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STUDIES ON SOME CHEMICAL CONSTITUENTS OF DIATOMS

By

EVA M. LOW

Department of Limnology
Academy of Natural Sciences of Philadelphia

ABSTRACT

In five species of diatoms 19 amino acids have been found. There was no qualitative difference between marine and freshwater species. Free amino acids were absent in aqueous and alcoholic extracts. The sterol content of three species was very low, from 0.009% of the dry weight in Nitzschia closterium to 0.6% in Navicula pelliculosa. The principal sterol of Navicula pelliculosa is a Δ7-stenol, provisionally identified as chondrillasterol, which is also known from Scenedesmus obliquus and certain sponges. It is reasonably certain that different sterols are found in the two species of Nitzschia.

INTRODUCTION

The diatoms represent a group of algae about whose physiology and chemistry very little information has been published. The fats of the marine diatom Nitzschia closterium have been studied by various investigators (Lovern, 1936; Clarke and Mazur, 1941) who fractionated the ether- or acetone-soluble fraction and isolated or identified the fatty acids and some of the other components. Clarke and Mazur (1941), who also studied the nonsaponifiable fraction of the ether extract, found a sterol with a melting point of 138°C and a rotation of $\[\alpha\]_D = -41.6^\circ$, which gave an acetate of m.p. 132°C, $\[\alpha\]_D = -22.9^\circ$. The amino acids of the freshwater diatom Navicula pelliculosa have been investigated by Fowden (cited by Fogg, 1953). Mazur and Clarke (1942) have determined some of the amino acids on what appears to be a mixture of diatoms.

Since diatoms produce fats rather than carbohydrates as storage products, and since it has been shown that there is a great diversity of sterols in lower plants and animals, it seemed advisable to begin a study of the fats with a consideration of sterols. In the present investigation the fats of three diatoms have been examined and the nonsaponifiable fractions have been studied. The amino acids of five diatoms have also been identified. This work represents the beginning of a study of the chemical composition of diatoms and it is hoped that the difference, if any, between closely related forms living in marine or fresh water may be determined.
METHODS

A. Culturing of Diatoms

*Navicula pelliculosa:* The diatoms were grown in 3-liter flasks in a constant temperature bath of about 22° to 24° C, illuminated from the bottom with fluorescent and neon lights. Between 10 and 15 flasks were used simultaneously for a period of 6½ months. Each flask contained two liters of culture medium. Approximately twice a week about one liter was harvested from each flask and this was replaced by one liter of double-strength medium. The medium consisted of modified Chu 14 (Chu, 1942), Hoagland’s A-5 solution — 2.5 ml/l (Hoagland and Arnon, 1938), and earth extract (1–2.5 ml) which was prepared by using 100 g of dry soil with 250 ml water which was filtered through a Seitz filter. The medium was autoclaved for 30 minutes. Either air or a mixture of air and 2 to 5% CO₂ was bubbled through the flasks. When CO₂ was used, 10 ml of 2M CaCO₃ was added. The harvested diatoms were centrifuged to remove the bulk of the water, then dried in a desiccator. A total of 213 g (dry weight) was obtained.

*Nitzschia linearis:* This organism was grown in the same manner as *N. pelliculosa,* with Chu 14 modified medium. A total of 27.2 g (dry weight) was obtained.

*Nitzschia closterium:* This diatom was grown in the same manner as the others. For a medium, sea water (obtained from the mouth of Shark River) was filtered and diluted with distilled water to give a salinity of approximately 1,700 ppm. To this water the following chemicals were added: 0.0466 g/l Na₂SiO₃.9H₂O; 0.5 ml/l Hoagland’s A-5 solution; 2.5 ml/l earth extract; 5 to 7.5 ml of a solution containing 25 g/l Na₂CO₃; 0.55 ml of Ketchum and Redfield’s (1938) medium. At most times marble chips were also placed in the flasks, which were illuminated with neon and fluorescent lights for 12 hours/day. During that time a mixture of air and 2 to 5% CO₂ was bubbled through the flasks. The diatoms were harvested every 10 days. About half of the medium and diatoms were siphoned off after which the volume was replaced with more medium. A total of 38 g (dry weight) was obtained.

*Nitzschia palea* and *Gomphonema parvulum* were obtained from stock cultures in the laboratory.

All cultures were free of algae and protozoa. Although there may have been some bacteria present, none could be observed under high power of the microscope. Any quantities of bacteria present would have been too small to interfere with chemical studies.
B. CHEMICAL STUDIES

The amino acid determinations were carried out on fresh material. One ml of packed cells was hydrolyzed by refluxing with 10 ml of 6N HCl for 24 hours. The hydrolysates were concentrated to dryness in vacuo; small amounts of water were added and distilled off until all acid was removed. The residues were then taken up in a known volume of distilled water. The method of two-dimensional paper chromatography of Consden, et al. (1944) was employed for the separation. Whatman No. 1 filter paper was used, and the solvents were phenol saturated with water and a mixture of one part collidine and one part lutidine saturated with water. The chromatograms were developed with a ninhydrin solution in water-saturated butanol. The amino acids were identified by comparing their $R_f$ values with those of known compounds and by using the map prepared by Dent (1948).

The fats were obtained by extracting the dried diatoms with acetone in a Soxhlet extractor for 48 hours. The acetone was evaporated and the residue was refluxed with 10% KOH in 75% ethanol for two hours. After diluting with water, the nonsaponifiable matter, extracted with ether, was then dried over anhydrous sodium sulfate, filtered and evaporated. The residue was repeatedly extracted with hot methanol and, since the sterols could not be obtained in satisfactory crystalline form, the residues were acetylated.

The acetylations were carried out by refluxing the nonsaponifiable residues with 10 ml of acetic anhydride for two hours, diluting with water, cooling in ice, and filtering. The precipitates were washed with water and recrystallized from methanol.

In the case of Navicula pelliculosa, the steryl acetate was saponified by refluxing with 10 ml of 5% alcoholic KOH for two hours, diluting with water, and extracting with ether. Recrystallization of the residue of the ether extract with methanol gave the sterol. The steryl benzoate was obtained by refluxing the sterol with 1 ml of benzoyl chloride in 3 ml of anhydrous pyridine for two hours. The mixture was then poured into 100 ml of hot methanol and cooled; the precipitate was recrystallized from methanol.

All melting points taken are corrected. Optical rotations were taken in chloroform. Ashings of the dried diatoms were carried out in a muffle furnace at 550° C.

RESULTS AND DISCUSSION

Table I gives the amino acids found in the freshwater diatoms Nitzschia palea, N. linearis, Gomphonema parvulum and Navicula pelliculosa and in the marine diatom Nitzschia closterium. There
appear to be no qualitative differences in the amino acid composition of the organisms investigated. All of the common naturally occurring acids were found in all organisms with the possible exception of hydroxyproline. In the diatoms where the presence of hydroxyproline was doubtful, several of the spots on the chromatograms overlapped, and it is possible that hydroxyproline was merely obscured. No difference in the amino acid composition of the marine and freshwater organisms was found. The amino acids found in *Navicula pelliculosa* are the same as those found by Fowden (Fogg, 1953). In all cases an aliquot of fresh cells was macerated with water or alcohol and then filtered, after which the filtrate was chromatographed. In this manner no free amino acids could be detected.

<table>
<thead>
<tr>
<th>Amino Acid</th>
<th><em>Gomphonema parvulum</em></th>
<th><em>Navicula pelliculosa</em></th>
<th><em>Nitzschia palea</em></th>
<th><em>Nitzschia linearis</em></th>
<th><em>Nitzschia closterium</em></th>
</tr>
</thead>
<tbody>
<tr>
<td>Glycine</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Alanine</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Serine</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Threonine</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
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</tr>
<tr>
<td>Valine</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
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<tr>
<td>Leucine</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Isoleucine</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
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<tr>
<td>Phenylalanine</td>
<td>+</td>
<td>+</td>
<td>+ (?)</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Tyrosine</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
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<td>Cystine (as cysteic acid)</td>
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<td>+</td>
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<td>Methionine</td>
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<td>Tryptophan</td>
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<td>+</td>
<td>+</td>
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<td>+</td>
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<td>Proline</td>
<td>+</td>
<td>+</td>
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<td>+</td>
<td>+</td>
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<tr>
<td>Hydroxyproline</td>
<td>?</td>
<td>?</td>
<td>+</td>
<td>?</td>
<td>+</td>
</tr>
<tr>
<td>Aspartic acid</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
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<tr>
<td>Glutamic acid</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
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<tr>
<td>Histidine</td>
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<td>+</td>
<td>+</td>
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<td>Arginine</td>
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<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Lysine</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
</tbody>
</table>

The fats, and in particular the sterols, were investigated in three of the diatoms, *Navicula pelliculosa, Nitzschia linearis,* and *N. closterium.* Unfortunately, rather large quantities of cells were required to obtain enough material for study. A large percentage of the dry weight of the cells is inorganic, in particular the siliceous skeleton. Although the total fat content has been found to be quite high, the sterol fraction, which represents almost the entire nonsaponifiable matter, is extremely small. The quantitative data are given in Table II.
The melting points and optical rotations of the sterols and their derivatives are given in Table III. Not enough material from *Nitzschia closterium* and *N. linearis* was available to determine the nature of the sterol or sterols present. The melting points and rotations of the acetates alone did not indicate whether there was a mixture or a homogeneous substance present. The melting point and the optical rotation of the acetate from *N. closterium* are similar to those given by Clarke and Mazur (1941), cited earlier. More information is available for *Navicula pelliculosa*. From the melting points and rotations of the sterol and its derivatives it appears without doubt that it is a homogeneous substance and a Δ7-stenol. Furthermore, its properties are similar to chondrillasterol found by Bergmann and Feeney (1950) in another alga, *Scenedesmus obliquus*. When the acetate was mixed with authentic chondrillasteryl acetate and a melting point was taken, no depression of the melting point could be observed. Unfortunately, not enough material was available to confirm these findings with an infrared spectrum. The sterol of *Navicula pelliculosa* clearly differs from those found in the two species of...
Nitzschia, and an extended study might bring out a relationship between taxonomic and chemical differences.

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