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Antineoplastic Evaluation of Pacific Basin Marine Algae

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Abstract □ Extracts of 107 marine alga specimens from Pacific islands were tested for P-388 lymphocytic leukemia and Ehrlich ascites tumor in mice. Several specimens showed high antitumor activity in both systems, with some featuring a notable lack of toxicity.

Keyphrases □ Antineoplastic activity—marine algae from Pacific islands, testing against P-388 lymphocytic leukemia and Ehrlich ascites tumor □ Marine algae—antineoplastic evaluation against P-388 lymphocytic leukemia and Ehrlich ascites tumor □ Antitumor activity—marine algae from Pacific islands, testing against P-388 lymphocytic leukemia and Ehrlich ascites tumor

There have been numerous reports of antitumor substances obtained from terrestrial plants, but no information on antitumor agents from marine plants was published prior to 1977 when Mynderse *et al.* (1) reported that chloroform extracts of some marine blue-green algae showed activity against P-388 lymphocytic leukemia in mice. A P-388 active compound was isolated from one alga, *Lyngbya majuscula* Gomont, and shown to be debromoaplysiatoxin. This finding prompted screening of other marine algae from the Pacific basin for activity against P-388 lymphocytic leukemia and Ehrlich ascites tumor in mice.

The 107 alga specimens were collected from Palau (Western Caroline Islands), Fanning Island (Line Islands), Enewetak Atoll (Marshall Islands), Johnston Island, and the Hawaiian Islands. Voucher samples were retained for most specimens, and the collection site of each sample was recorded carefully.

This paper is a preliminary report of the antitumor activity of crude extracts and some partially purified fractions from these algae.

EXPERIMENTAL

Most algal samples were refrigerated or frozen soon after collection. When refrigerator or freezer facilities were not available, the samples were air dried. The frozen samples were freed from extraneous matter and freeze dried before extraction. The dried samples were powdered in a mortar or blender[1].

There were two general extraction procedures. In Method I, the dried alga was extracted initially with a succession of organic solvents of increasing polarity followed by a final extraction with water. In Method II, the sample was subjected to extraction with 30% ethanol followed by extractions (liquid–liquid) with a succession of organic solvents of the aqueous concentrate.

In Method I, the sample was homogenized in a blender[1] for 5 min with a volume of organic solvent weighing five to 10 times the sample weight. The mixture then was allowed to stand for 24 hr or was stirred for 4–5 hr. The resultant homogenate was centrifuged, and the supernate was

[1] Waring.

Table I—Active Materials

Alga Sample	Location	Extracting Solvent and Purity of Fraction[a]	P-388 Lymphocytic Leukemia[b]: Dose, mg/kg	P-388: Percent Activity[d]	P-388: Number of Mice	P-388: Toxicity, deaths[e]	Ehrlich Ascites[c]: Dose, mg/kg	Ehrlich Ascites, Survivors at 30 Days: Percent Alive	Ehrlich Ascites, Survivors at 30 Days: Nonascitic
Aphanococcus biformis A. Br., 30%; *Chroococcus minor* (Kütz.) Nag., 30%; *Oscillatoria foreaui* Fremy, 30%	Palau	*n*-Butanol, I	29.0	125	5	0	23.2	0	0
Bangia sp.	Palau	30% ethanol, I	17.5	230	4	0	14.0	100	100
		30% ethanol, II	21.8	224	5	0	12.4	80	80
		30% ethanol, II	12.4	196	5	0	11.9	100	100
		30% ethanol, III	<0.5	186	5	0	<0.5	100	100
Caulerpa racemosa var. *peltata* Lam.	Palau	30% ethanol, I	50.0	151	4	2	40.0	100	80
		30% ethanol, II	—	—	—	—	1.3	100	80
		30% ethanol, III	—	—	—	—	0.8	80	80
Crinalium sp.	Fanning Island	Ethyl acetate, I	—	—	—	—	6.0	50	50
		Ethyl acetate, I	17.5	178	6	1	14.0	80	80
		Water, III	0.5	210	4	0	—	—	—
Cryptonemia crenulata J. Ag.	Palau	30% ethanol, I	100	129	4	0	80.0	0	0
Dictyota crenulata J. Ag.	Oahu, Hawaii	Methylene chloride, I	153	129	4	0	122	0	0
		Methylene chloride, I	265	135	4	0	212	0	0
		Methylene chloride, I	—	—	—	—	68	80	80
Ectocarpus breviarticulatus J. Ag.	Fanning Island	Ethyl acetate, I	25	170	5	1	20	0	0
Gracilaria salicornia (C. Ag.) Dawson	Palau	30% ethanol, I	0.5	137	3	0	0.4	0	0
Halimeda sp.	Palau	Butanol, I	21.5	154	4	0	17.2	0	0
		Hexane, I	26.0	131	4	0	20.8	0	0
Herposiphonia arcuata Hollenberg	Fanning Island	30% ethanol, I	—	—	—	—	120	100	100
		30% ethanol, II	—	—	—	—	10	80	60
Lobophora variegata (Lamx.) Wom.	Palau	Butanol, I	21.5	127	4	0	17.2	0	0
		Hexane, I	10.0	127	5	0	8.0	0	0
		Chloroform, I	11.0	144	5	0	8.8	0	0
Lyngbya convervoides C. Ag.	Palau	30% ethanol, I	—	—	—	—	20	100	100
Lyngbya majuscula Gomont	Palau	Water, I	25.0	166	5	1			
		Water, II	10.0	164	5	0	8	60	40
		Water, III	<0.5	166	5	0	<0.4	100	100
	Oahu, Hawaii	Chloroform, I	4.2	134	5	1	3.2	20	20
		Chloroform, I	0.5	144	5	0	0.4	0	0
Lyngbya sp.	Hawaii, Hawaii	Methanol, I	—	—	—	—	27.2	100	100
		Methanol, II	150	182	5	0	—	—	—
		Methanol, III	—	—	—	—	1.6	100	100
Oscillatoria annae van Goor	Palau	30% ethanol, I	415	125	5	3	332	0	0
Oscillatoria sp.	Palau	30% ethanol, I	5.0	205	5	0	4.0	100	100
		30% ethanol, III	—	—	—	—	0.4	100	100
Phormidium crosbyanum Tilden	Johnston Island	Methylene chloride, I	7.5	134	5	3	6.0	0	0
Phormidium sp.	Molokai, Hawaii	Methanol, I	12.5	210	5	0	10	100	100
Sargassum polycystum C. Ag.	Palau	30% ethanol, I	250	129	4	0	200	0	0
Schizothrix calcicola (Ag.) Gomont	Fanning Island	Ethyl acetate, I	25	255	5	3	2.5	100	100
		Water, II	2.5	209	5	1	3.6	100	80
Rivularia atra Born. + Fla.; *Entophysalis deusta* (J. Ag.) Dr. + Dail.; *Anacystis dimidata* (Kütz.) Dr. + Dail.	Enewetak	Methanol, I	22	125	5	1	—	—	—
Schizothrix sp.	Enewetak	Methanol, I	—	—	—	—	8.0	40	40
Spyridia filamentosa (Wulfen) Harvey	Oahu, Hawaii	Methylene chloride, I	90	128	5	1	—	—	—
Tolypothrix conglutinata var. *clorata* Ghose	Fanning Island	Ethyl acetate, I	13.5	233	5	3	10.8	0	0

Table I—*Continued*

Alga Sample	Location	Extracting Solvent and Purity of Fraction[a]	P-388 Lymphocytic Leukemia[b]: Dose, mg/kg	Percent Activity[d]	Number of Mice	Toxicity, deaths[e]	Ehrlich Ascites[c]: Dose, mg/kg	Survivors at 30 Days: Percent Alive	Nonascitic
Tydemannia expeditionis Weber van Bosse	Palau	30% ethanol, I	250	130	4	0	200	0	0
	Enewetak	Chloroform, I	19	156	5	3	15.2	0	0
Udotea geppii Yamada	Enewetak	Methylene chloride, I	—	—	—	—	47.6	100	100

[a] I = crude; II = fraction chromatographed through Sephadex G-25; and III = fraction chromatographed through CM Sephadex C-25. [b] Standard screening procedure used in this laboratory (2). [c] Procedure used in this laboratory described by Tabrah *et al.* (3). [d] Mean survival time as percent of untreated, diseased controls. [e] Number of mice that died within 7 days after tumor cell inoculation.

Table II—Nonactive Samples

Sample	Location
Amphiroa fragilissima (L.) Lamour.	Palau
Aphanocapsa biformis A. Br.	Palau
Asparagopsis taxiformis (Del.) Coll. + Herr.	Enewetak
Boodlea composita (Harv.) Brand.	Palau
Bornetella nitida (Harv.) Mun.	Palau
Bryopsis sp.	Fanning Island
Calothrix crustacea Born. + Fla.	Enewetak
Caulerpa racemosa var. *laetevirens* (Mont.) Weber van Bosse	Palau
Caulerpa racemosa var. *occidentalis* (J. Ag.) Boerg.	Palau
Caulerpa racemosa var. *peltata* Lam.	Palau
Caulerpa urvilliana Mont.	Fanning Island
Centroceras clavulatum (C. Ag.) Mont.	Fanning Island
Centroceras minimum Yamada	Enewetak
Chlorodesmis hildebrandtii Gepp et Gepp	Palau
Chondrococcus hornemanni (Lyng.) Kütz.	Palau
Codium geppei Schmidt	Enewetak
Crinalium sp.	Palau
Dactylococcopsis raphidioides Hansg.	Fanning Island
Derbesia sp.	Palau
Dictyopteris repens (Okamura) Boerg.	Palau
Dictyosphaeria sp.	Oahu, Hawaii
Dictyota acutiloba J. Ag.	Oahu, Hawaii
Dictyota divariacata Lamour.	Enewetak
Dictyota friabilis Setchell	Fanning Island
Dictyota patens J. Ag.	Palau
Enteromorpha sp.	Oahu, Hawaii
Eucheuma striatum Schmidt	Oahu, Hawaii
Falkenbergia rufanolosa Harvey	Enewetak
Gloeocapsa decorticans (A. Br.) Richter	Fanning Island
Gracilaria salicornia (C. Ag.) Dawson	Palau
Halimeda cylindracea Decaisne	Palau
Halimeda discoidea Decaisne	Palau
Halimeda macrophysa Barton	Enewetak
Halimeda monile (Sol.) Lamour.	Enewetak
Halimeda opuntia (L.) Lamx.	Palau
Halophila ovata Gaud.	Palau
Halymenia maculata J. Ag.	Palau
Herposiphonia arcuata Hollenberg	Fanning Island
Hyella caespitosa Born. et Flah.	Fanning Island
Hypnea nidifica J. Ag.	Palau
Hypnea pannosa J. Ag.	Palau
Liagora farinosa Lamour.	Enewetak
Liagora maxima Butters	Oahu, Hawaii
Lobophora sp.	Enewetak
Lobophora variegata (Lamx.) Wom.	Palau
Lyngbya convervoides C. Ag.	Palau
Lyngbya majuscula Gomont	Palau
Lyngbya porphyrosiphonis Fremy	Palau
Lyngbya semiplena (C. Ag.) J. Ag.	Palau
Martensia fragilis Harvey	Oahu, Hawaii
Microcoleus acutissimus Gardner	Fanning Island
Microcoleus sp.	Fanning Island
Microcoleus tenerrimus Gomont	Enewetak
Microcoleus vaginatus (Vaucher) Gomont	Palau
Nostoc calcicola Born. et Flah.	Palau
Oscillatoria hamelii Fremy	Palau
Oscillatoria nigroviridis Gomont	Enewetak
Oscillatoria sp.	Fanning Island
Padina commersonii Bory	Palau
Polysiphonia quadrata Hollenberg	Fanning Island
Pterocladia sp.	Oahu, Hawaii
Rhipilia orientalis A. + E. Gepp	Enewetak
Scytonema pascheri Bharaduaja	Fanning Island
Spyridia filamentosa (Wulfen) Harvey	Oahu, Hawaii
Symploca hydnoides Kütz.	Palau
Symploca sp.	Palau
Turbinaria ornata (Turn.) J. Ag.	Palau
Tydemannia expeditionis Weber van Bosse	Palau
Valonia aegagropila C. Ag.	Fanning Island
Valonia ventricosa J. Ag.	Palau

evaporated *in vacuo* to yield the crude lipophilic extract. The marc was dried and then soaked in a volume of water weighing five to 10 times the marc weight for 4–5 hr with stirring and for an additional 24 hr or more without stirring. After centrifugation, the supernate was freeze dried to give the water-soluble extract. For initial fractionation, the water-soluble extract was subjected to gel filtration[2] with 0.1 *M* ammonium bicarbonate as the eluent. This procedure was followed by cation-exchange chromatography[3] with a gradient elution of the active compound with 0.03–3.0 *M* ammonium acetate. All column separations were monitored by UV spectroscopy at 280 nm[4].

In Method II, a sample was extracted initially with 30% ethanol in the same manner as described for Method I for the extraction of water-soluble constituents from the marc. After the ethanol was eliminated from the supernate by flash evaporation, this crude extract was extracted further with either *n*-butanol or chloroform to remove lipophilic constituents. The water-soluble fraction then was purified by procedures similar to those described for Method I for the water-soluble crude extract.

The isolation of the purified lipophilic antitumor agents will be reported later.

RESULTS AND DISCUSSION

The results of the screening tests are listed in Tables I and II. Table I shows that eight marine algal extracts had T/C (test/control) values of ≥170% in the P-388 lymphocytic leukemia test. Of these extracts, six species showed T/C activity of >200%. In the Ehrlich ascites tumor system, nine species of algae showed a 100% survival rate at 30 days with no ascitic condition. Several of the groups of mice that survived for 30 days with a nonascitic condition were kept alive for an additional 30 days with no tumor recurrence at the end of the 60-day period.

[2] Sephadex G-25, Pharmacia Fine Chemicals, Piscataway, NJ 08854.
[3] CM Sephadex C-25, Pharmacia Fine Chemicals, Piscataway, NJ 08854.
[4] Model UA-5 absorbance monitor, Instrument Specialties Co., Lincoln, NE 68505.

Some of the algal extracts active against both P-388 leukemia and Ehrlich ascites tumor were subjected to preliminary isolation work (Table I). The finding that some of these crude extracts as well as partially purified fractions showed excellent activity at relatively low dosages with no evidence of toxicity is most encouraging.

Table II lists the marine algae that showed a T/C activity of <125% against P-388 lymphocytic leukemia and <20% survivors at 30 days in the Ehrlich ascites tumor system.

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COMMUNICATIONS

Ocular Absorption of Propranolol in Rabbits

Keyphrases □ Propranolol—ocular absorption in rabbits □ β-Adrenergic blocking agents—propranolol, ocular absorption in rabbits □ Absorption, ocular—propranolol in rabbits

To the Editor:

The topical application of β-adrenergic blocking agents to the eye has been found to be effective in the control of glaucoma (1). However, the precise rate and extent of disposition of these compounds in the various ocular tissues have not been fully established. The purpose of this report is to compare and contrast the ocular absorption of a model β-blocking agent, propranolol, to what is known about the widely used miotic pilocarpine.

Male New Zealand albino rabbits, 3.0–3.6 kg, were minimally restrained in wooden boxes; topical and local anesthetics were not used. A 50-μl dose of 0.5% propranolol hydrochloride in isotonic buffer (pH 7.4) was instilled onto the cornea and allowed to distribute normally within the cul-de-sac. All tissue sampling procedures were performed as outlined previously (2, 3). The tissue samples collected were the whole intact cornea, aqueous humor, iris, and lens. The amount and concentration of propranolol in these tissues were determined spectrophotofluorometrically (4). The minimum detection limit for the drug was ~5 ng.

Figure 1 shows the propranolol concentration in ocular tissues as a function of time. The data indicate that propranolol reached a peak concentration in the aqueous humor at ~30 min. This result corresponds well with previous data for pilocarpine, which has a peak time of 20–30 min. This peak time was anticipated in the current studies since it was shown previously that the apparent ocular pharmacokinetic parameters are largely determined by the parallel first-order loss process in the precorneal area, so that most drugs show similar peak times in the aqueous humor (2, 5). The elimination characteristics of propranolol also are very similar to pilocarpine and suggest that both drugs are lost from the eye *via* the same mechanism, namely, aqueous humor turnover. The rate constant associated with this process for pilocarpine in rabbits is 0.017 min^{-1} (2), and this value is nearly identical to the elimination rate of propranolol from the aqueous humor in the present studies (0.019 min^{-1}).

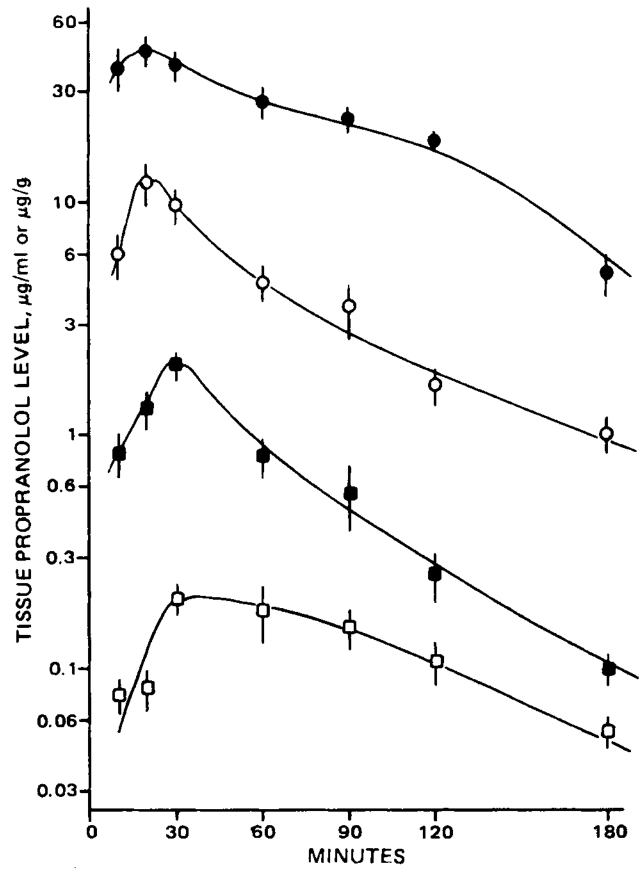

Figure 1—*Concentration of propranolol in various ocular tissues after topical application of propranolol solution. Key: ●, cornea; ○, iris; ■, aqueous humor; and □, lens.*

Concentration–time profiles such as those depicted in Fig. 1 can be somewhat misleading in ocular studies of this type which deal with tissues of greatly different distribution volumes. For this reason, it often is useful to consider the amount of drug represented by the peak drug concentration for each tissue. These data are presented in Table I. The rank order for tissue concentration was cornea > iris > aqueous humor > lens, whereas the rank order for tissue amounts was cornea > aqueous humor > iris > lens. The change in rank order for the iris and aqueous humor was due to the 12-fold difference in the wet weights of these

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