The *Journal of Marine Research* is an online peer-reviewed journal that publishes original research on a broad array of topics in physical, biological, and chemical oceanography. In publication since 1937, it is one of the oldest journals in American marine science and occupies a unique niche within the ocean sciences, with a rich tradition and distinguished history as part of the Sears Foundation for Marine Research at Yale University.

Past and current issues are available at [journalofmarineresearch.org](http://journalofmarineresearch.org).

Yale University provides access to these materials for educational and research purposes only. Copyright or other proprietary rights to content contained in this document may be held by individuals or entities other than, or in addition to, Yale University. You are solely responsible for determining the ownership of the copyright, and for obtaining permission for your intended use. Yale University makes no warranty that your distribution, reproduction, or other use of these materials will not infringe the rights of third parties.

This work is licensed under the Creative Commons Attribution-NonCommercial-ShareAlike 4.0 International License. To view a copy of this license, visit [http://creativecommons.org/licenses/by-nc-sa/4.0/](http://creativecommons.org/licenses/by-nc-sa/4.0/) or send a letter to Creative Commons, PO Box 1866, Mountain View, CA 94042, USA.
HALOGENATED AMINO ACIDS OF THE BATH SPONGE

BY

EVA M. LOW

Osborn Zoological Laboratory, Yale University
and
Survey of Contemporary Knowledge of Biochemistry
American Museum of Natural History

ABSTRACT

The amino acid composition and halogen content of the bath sponge, Spongia officinalis obliqua (D. & M.), collected in the Gulf of Mexico, was studied to determine the nature of the halogenated amino acids present. The sponge was found to contain 1.06% tyrosine, 0.38% iodine, and 0.80% bromine. It is believed that the major halogenated amino acids are the mono- (3-) and di- (3, 5-) bromo- and iodo-tyrosines.

INTRODUCTION

In 1910 Wheeler and Mendel (12) isolated an iodine-containing amino acid, 3, 5-diiodotyrosine, from the bath sponge. Previously this compound had been found in gorgonians by Drechsel (5), who named it iodogorgoic acid. In 1941 it was shown by Ackermann and Muller (2) that although halogen analyses of the “3, 5-diiodotyrosine” from the bath sponge always agreed with the calculated values, carbon analyses were too high by 5%. Furthermore, the x-ray diffraction pattern of the natural “diiodotyrosine” was different from that of the synthetic d, l-compound. Careful halogen analysis showed that it was a mixture of dibromotyrosine and diiodotyrosine in a ratio of 4 to 1. Although Ackermann and Müller were unable to separate the mixture from the bath sponge, they had no difficulties in separating synthetic mixtures. They believed that racemization, which accompanies hydrolysis, made the separation impossible.

Ackermann and Burchard (1) found that bath sponge spong in contained 0.79% diiodo- and dibromotyrosine. On the basis of their determinations of the iodine and bromine contents of unhydrolyzed spong in (1.41% I and 2.93% Br), 11.4% of such a mixture might be expected if all halogen were bound to tyrosine. Even if substantial losses are encountered during hydrolysis and isolation, this would not account for the discrepancy in the results. Furthermore, only 1–2% tyrosine was found in spong in which had been dehalogenated with

1 Contribution to the study of Marine Products. XXXIII.
2 Present address: Eva L. Verplanck, Dept. of Limnology, Academy of Natural Sciences, Philadelphia, Penn.

239
hydrochloric acid instead of the 4.3% needed to bind all iodine and bromine. From this, Ackermann and Burchard concluded that there must be other halogenated amino acids in sponges.

The present author (6) found much less halogen than Ackermann in several sponges, some of which were closely related to those investigated by Ackermann. Roche and Lafon (7) found much less iodine than Ackermann and no relation between the iodine, diiodotyrosine and tyrosine contents; they did not investigate the bromine and dibromotyrosine contents. Therefore it seemed pertinent to reinvestigate the amino acid composition of the bath sponge and relate it to its halogen content. All experiments were carried out on the common bath sponge, *Spongia officinalis obliqua* (D. & M.), which was collected in the Gulf of Mexico by courtesy of F. G. Walton Smith and kindly identified by Willard Hartman.

**METHODS**

The sponges had been cleaned and dried for market but were not bleached. They were washed free of sand with water, then dried at about 100° C. and kept in a desiccator. The iodine and bromine analyses were carried out according to the methods described in a previous paper (6). The methods were checked with known amounts of diiodo- and dibromotyrosine.

The sponges were hydrolyzed with either 5N Ba(OH)$_2$ • 8H$_2$O or 20% HCl in a sealed tube at 100° for 24 hours. The acid hydrolyzates were concentrated to dryness in vacuo; small amounts of water were added and distilled off until the acid was removed. The residue was then taken up in a known volume of distilled water. The basic hydrolyzates were neutralized with CO$_2$, filtered, and concentrated in vacuo.

Nitrogen analyses were done by the Kjeldahl method.

Tyrosine was determined according to the method of Thomas (10), which is a color reaction with α-nitroso-β-naphthol. A Beckmann spectrophotometer was used for the color readings.

Aromatic and aliphatic amino acids were separated on charcoal columns (11). Darco G-60 charcoal was washed with ethyl acetate and water, after which it was dried at 100°. The charcoal was mixed with two parts of filter-paper pulp; the mixture was then made into a slurry with water and poured into a column. A solution of amino acids was poured onto the column and the column was washed with water until the eluate gave a negative ninhydrin test. The column was then washed with ethyl acetate saturated with water to elute the aromatic and other ring-containing amino acids. Nitrogen analyses showed a quantitative recovery of amino acids (98.6%).
The method of paper chromatography of Consden, Gordon and Martin (3) was employed for the separation of amino acids. Whatman No. 1 filter paper and the solvents phenol-water, collidine-water (Eastman Technical grade), and o- and p-cresol-water were used. The chromatograms were developed with a ninhydrin solution in water-saturated butanol. The amino acids were identified by comparing their flow rates with those of known compounds and by using the map prepared by Dent (4).

Diodotyrosine was prepared according to the method of Savitzki (9), dibromotyrosine according to the method of Zeyneck (13). A sample of 3-iodotyrosine was obtained through the courtesy of R. M. Herriot.

RESULTS AND DISCUSSION

Each series of determinations was carried out on one sponge. Each determination was carried out several times, and each series was done on several sponges of the same species collected in the same general area. The figures given are means of these values.

In a typical experiment, 0.51 g. dried sponge was hydrolyzed with hydrochloric acid to dehalogenate tyrosine. During hydrolysis some material is generally lost, some of the loss being due to an actual decomposition of amino acids and some to mechanical losses. To ascertain the amount lost, nitrogen determinations were done on the unhydrolyzed sponge and on aliquots of the hydrolysate. It was found that the total sponge had 15.4% N, whereas the hydrolysate had the equivalent of 14.5% N. Tyrosine determinations were carried out on the hydrolysate, and the values were corrected for the nitrogen loss (5.8%). It was found that the sponge had an average of 1.06% tyrosine (including halogenated tyrosine).

Iodine and bromine analyses were done on a part of the above sponge. A mean of five analyses gave 0.38% I and 0.80% Br.

The above figures indicate that 1 gram of this bath sponge has 0.0587 millimols tyrosine, 0.10 millimols bromine, and 0.0294 millimols iodine (0.129 millimols halogen), or about two halogen molecules for each tyrosine molecule. In this sponge, therefore, almost all of the halogen molecules could be bound to tyrosine.

Amino acid analyses were done on the barium hydroxide hydrolysate of the sponge. This method racemizes the amino acids but does not dehalogenate tyrosine; the amino acids serine, arginine, cystine and cysteine, and threonine are destroyed to a large extent. Paper chromatography with phenol and collidine showed the presence of the paper.
aspartic acid, glutamic acid, lysine, glycine, alanine, proline and hydroxyproline, $\alpha$-aminobutyric acid, leucine, valine, methionine, and tyrosine.

The most important amino acids that could combine with iodine are the aromatic amino acids and histidine. Since they are present in much smaller amounts than the aliphatic amino acids, they cannot be seen easily on the chromatograms. Therefore the amino acids were first separated on charcoal and each fraction was chromatographed on paper.

The chromatograms of the aliphatic fraction showed no amino acids which had not been on the chromatograms of the total hydrolysate. The aromatic fraction gave seven spots when a concentrated

Figure 1. Schematic representation of a paper chromatogram of the "aromatic" fraction of a basic hydrolysate of the bath sponge. 1 = tyrosine; 2 = 3-iodotyrosine; 3 = histidine; 4 = dibromotyrosine; 5 = tryptophan; 6 = phenyl alanine; 7 = diiodotyrosine.
solution was chromatographed with phenol and collidine; they could be identified as tyrosine, moniodotyrosine, diiodotyrosine, dibromotyrosine, phenylalanine; the very faint spots are probably tryptophan and histidine (see Fig. 1). The flow rates of diiodo- and dibromotyrosine are similar (see Table I), and they were separated only when large sheets of paper were used. The moniodotyrosine spot was always diffuse. No obvious indication of a spot that might be referable to iodo bromotyrosine was observed, though the occurrence of such a compound would surely be expected.

Since Roche, Rand and Yagi (8) recently found monobromotyrosine in some gorgonians, it is not unlikely that it is present also in sponges. No pure bromotyrosine could be obtained; it is probable, however, that it has similar flow rates to those of iodotyrosine in phenol and collidine and that the spot labeled moniodotyrosine is really a mixture of iodo- and bromotyrosine. Roche and co-workers found that the flow rates for the two were similar in butanol containing acetic acid or ammonia.

The present author was unable to find any differences in the behavior of racemized and nonracemized diiodo- and dibromotyrosine mixtures. Mixtures of synthetic dibromo- and diiodotyrosine, when chromatographed, had the same flow rates whether they were racemized with barium hydroxide or not. They could be easily separated with o cresol and with phenol and collidine when allowed to run for a long time. Table I gives the flow rates ($R_f$ values) in several solvents.

TABLE I. FLOW RATES OF TYROSINE AND SOME HALOGENATED TYROSINE DERIVATIVES

<table>
<thead>
<tr>
<th>Solvent</th>
<th>Tyrosine</th>
<th>3-Iodotyrosine</th>
<th>Nonracemized Mixture</th>
<th>Racemized Mixture</th>
</tr>
</thead>
<tbody>
<tr>
<td>(water-saturated)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Phenol</td>
<td>0.60</td>
<td>0.64</td>
<td>0.90</td>
<td>0.76</td>
</tr>
<tr>
<td>o-Cresol</td>
<td>0.17</td>
<td>—</td>
<td>0.72</td>
<td>0.53</td>
</tr>
<tr>
<td>p-Cresol</td>
<td>0.33</td>
<td>—</td>
<td>0.82</td>
<td>0.74</td>
</tr>
<tr>
<td>Collidine</td>
<td>0.50</td>
<td>0.66</td>
<td>0.62</td>
<td>0.49</td>
</tr>
</tbody>
</table>

From the experiments presented in this paper it appears likely that diiodotyrosine, dibromotyrosine, monoidotyrosine, and probably monobromotyrosine are the main halogenated amino acids in the bath sponge, *Spongia officinalis obliqua*, from the Gulf of Mexico. The tyro-

4 Absolute figures are of little value unless all observations are made at the same time [see Dent (4)]. In these experiments, all amino acids were run in the same solvent simultaneously.
sine and halogen determinations indicate that all halogen could be attached to tyrosine. But the possibility exists that there are small amounts of other halogenated amino acids. The most likely amino acids to take up halogen would be histidine and phenyl alanine. However, preliminary experiments with radioactive iodine did not give any indication of their presence.

The author wishes to thank Werner Bergmann for stimulating her interest in sponge chemistry, Harold G. Cassidy for his help with the amino acid chemistry, and G. Evelyn Hutchinson for his constant help, interest and encouragement.

SUMMARY

The bath sponge, *Spongia officinalis obliqua* (D. & M.), collected in the Gulf of Mexico, was found to contain 1.06% tyrosine, 0.38% iodine, 0.80% bromine, and 15.4% nitrogen.

The amino acid composition of the sponge was studied with special emphasis on the halogenated amino acids.

It is believed that the major halogenated amino acids are the mono- (3-) and di- (3, 5-) bromo- and iodo-tyrosines.

REFERENCES

(1) Ackermann, Dankwart and Charlotte Burchard.

(2) Ackermann, Dankwart and Ernst Müller.


(4) Dent, C. E.

(5) Drechsel, H. F. E.

(6) Low, E. M.

(7) Roche, Jean and Michel Lafon.

(8) Roche, Jean, Michel Rand and Yasuo Yagi.
(9) Savitskii, A. Y.

(10) Thomas, L. E.

(11) Tiselius, Arne, B. K. Drake and Lennart Hagdahl.


(13) Zeynek, R.