunicate *Lissoclinum patella*.

INTRODUCTION

In early 1981 a program was initiated in the University of Hawaii's chemistry department to isolate hundreds of blue-green algae from terrestrial, fresh-water, and marine environments and to screen hydrophilic and lipophilic extracts of the cultured cyanophytes for pharmacological activity. Based on results accumulated in this laboratory over the past seven years, blue-green algae appear to be an excellent source for new cytotoxins and fungicides (ref. 1). More recently extracts of marine animals suspected of harboring symbiotic algae have been tested for cytotoxicity and antifungal activity and studies have been initiated on evaluating the role of the symbionts in the production of the active agents. A status report is presented here.

CULTURED BLUE-GREEN ALGAE

Between January 1, 1984 and October 31, 1987, 10 liter cultures of 516 isolates of blue-green algae (mostly non-axenic and mostly of terrestrial origin) were grown for anticancer evaluation. Typically 3-5 grams of dried cells were obtained from each 10 L production culture. Each alga was extracted successively with 3:7 ethanol/water (A) and 1:1 dichloromethane/2-propanol (B) and the extracts were screened for cytotoxicity against the KB cell line. Thirty-four extracts, representing 30 (5.8%) of the isolates and mostly species belonging to the Scytonemataceae and Noctocaceae, showed MICs of <20 μg/mL (see Table 1). Nine percent of the extracts (i.e. 13% of the isolates) showed antifungal activity against one or more of five test organisms, viz. *Aspergillus oryzae*, *Candida albicans*, *Penicillium notatum*, *Saccharomyces cerevisiae*, and *Trichophyton mentagrophytes*.

Several of the cytotoxic, hydrophilic (A) extracts of species belonging to the Scytonemataceae listed in Table 1 exhibited antifungal activity also. Two classes of compounds were found to be responsible for this dual activity, viz. scytophycin-type macrolides and tubercidin/toyocamycin-type nucleosides. Scytophycins A and B (1) were the potent cytotoxic, fungicidal agents in *Scytonema pseudohofmanni* (BC-1-2), showing minimum toxicity against KB cells at 1 ng/mL (ref. 2) and broad spectrum antifungal activity at 10 ug/mL or 0.3 μg/disk in an *in vitro* agar diffusion assay (ref. 3). Both compounds also exhibited moderate activity *in vivo* against P-388 lymphocytic leukemia and

Lewis lung carcinoma in mice (ref.2). Tolytoxin, the cytotoxin (KB MIC = 1 ng/mL) in field-collected Tolypothrix conglutinata var. colorata (ref.3), was shown to be the major cytotoxic, fungicidal agent in Scytonema mirabile (BY-8-1) and S. ocellatum (DD-8-1) (ref. 4) and its gross structure tentatively established as 6-methoxyscytophycin B (ref. 3).

TABLE 1. Cytotoxicity of extracts of cultured blue-green algae against the KB cell line.

Isolate #	Extract	Identification of alga	Origin of alga	MIC µg/mL
BC-1-2	A	Scytonema pseudohofmanni	Oahu	0.2[a]
BL-11-2	A	Tolypothrix distorta	Oahu	18
BN-7-4	A	Tolypothrix tenuis	Oahu	12
BN-15-1	A		Oahu	3
BY-8-1	A	Scytonema mirabile	Oahu	<18
CN-8-1	A		Palau	9
CN-10-1	A		Palau	11
CS-10-2	A		Maui	10
CV-14-1	A	Scytonema saleyeriense var. indica	South Africa	2
CV-14-2	A/B		South Africa	7/9
CV-15-3	B		South Africa	11
CW-3-1	B		Oahu	17
DC-2-1	A		St. Thomas	16
DD-4-3	A		Guam	19
DD-8-1	A/B	Scytonema ocellatum	Guam	1.1/0.45
DF-6-1	A	Plectonema radiosum	Fiji	3
DO-2-1	B	Microchaete violacea	Okinawa	10
DO-4-1	A/B	Scytonema burmanicum	Okinawa	5/1
DO-32-2	A/B	Plectonema fortii	Okinawa	0.12/0.06
DT-65-1	B	Plectonema radiosum	Phillipines	5
DT-80-1	B	Nostoc commune	St. Thomas	11
DU-18-2	A	Nostoc ellipsosporum	Tahiti	10
MWQ#6	B	Synechococcus elongatus	California	5
UTEX-B1617	B	Anabaena subcylindrica	Unknown	10
UTEX-B1623	A	Nostoc ellipsosporum	Unknown	17
UTEX-B1932	B	Nostoc linckia	Unknown	10
UTEX-2349	B	Scytonema hofmanni	Unknown	10
UTEX-2380	B	Synechococcus sp.	Unknown	7
ATCC-29204	B	Cylindrospermum sp.	Unknown	10
ATCC-29412	B	Cylindrospermum licheniforme	Unknown	10

[a]Cytotoxicity determined against NIH3T3 cell line

Tubercidin is the major cytotoxin (KB MIC = 70 ng/mL) in cultured Tolypothrix byssoidea (H-6-2) (ref. 5) and Scytonema saleyeriense var. indica (CV-14-1) (ref. 4). This well-known nucleoside accounts for some of the antifungal activity associated with these two cyanophytes. Tubercidin is only a minor constituent in Plectonema radiosum (DF-6-1) and T. distorta (BL-11-2), but interestingly the α-D-glucoside derivative (2) (KB MIC = 560 ng/mL) is a major metabolite. Two related water-soluble nucleosides have been isolated from Tolypothrix tenuis (strain BN-7-4) and identified as toyocamycin (minor metabolite) and the α-D-glucoside derivative (3, major metabolite, KB MIC = 0.3 µg/mL).

Studies on the other blue-green algae listed in Table 1 are in progress.

FIELD-COLLECTED MARINE ANIMALS POSSESSING ALGAL SYMBIONTS

Sixty-seven marine animals (mostly sponges and tunicates) suspected of having symbiotic microalgae were collected. Lipophilic and hydrophilic extracts were prepared by extracting each freeze-dried animal successively with 50% ethanol in dichloromethane (C) and 30% ethanol in water (D). Each extract was then evaluated for cytotoxicity and antifungal activity. Forty-six percent of the organisms showed cytotoxicity against the KB cell line at 10 µg/mL or less and 37% showed antifungal activity against one or more of five test organisms at 250 µg/disk.

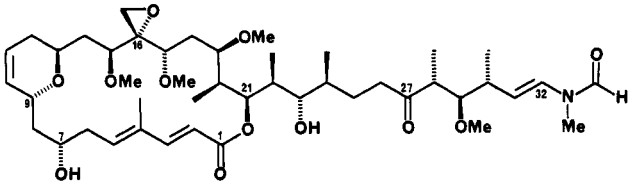
The major cytotoxin(s) in five of the most active samples (Table 2) were isolated and identified. The cytotoxicity of the Sarcotragus sp. from Guam (SG-2) was attributed to a mixture of variabilin oxidation products. In 1973 Faulkner had isolated variabilin from the sponge Ircinia variabilis and determined its structure (ref.6). Recently Barrow et al. have reported that variabilin is responsible for the antiviral activity of a Sarcotragus sp. from New Zealand (ref.7). Pure variabilin is not cytotoxic, but solutions of the sesterterpene become cytotoxic on standing. The structures of the variabilin oxidation products appear to be similar to those of oxidized furanoterpenes related to furospongin-1 (ref. 8).

TABLE 2. Cytotoxicity and antifungal activity of extracts of field-collected marine invertebrates possessing algal symbionts.

Iden. #	Phylum	Site of collection	Identification	Ext.	KB MIC (μg/mL)	Antifungal activity
SG-2	sponge	Guam	Sarcotragus sp.	C	0.1	Sc,Tm
SG-6	sponge	Indonesia	Hyattella sp.	C	0.05	Ao,Pn,Sc,Tm
SG-12	sponge	Guam		C	5	-
SG-37	tunicate	Guam	Lissoclinum patella	C	0.015	-
SG-51	tunicate	Ponape	Lissoclinum patella	C	1	-

The cytotoxicity of the Guamanian sponge SG-12 was shown to be due to a mixture of known 2-polyprenylated 1,4-hydroquinones (ref. 9). The major compound in the mixture, 2-heptaprenyl-1,4-hydroquinone (KB MIC = 0.3 μg/mL), proved to be five times more active than the hexa- and octaprenyl homologs.

The potent cytotoxin and fungicide in the Hyattella sp. sponge from Indonesia (SG-6) was isolated and shown to be a novel macrolide, laulimalide (4) (KB MIC = 15 ng/mL) (ref. 10). Laulimalide, which curiously does not fully conform to polyketide biogenetic principles, is accompanied by an inactive, structurally-related compound, isolaulimalide (KB MIC >200 ng/mL).



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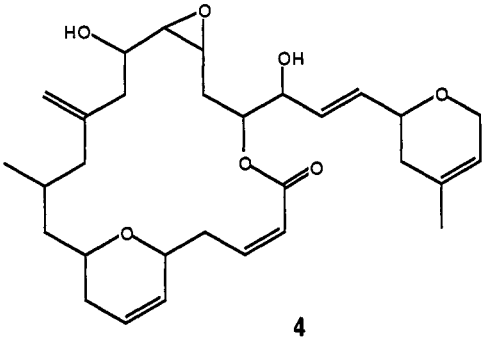

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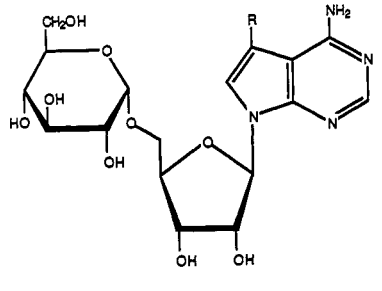
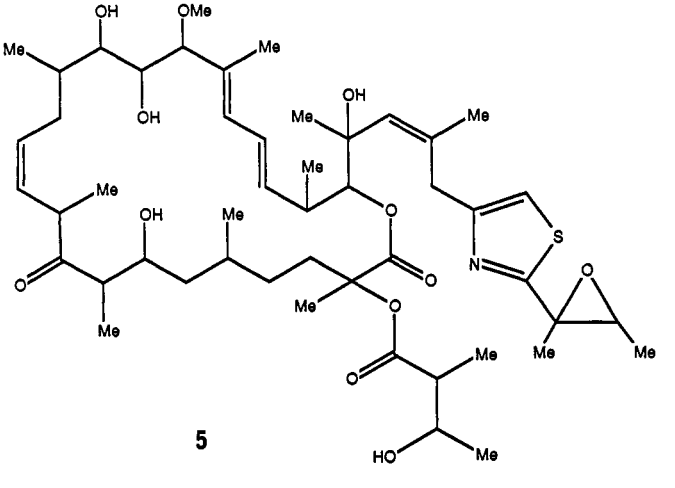


2 R = H

3 R = CN



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The Lissoclinum patella from Ponape (SG-51) contains several known, weakly cytotoxic cyclic peptides, e.g. patellamide A (ref. 11), but the potent cytotoxin associated with this tunicate appears to be a new, previously undescribed compound. It is not ulithiacyclamide.

The extremely potent cytotoxin in the Lissoclinum patella from Guam (SG-37), patellide (KB MIC = 0.3 ng/mL), was isolated and shown to be a novel thiazole-containing macrolide having the gross structure 5 (ref. 12). Patellide lacks antifungal activity, but it may be responsible for the potent activity of the crude extract against respiratory syncytial virus.

IMPORTANCE OF FINDINGS

The discovery of the scytophycins provides circumstantial evidence that symbiotic blue-green algae may be responsible for the production of bioactive compounds in certain marine organisms. The scytophycins have molecular structures and biological activities that are comparable with those of swinholide A (ref. 13), kabiramide C (ref. 14), and halichondramide (ref. 15). Interestingly Theonella swinhoei, the Red Sea sponge that contains swinholide A, harbors a unicellular blue-green alga (ref. 16), but the kabiramide and halichondramide-producing sponges (Halichondria spp.) do not possess algal symbionts (D.J. Faulkner, personal communication).

The discovery of 5'-glucosylated nucleosides in blue-green algae could be a very important finding. Clearly one serious problem with many nucleosides in clinical use today is their generally poor solubility in water. The 5'-glucosylated nucleosides, on the other hand, are much more soluble in water and may be useful pro-drugs. We have shown that a cell-free extract of Tolypothrix tenuis (BN-7-4) contains an enzyme that catalyzes the glycosylation of toyocamycin at the 5'-OH position (ref. 4). It may be possible to use this glucosyltransferase enzyme to glycosylate synthetic nucleosides such as ribavirin, cyclaradine, acyclovir and vidarabine (ara-A) to increase their solubilities in water. Surprisingly, 5'-glucosylated nucleosides are not described in the chemical literature.

Acknowledgements

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