Isolation of 2,6-Dibromophenol from the Marine Hemichordate, Balanoglossus biminiensis

Abstract. *2,6-Dibromophenol has been isolated from a luminous marine enteropneust,* Balanoglossus biminiensis, *found on intertidal beach areas at Sapelo Island, Georgia. This compound, responsible for the characteristic "iodoform-like" odor of these animals, is present in relatively large amounts; the estimated quantity per organism is 10 to 15 milligrams. Identity of the isolated substance as 2,6-dibromophenol is based on analyses of ultraviolet, infrared, and nuclear magnetic resonance spectra, mass spectrometry analysis, and on melting-point data.*

Balanoglossus biminiensis, commonly referred to as an acorn worm, is a marine hemichordate which lives in the intertidal waters along the coast of the southern United States. This organism is about 25 to 30 cm in length, orange-yellow in color, and possesses a strong odor that has been characterized as "iodoform-like" (*1*). In addition, the organism produces a brilliant bluish luminescence when excited mechanically or electrically (*1*). The requirements for bioluminescence and the mechanism of the light reaction have been described (*2–4*).

We isolated the odor-producing compound because it occurred in rather large amounts and because it might be related to luciferin or its oxidized form. Actually, any relationship of the isolated compound to Balanoglossid luciferin appears doubtful. However, the compound is most unusual because of its brominated character and the large quantities occurring in these organisms (calculated minimum content is 10 to 15 mg of compound per organism).

Balanoglossid worms were obtained by digging in areas marked by cone-like structures found at burrow entrances of the organism. When whole worms were lyophilized, small amounts of luciferin activity were found in the melted condensate (from the cone) which also contained fairly large quantities of the compound responsible for the characteristic odor of these animals.

The Balanoglossid compound was obtained by direct extraction with chloroform at acid and alkaline *p*H's from the condensate of the cone. Initially, 100 ml of this condensate was adjusted to *p*H 10 with 1*N* NaOH and extracted with 10-ml portions of chloroform. Efficiency of the extraction was judged by the absorption of the chloroform solution at 305 mμ. Four successive 10-ml extractions usually brought the absorption at 305 mμ to readings of less than 0.1 unit.

The *p*H of the aqueous phase was then adjusted to *p*H 3 with 1*N* HCl, and the extraction with 10-ml portions of chloroform was repeated. Again, four successive 10-ml extractions were sufficient to complete the extraction. Efficiency of extraction in the acid system was followed by monitoring ultraviolet absorption at 280 mμ in a Model 14 Cary recording spectrophotometer. Examination of the aqueous phase after the final extraction showed that essentially all ultraviolet-absorbing material had been extracted.

Extracts from the acid and alkaline systems were then pooled in separate containers and evaporated with a gentle stream of air. Crystalline material separated from the pooled evaporated acid extract which had a strong odor reminiscent of iodoform. The alkaline-extracted material possessed only a faint odor and was not further examined. Qualitative tests on the isolated compound with nitrous acid and bromine water indicated that the compound was a phenol. An initial melting point (uncorrected) was 50° to 51°C. Due to the fact that the compound was volatile, it could be purified by sublimation in a vacuum. Accordingly, the crystals obtained from the chloroform extract were dissolved again in a small volume of chloroform and quantitatively transferred to a sublimation tube of the type described by Mills (*5*), except that the inner and outer tubes were of conical-tip design. After the chloroform was gently evaporated, the compound sublimed onto the cold finger of the apparatus. It was then removed from the cold-finger tip by small amounts of chloroform applied with an eye dropper, and the solution was transferred to a small test tube. After evaporation of the chloroform, white needle-like crystals with a very powerful odor, now somewhat reminiscent of cresol, were obtained.

Ultraviolet spectra of the compound were obtained with a Coleman-Hitachi spectrophotometer, Model EPS-3T. Two molecular species were found (Fig. 1). At *p*H values below 4, only the acid form was seen with two peaks at 279 and 286 mμ, with estimated molar absorptivities of 2184 and 2135, respectively. From *p*H 4 to about 5.8 a transitional form was evident, having maxima at 279, 286, and 305 mμ. Between *p*H 4 and 7, the maximum at

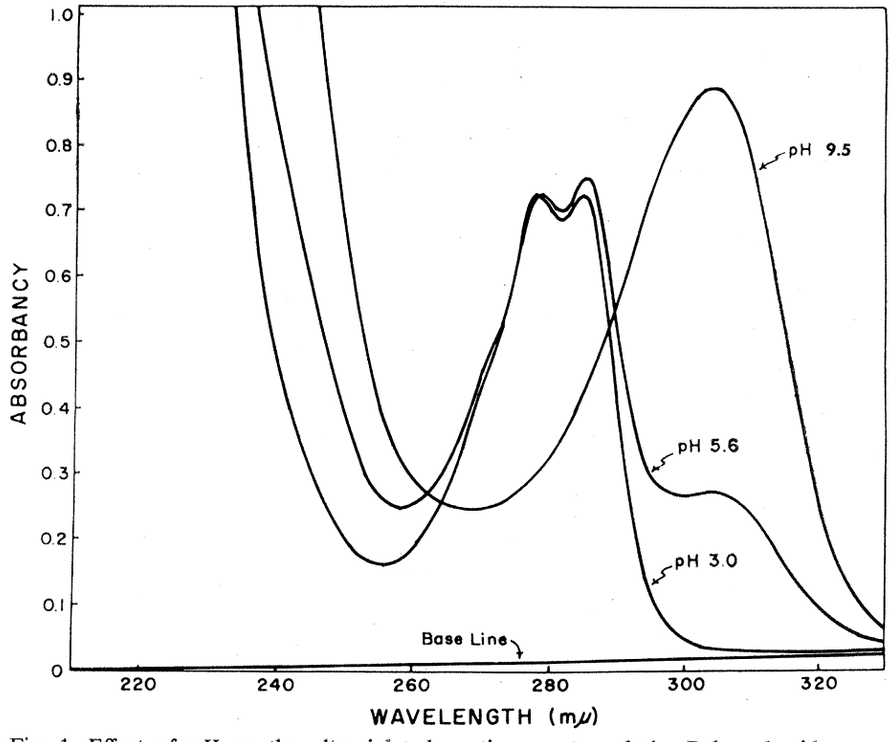

Fig. 1. Effect of *pH* on the ultraviolet-absorption spectra of the Balanoglossid compound. Concentrations were 0.33 μmole/ml at *p*H 3.0 and 5.6, and 0.165 μmole/ml at *p*H 9.5.

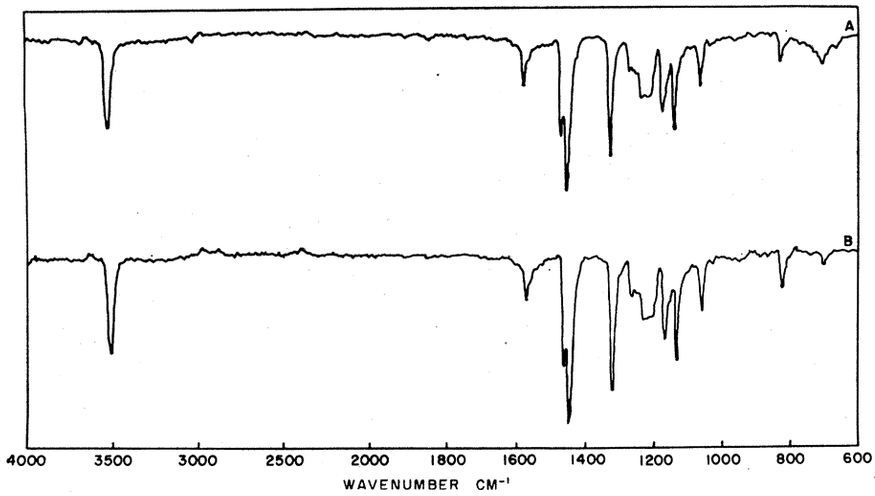

Fig. 2. Comparison of the infrared spectra of the Balanoglossid compound (A) to that of 2,6-dibromophenol (B). Concentrations were 1.2 percent in $CHCl_3$ in each case.

279 mμ disappeared entirely, the maximum at 286 mμ diminishing more slowly. This maximum was almost obliterated by the time *p*H 7.2 was reached. The maximum at 305 mμ increased slowly over the range of *p*H 3 to 5.6. Between *p*H 5.6 and 6.2 there was a marked increase in absorption at 305 mμ, the molar absorptivity for this peak approximately doubling. At *p*H 7.5 the maxima at 279 and 286 mμ were completely obliterated, and only a species having an absorption at 305 mμ was evident. Estimated molar absorptivity for this form was 5040. This bathochromic shift is typical of phenols. When changes in absorption at 305 mμ were plotted against *p*H, the *p*K of the compound was estimated to be at *p*H 6.2. The di-ortho positions of the bromine atoms are undoubtedly the reason for the strong acidity. Ultraviolet-absorption spectra for the compound from *Balanoglossus* and the authentic 2,6-dibromophenol were identical in every respect.

The infrared and nuclear magnetic resonance spectra obtained for the Balanoglossid compound and 2,6-dibromophenol were identical and superimposable (Figs. 2 and 3). Mass spectral analysis indicated that the compound had a molecular weight of 252 and a fragmentation pattern typical of dibromophenol. For example, the molecular ions 250, 252, and 254 were in the correct distribution for two bromine atoms.

A comparison of melting points for dibrominated phenols ruled out all dibromophenols except the 2,6-substituted compound. A melting point higher than 52°C (uncorrected) could not be obtained either for the compound isolated from *Balanoglossus* or for authentic 2,6-dibromophenol (*6*) purified by sublimation and recrystallized from water. However, the two melting points were within 1°C of each other, and a mixed melting point was 51° to 52°C. Both compounds were extremely volatile and sublimed readily. These data leave no doubt that the Balanoglossid compound is 2,6-dibromophenol.

The presence of large amounts of 2,6-dibromophenol in a living organism is hard to understand in view of the general toxicity of such compounds. For example, the related chlorinated phenols are well known for their potency as disinfectants. Unless Balanoglossids must continuously surround themselves with a disinfectant for their own survival, it is difficult to imagine the function that 2,6-dibromophenol serves in these animals.

Nothing is known about the biosynthesis of this compound in Balanoglossids. However, the luciferase of these organisms may play a role here in view of the fact that luciferase is a peroxidase (*3*) and that peroxidases that catalyze the bromination of phenols have been isolated (*7*). Brominated compounds have been isolated from nature only in rare instances, such as the isolation by Morner (*8*) of dibromotyrosine from two species of coral and that of some brominated compounds of ill-defined structure from several species of red algae (*9*).

RAYMOND B. ASHWORTH
MILTON J. CORMIER
Department of Biochemistry, University of Georgia, Athens 30601

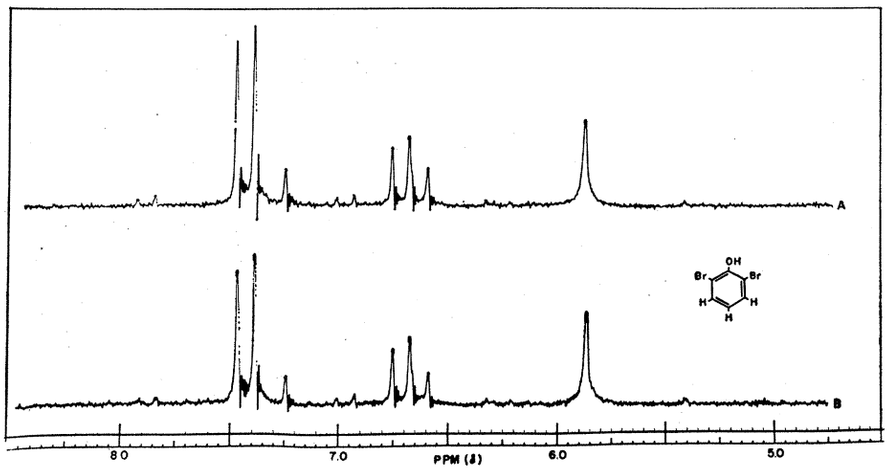

Fig. 3. Comparison of the nuclear magnetic resonance spectra of the Balanoglossid compound (A) to that of 2,6-dibromophenol (B). A 30-mg sample dissolved in $CDCl_3$ was used in each case. The small peak where δ is about 7.2 is due to traces of $CHCl_3$ in the $CDCl_3$.

References and Notes

1. E. N. Harvey, *Bioluminescence* (Academic Press, New York, 1952), pp. 202–205.
2. M. J. Cormier and L. S. Dure, *J. Biol. Chem.* **238**, 785 (1963).
3. L. S. Dure and M. J. Cormier, *ibid.*, p. 790.
4. ———, *ibid.* **239**, 2351 (1964); M. J. Cormier, P. Kreiss, P. M. Prichard, in *Bioluminescence in Progress*, F. H. Johnson and Y. Haneda, Eds. (Princeton Univ. Press, Princeton, N.J., 1966), p. 363.
5. G. L. Mills, *Biochem. J.* **50**, 707 (1952).
6. Obtained from Eastman Chemical Co.
7. L. P. Hager, D. R. Morris, F. S. Brown, H. Eberwein, *J. Biol. Chem.* **241**, 1769 (1966).
8. C. T. Morner, *Z. Physiol. Chem.* **88**, 138 (1913).
9. M. Peguy, in *Comptes Rendus du IV^e Congrès International des Algues Marines*, A. D. Virville and J. Feldmann, Eds. (Macmillan, New York, 1964), p. 366.
10. Supported in part by NSF and AEC. M.J.C. holds career development award 1-K3-GM-3331-01. We thank Dr. M. Grostic of the Upjohn Company for performing the mass spectrometry analysis, and Dr. L. Keith of the Southeast Water Research Laboratory, Athens, Georgia, for the nuclear magnetic resonance analysis. Contribution 124 from the University of Georgia Marine Institute, Sapelo Island, Georgia.

13 February 1967