Physiologically Active Substances from Marine Sponges V: Isolation of Physiologically Active Compounds from the Sponge *Verongia archeri*

JOGINDER S. CHIB ˣ, MARTIN F. STEMPIEN, Jr., RONALD A. MIERZWA, GEORGE D. RUGGIERI, and ROSS F. NIGRELLI

Received November 26, 1976, from the *Osborn Laboratories of Marine Sciences, New York Aquarium, New York Zoological Society, Brooklyn, NY 11224.* Accepted for publication April 19, 1977.

Abstract □ Methanolic extracts of hard and soft varieties of the sponge *Verongia archeri* were found to contain similar compounds. An isolation procedure and the structures of 2-(3′,5′-dibromo-4′-hydroxyphenyl)-acetamide, 1-(3′,5′-dibromo-1′,6′-dihydroxy-4′-methoxycyclohexa-2′,4′-diene)acetonitrile, and 2-oxo-5,7-dibromo-6-methoxy-9-hydroxy-8,9-dihydrocoumarin from one variety are described.

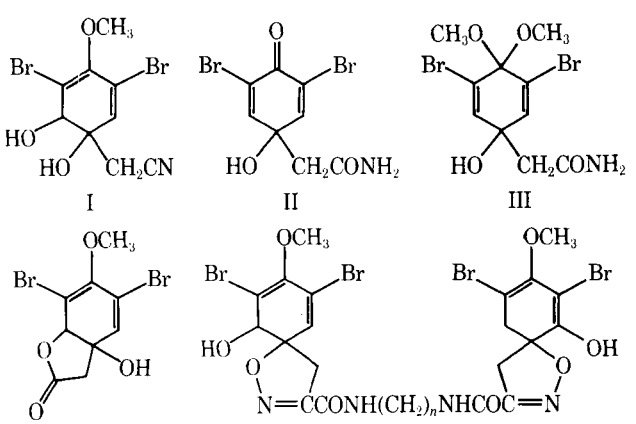
Keyphrases □ *Verongia archeri*—methanolic extracts of hard and soft sponges, various compounds isolated and identified, antibacterial activity evaluated □ Antibacterial activity—evaluated in various compounds isolated from methanolic extracts of hard and soft sponge *Verongia archeri*

Sponges of the genus *Verongia* have attracted considerable attention because of the strong antibacterial activity of their chemical constituents. The sponges *V. aerophobia* (1–3), *V. cauliformis* (4, 5), *V. fistularis* (6), *V. thiona* (7–9), and *Ianthella ardis* (10, 11) have yielded several antibiotics (I–VI). A 3,4-dihydroxyquinoline derivative (VII) was also identified as one metabolite in *V. aerophobia* (12).

In a continuing investigation (13, 14) of marine natural resources, the sponges *V. archeri* (hard and soft) were studied. The isolation and identification of various antibiotics from these two species are reported.

EXPERIMENTAL[1]

Materials—The hard form of *V. archeri*[2] was collected as isolated cylinders from the north shore of Jamaica. The soft form was collected either as isolated cylinders or as groups of anastomosing cylinders, up to 40 cm in height, from the British Virgin Islands. Both varieties lose their antibiotic activity after freeze drying, but the activity is retained when the sponges are oven dried at 60–80°. A similar phenomenon was observed with the sponge *V. fistularis*.



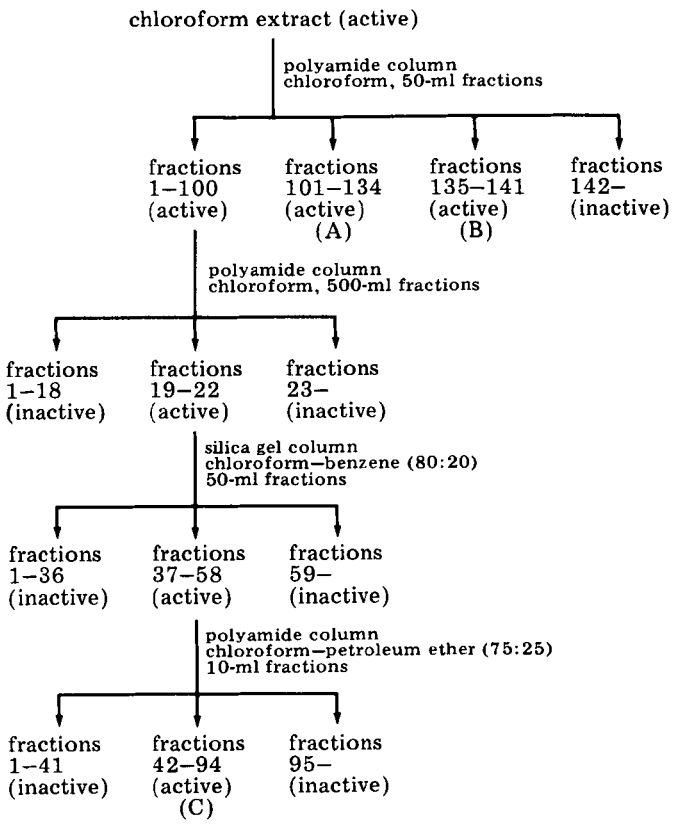
Both varieties of *V. archeri* exhibit the same antibiotic profile, and the same active compounds were isolated from each species. The methods described in this report are for the hard species of *V. archeri*.

Preparation of Crude Antibiotic Fraction—Powdered sponge (7 kg) was extracted repeatedly with methanol (3 × 5 liters) at room temperature until the extract showed no antibiotic activity. A total of 1580 g was obtained after solvent removal. This product was extracted with ethyl acetate to obtain 278 g of ethyl acetate-soluble material and then was extracted with ether to obtain 240 g of material.

The ether-soluble material was partitioned between acetonitrile and petroleum ether (1:1). The acetonitrile-soluble fraction (210 g) contained the antibiotic active substances, while the petroleum ether-soluble material was inactive against *Escherichia coli*. The acetonitrile-soluble fraction was then extracted with chloroform. Evaporation of the chloroform yielded 30 g of an antibiotic-containing mixture. The purification of Compounds A, B, and C from this mixture is outlined in Scheme I.

RESULTS AND DISCUSSION

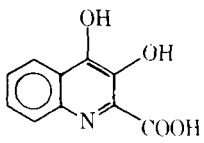
Compound A—This compound from pooled fractions 101–134 (40 mg) was first crystallized from 95% ethanol and then recrystallized from chloroform–methyl ethyl ketone.



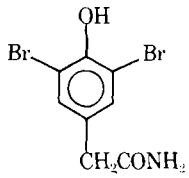
Scheme I

[1] The following instruments were used: a Perkin-Elmer model 421 grating IR spectrometer, a Cary 14 UV spectrophotometer, a Perkin-Elmer model 141 polarimeter, an A-60A Varian NMR spectrometer, and a Hitachi Perkin-Elmer model 107 mass spectrometer. Microanalyses were performed by Schwarzkopf Microanalytical Laboratories, Woodside, N.Y.

[2] The sponge was identified by Dr. Willard D. Hartman, Department of Zoology, Yale University, New Haven, Conn.



VII



VIII

The IR spectrum in a potassium bromide pellet showed absorbances at 3425 (amine, hydroxyl) and 1625 (amide carbonyl) cm^{-1}. The spectrum in deuterated dimethyl sulfoxide gave proton resonances at δ 7.45 (s, 2H), 7.1–6.80 (broad, 3H), and 3.31 (s, 2H) ppm. The mass spectrum had molecular ions at *m/e* 307, 309, and 311 (1:2:1 ratio) and prominent peaks at 263, 265, and 267 (1:2:1 ratio, $M^+ - 44$). The compound, mp 190–191°, gave an elemental analysis of: C, 31.31; H, 2.17; and N, 4.67.

These data are indicative of Structure VIII, 2-(3′,5′-dibromo-4′-hydroxyphenyl)acetamide, with a molecular formula of $C_8H_7Br_2NO_2$. Confirmation of this structure for Compound A was achieved by treatment with diazomethane followed by a study of the mass spectrum of the resulting methyl ether. This spectrum showed the molecular ion peak at *m/e* 321, 323, and 325 (1:2:1 ratio) and another prominent triplet at 277, 279, and 281 (1:2:1 ratio, $M^+ - 44$). The identity was also established by synthesis from *p*-hydroxyphenylacetic acid (5).

Compound B—Evaporation of solvent from pooled fractions 135–141 gave 6.1 g of a white crystalline substance, which was crystallized from chloroform–methyl ethyl ketone as an analytically pure compound (5.5 g), mp 120–122°.

The IR spectrum in a potassium bromide pellet showed absorptions at 3370 (hydroxyl) and 2265 (nitrile) cm^{-1}. The NMR spectrum in deuterated dimethyl sulfoxide gave proton resonances at δ 6.49 (s, 1H), 6.12 (d, 1H, J = 7 Hz), 6.05 (s, 1H), 3.96 (d, 1H, J = 7 Hz), 3.66 (s, 3H), and 2.77 (s, 2H) ppm and an optical rotation of $[\alpha]_D$ +186° (c, 0.5 g/liter in methanol). The mass spectrum had molecular ions at *m/e* 337, 339, and 341 (1:2:1 ratio). The compound gave an elemental analysis of: C, 31.50; H, 2.82, Br, 46.94; and N, 4.23.

On the basis of its spectral and elemental analysis, this component was identified as 1-(3′,5′-dibromo-1′,6′-dihydroxy-4′-methoxycyclohexa-2,4-diene)acetonitrile (I), previously isolated (1) from *V. cauliformis*.

Compound C—This compound was crystallized from hexane–ethyl acetate as an analytically pure substance (92 mg), mp 129–130°.

The IR spectrum in a potassium bromide pellet gave absorbances at 3310 (hydroxyl) and 1760 cm^{-1}, and the UV spectrum had the λ_{max} at 282.2 nm (ϵ 4680). The NMR spectrum in deuterated chloroform gave proton resonances at δ 6.3 (s, 1H), 5.17 (s, 1H), 3.77 (s, 3H), 3.50 (broad, 1H), and 2.9 (s, 2H) ppm. The mass spectrum had molecular ions at *m/e* 338, 340, and 342 (1:2:1 ratio) and peaks at 320, 322, and 324 (1:2:1 ratio, $M^+ - H_2O$), indicating the presence of two bromine atoms in the compound. The compound gave an elemental analysis of: C, 31.76; H, 2.35; and Br, 47.06.

The data suggest that this antibiotic is 2-oxo-5,7-dibromo-6-methoxy-9-hydroxy-8,9-dihydrocoumarin (aeroplysinin-2) (IV), described by Minale *et al.* (3).

Biological Activity—The natural products were tested for their antibacterial activity against *E. coli*[3] in the following manner. The compound, 0.003 g, was applied to a paper disk and incubated for 18 hr at 35°. Zones of bacterial inhibition were measured and indicated by: +, less than 15 mm; 2+, 15–18 mm; 3+, 18–22 mm; and 4+, greater than 22 mm. The results were: I, 4+; IV, +; and VIII, 3+.

REFERENCES

(1) E. Fattorusso, L. Minale, and G. Sodano, *Chem. Commun.*, **1970,** 751.

(2) E. Fattorusso, L. Minale, and G. Sodano, *J. Chem. Soc. Perkin I*, **1972,** 16.

(3) L. Minale, G. Sodano, W. R. Chan, and A. M. Chen, *J. Chem. Soc. Chem. Commun.*, **1972,** 674.

(4) G. M. Sharma and P. R. Burkholder, *J. Antibiot., Ser. A*, **20,** 200 (1967).

(5) G. M. Sharma and P. R. Burkholder, *Tetrahedron Lett.*, **1967,** 4147.

(6) G. M. Sharma, B. Vig, and P. R. Burkholder, *J. Org. Chem.*, **35,** 2823 (1970).

(7) E. Fattorusso, L. Minale, K. Moody, G. Sodano, and R. H. Thomson, *Gazz. Chim. Ital.*, **101,** 61 (1971).

(8) K. Moody, R. H. Thomson, E. Fattorusso, L. Minale, and G. Sodano, *J. Chem. Soc. Perkin I*, **1972,** 18.

(9) R. J. Anderson and D. J. Faulkner, *Tetrahedron Lett.*, **1973,** 1175.

(10) W. Fulmor, G. E. V. Lear, G. O. Morton, and R. D. Mills, *ibid.*, **1970,** 4551.

(11) D. B. Cosulich and F. M. Lovell, *J. Chem. Soc. Chem. Commun.*, **1971,** 397.

(12) E. Fattorusso, S. Forenza, L. Minale, and G. Sodano, *Gazz. Chim. Ital.*, **101,** 104 (1971).

(13) M. F. Stempien, Jr., J. S. Chib, R. F. Nigrelli, and R. A. Mierzwa, in "Food-Drugs from the Sea Proceedings, Marine Technology Society," L. R. Worthen, Ed., Marine Technology Society, Washington, D.C., 1972, pp. 110–115.

(14) J. S. Chib, R. A. Mierzwa, R. F. Nigrelli, and M. F. Stempien, Jr., paper presented at the National Conference of Indian Science Congress, Chandigarh, India, 1973, p. 124.

ACKNOWLEDGMENTS

Supported by Sea Grant Project Grant 1-35263 to the New York Zoological Society.

The authors thank Professor A. K. Bose of Stevens Institute of Technology, Hoboken, N.J., for helpful discussions during this work and for providing facilities for the recording of mass and NMR spectra.

[3] The American Type Cuture Collection, *Escherichia coli* 4157 ← NC TC 86 (original *Escherichia* strain) containing both smooth and rough colonies.