107(1970).
(17) D. Sampson, I. Harasymiv, and W. J. Hensley, *ibid.*, **17**, 382(1971).
(18) D. Chin, E. Fastlich, and B. Davidow, *J. Chromatogr.*, **71**, 545(1972).
(19) D. H. Sandberg, G. L. Resnick, and C. Z. Bacallao, *Anal. Chem.*, **40**, 736(1968).
(20) H. J. Kupferberg, *Clin. Chim. Acta*, **29**, 283(1970).
(21) E. M. Baylis, D. E. Fry, and V. Marks, *ibid.*, **30**, 93(1970).
(22) T. Chang and A. J. Glazko, *J. Lab. Clin. Med.*, **75**, 145(1970).
(23) J. MacGee, *Anal. Chem.*, **42**, 421(1970).
(24) R. H. Hammer, B. J. Wilder, R. R. Streiff, and A. Mayersdorf, *J. Pharm. Sci.*, **60**, 327(1971).
(25) G. E. Simon, P. I. Jatlow, H. T. Seligson, and D. Seligson, *Amer. J. Clin. Pathol.*, **55**, 145(1971).

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# Antitumor Activity and Cardiac Stimulatory Effects of Constituents of *Anthopleura elegantissima*

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**Abstract** □ A crude aqueous ethanolic extract of the sea anemone, *Anthopleura elegantissima* (Brandt), displayed activity against two experimental mouse tumors (P-388 lymphocytic leukemia and Ehrlich ascites tumor). In addition, it displayed a positive inotropic effect on isolated rat atria. Preliminary separations established that the three biological activities are due to three different constituents of this marine organism.

**Keyphrases** □ *Anthopleura elegantissima* (Brandt)—separation of constituents, screened for antitumor activity and cardiac stimulatory effects □ Sea anemone—separation of constituents, screened for antitumor activity and cardiac stimulatory effects □ Antitumor activity—constituents of *A. elegantissima* screened □ Cardiac stimulatory activity—constituents of *A. elegantissima* screened

The antitumor activity of a crude extract of the sea anemone, *Anthopleura xanthogrammica* (Brandt) (1), and the cardiac stimulatory effects of extracts of both *A. xanthogrammica* and *A. elegantissima* (2) have been reported. An aqueous ethanolic extract of *A. elegantissima*[1] has been found active against P-388 lymphocytic leukemia (P-388) and Ehrlich ascites tumor in mice and produces a positive inotropic effect on isolated rabbit auricles (Table I). These three effects are due to three different constituents obtained in a preliminary fractionation of the crude extract.

Gel permeation chromatography using Resin 1[2] separates the P-388 active material, which is not retarded, from the Ehrlich ascites and heart stimulant substances, which are retarded. The P-388 active material is further purified using Resin 2[3] and the Ehrlich ascites and heart stimulant substances are separated from each other using cellulose chromatography.

Taurine was also isolated from the *A. elegantissima* extract. Taurine was first isolated from a marine organism from the red algae *Ptilota pectinata, Porphyra umbilicalis,* and *Gelidium cartilagineum* (3). *N,N*-Dimethyltaurine was isolated previously from the red alga *Furcellaria fastigiata* (4), while both the *N*-methyl- and *N,N*-dimethyltaurines were shown to be constituents of the sponge *Calyx nereis* (5); the trimethyl derivative, taurobetaine, was found in the gorgonian *Briareum asbestinum* (6).

## RESULTS AND DISCUSSION

A typical chromatogram, monitored at 280 nm, of the crude extract on Resin 1 is shown in Fig. 1. Fraction A ($V_e/V_0$ 0.86–1.50) was active against P-388, Fraction C ($V_e/V_0$ 1.73–1.97) displayed a strong positive inotropic effect on isolated rat atria (2) and some inhibition of Ehrlich ascites, and Fraction D ($V_e/V_0$ 1.97–2.25) inhibited Ehrlich ascites tumor.

Further gel permeation chromatography of Fraction A was achieved on Resin 2 (Fig. 2). The P-388 active constituent separated from the large amount of 280-nm absorbing material at $V_e/V_0$ 0.83–1.31 but was distributed over a large region (Fraction H, $V_e/V_0$ 1.31–2.35). In comparison to the crude extract, Fraction H showed a marked improvement in activity against P-388.

Fraction C was separated further by cellulose chromatography (Fig. 3). The Ehrlich ascites active material was eluted almost at

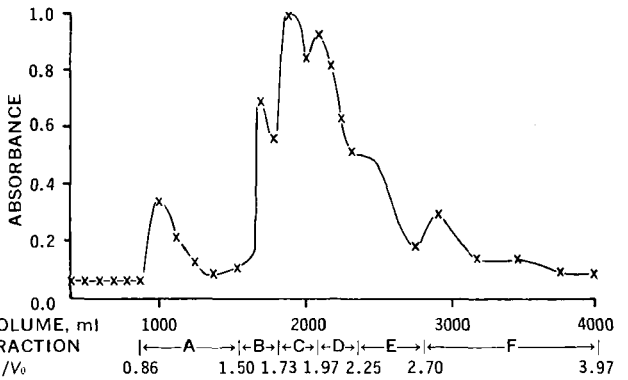


**Figure 1**—*Chromatography of crude extract on Resin 1.*

[1] The authors thank Dr. Cadet Hand, Director, Bodega Marine Laboratory, Bodega Bay, Calif., for supplying a collection of the animals.
[2] Sephadex G-25.
[3] Sephadex G-75.

**Table I—Biological Results**

| Fraction | P-388 Leukemia[a,b] Dose, mg/kg | P-388 Leukemia[a,b] T/C, % | Ehrlich Ascites[b] Dose, mg/kg | Ehrlich Ascites[b] Survivors at 30 Days % Alive | Ehrlich Ascites[b] Survivors at 30 Days % Nonascitic | Heart Stimulant[c] |
|---|---|---|---|---|---|---|
| Crude extract | 300 | Toxic | 2.5 | 80 | 60 | Strong positive inotropic effect at 100 μg/ml |
| | 200 | 125[a] | 1.25 | 40 | 40 | |
| | | 129[a] | 0.8 | 40 | 20 | |
| | | 107[b] | 0.4 | 0 | 0 | |
| | 160 | 155[b] | | | | |
| | 133 | 133[a] | | | | |
| | | 116[a] | | | | |
| | 106 | 114[b] | | | | |
| | 100 | 118[b] | | | | |
| | 88 | 125[a] | | | | |
| | | 129[a] | | | | |
| | 70 | 102[b] | | | | |
| A | 320 | Toxic (0.5 hr) | 50 | 50 | 50 | Weak effect at 91 μg/ml |
| 12% of crude | 160 | 140[b] | 25 | 50 | 25 | |
| extract | 80 | 128[b] | 3.0 | 0 | 0 | |
| | 50 | 125[b] | 0.76 | 0 | 0 | |
| | 40 | 137[b] | | | | |
| | 25 | 137[b] | | | | |
| | 12.5 | 126[b] | | | | |
| C | B,C,D,E,F combined | | 8 | 25 | 25 | Threshold 2.58 μg/ml, maximum 25.8 μg/ml |
| 33% of crude | 310 | 106[b] | 2.56 | 60 | 40 | |
| extract | | | 2.2 | 40 | 20 | |
| | | | 2 | 0 | 0 | |
| | | | 0.64 | 80 | 40 | |
| D | B,C,D,E,F combined | | 3.3 | 50 | 25 | Weak at 129 μg/ml |
| 16% of crude | 310 | 106[b] | 0.83 | 25 | 25 | |
| extract | | | | | | |
| H | 22.5 | 157[b] | | | | |
| 16.6% of A | 6.75 | 146[b] | | | | |
| 2% of crude | 2.0 | 131.5[b] | | | | |
| extract | | | | | | |
| L | | | 2.2 | 80 | 80 | Inactive |
| | | | 0.43 | 40 | 40 | |
| M | | | 3.7 | 40 | 40 | Inactive |
| | | | 0.74 | 40 | 40 | |
| O–R | | | O-9.5 | 0 | 0 | Positive inotropic effect |
| | | | P-12.3 | 0 | 0 | |
| | | | R-25.4 | 0 | 0 | |

[a] Data from the National Cancer Institute screening program through the courtesy of Dr. Jonathan L. Hartwell. Schedule of 1 dose/day × 9 days. [b] Standard screening procedure used in this laboratory; for general procedure, see Ref. 7. Schedule of 1 dose/day × 10 days. [c] Determined using the isolated atria of live rat hearts as described in Ref. 2.

the solvent front (Fractions L and M), while the heart stimulant was eluted later (Fractions O–R). The bioassay results are given in Table I. Fraction L gave the best observable inhibition of Ehrlich ascites in that 80% of the treated mice were cured, *i.e.*, no abdominal distension at 30 days. Fraction M showed less Ehrlich ascites activity. TLC of Fractions L and M on cellulose HF[4] with 76% aqueous ethanol gave two ninhydrin-positive spots at $R_f$ 0.64 and 0.56. Examination of the Ehrlich ascites active Fraction D revealed the presence of two faint spots with the same $R_f$ values.

The P-388 active material is excluded from Resin 1 and, therefore, should have a molecular weight greater than 5000. But the Ehrlich ascites active and heart-stimulating constituents may be small molecules since Fraction C was retarded by Resin 1 and contains taurine, which is isolated from Fraction S from the cellulose chromatography.

The three biological activities displayed by a crude extract of *A. elegantissima*, as described here, have been shown to be due to three different constituents. Further purification of these drugs is continuing.

## EXPERIMENTAL

*A. elegantissima* specimens were collected from Bodega Bay, Calif.[1], preserved in 95% ethanol, and stored at −10° prior to extraction.

Wet, drained anemones (8.7 kg) were homogenized batchwise in a blender with 36 liters of 28.5% ethanol, combined with the 4.6 liters of storage liquor, and allowed to stand at room temperature for 2 days. Most of the supernatant liquid was decanted, and the remaining extract was separated from the solids by centrifugation (2000 rpm, 5 min).

The combined liquors were concentrated under reduced pressure at ≦50° to 7 liters. The concentrate was clarified by centrifugation (20,000×*g*, 10 min), decanted through a plug of glass wool to remove a small amount of orange oil floating on the surface, and lyophilized to give 500 g of a yellow hygroscopic solid.

The combined marc from both centrifugation steps was resuspended in 7 liters of 28.5% ethanol and allowed to stand, with occa-

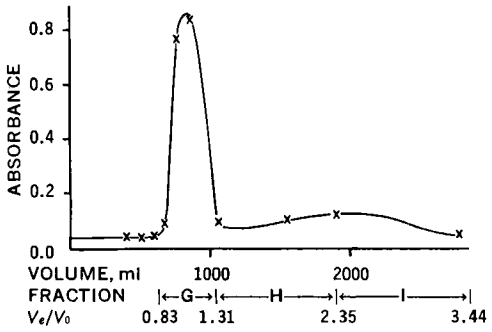


**Figure 2**—*Chromatography of Fraction A on Resin 2.*

[4] Merck.

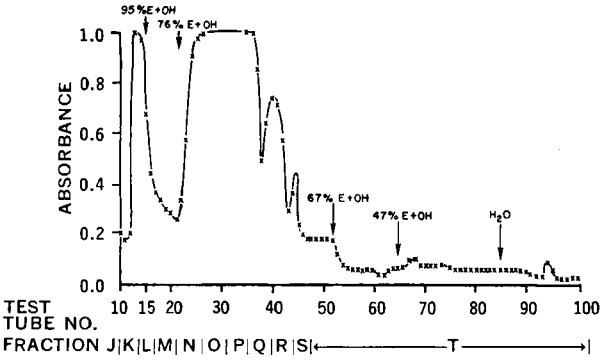


**Figure 3**—*Chromatography of Fraction C on cellulose.*

sional shaking, for 2 weeks. After decantation, centrifugation (2000 rpm, 10 min), and filtration through a plug of glass wool, the solution was lyophilized to give 79 g of a yellow hygroscopic solid.

The crude extract (20 g) was dissolved in 100 ml of water with the aid of sonication for 2 min and shaken vigorously with 100 ml of chloroform. Centrifugation (20,000×*g*, 15 min) of this mixture gave a brown aqueous layer, a yellow chloroform layer, and some insoluble solid at the interface. The aqueous layer was removed, recentrifuged, and then lyophilized to give the water-soluble portion of the crude extract, 16.5 g of a yellow solid.

**Chromatography on Resin 1**—The water-soluble portion of the crude extract (3.00 g) in 20 ml of water was chromatographed on a 49 × 8-cm column of Resin 1 (fine, 500 g). The void volume was determined to be 1025 ml with 20 ml of 0.15% blue dextran. Elution was with water saturated with chloroform at a rate of 6.5 ml/min (7.7 ml $hr^{-1}$ $cm^{-2}$). The column effluent was monitored using a recording UV monitor at 280-nm wavelength. The collected fractions were flash evaporated (≤50°) to reduce volume and lyophilized (Fig. 1). The weights of the active fractions were: A, 441 mg; C, 1220 mg; and D, 589 mg.

**Chromatography on Resin 2**—Fraction A (2.00 g in 20 ml of water) was chromatographed on a 49 × 8-cm column of Resin 2 (180 g) with a 810-ml void volume. Elution was with water saturated with chloroform at a rate of 5.25 ml/min (6.3 ml $hr^{-1}$ $cm^{-2}$). The column effluent was monitored at 280 nm. Fraction H fluoresced yellow green with longwave UV light and Fraction I fluoresced blue. The fractions collected were flash evaporated (≤50°) to reduce volume and lyophilized (Fig. 2). The P-388 activity was found in Fraction H, which weighed 333 mg.

**Chromatography on Cellulose**—A slurry of 1.00 g of Fraction C and about 0.5 g of cellulose in 10 ml of water was lyophilized. This mixture, slurried in 95% ethanol, was mounted on top of a 38 × 1.5-cm column of 20 g of cellulose MN 300[5] packed in 95% ethanol. Elution was initiated with 300 ml of 95% ethanol, followed successively with 500 ml of 76% ethanol, 200 ml of 67% ethanol, 322 ml of 47% ethanol, and 200 ml of water (Fig. 3).

Fraction S, on standing, deposited long colorless needles, which were collected by filtration, washed with 95% ethanol, and dried to give 16.3 mg of solids. The NMR and IR spectra were superimposable with an authentic sample of taurine.

The collected fractions were evaporated to remove the ethanol and lyophilized. The weights of the active fractions were: L, 27.1 mg; and M, 45.9 mg.

## REFERENCES

(1) G. R. Pettit, J. F. Day, J. L. Hartwell, and H. B. Wood, *Nature*, **227**, 962(1970).

(2) S. Shibata, D. F. Dunn, M. Kuchii, M. Kashiwagi, and T. R. Norton, *J. Pharm. Sci.*, **63**, 1332(1974).

(3) B. Lindberg, *Acta Chem. Scand.*, **9**, 1323(1955).

(4) *Ibid.*, **9**, 1093(1955).

(5) D. Ackermann and R. Pant, *Naturwissenschaften*, **48**, 646(1961).

(6) L. S. Cereszko, P. K. Odense, and R. W. Schmidt, *Ann. N.Y. Acad. Sci.*, **90**, 920(1960).

(7) R. J. Quinn, M. Kashiwagi, R. E. Moore, and T. R. Norton, *J. Pharm. Sci.*, **63**, 257(1974).

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