Studies of the dissolved organic compounds in the sea

A preliminary report on the isolation, separation and identification of free amino acids

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INTRODUCTION

The nature of the dissolved organic compounds in the sea is a subject about which relatively little is known and, as Vallentyne (1957) has pointed out, is one that is just beginning to be explored. The major difficulty in isolating these compounds arises from the great preponderence of inorganic salts in sea water. Thus, from the values of Duursma (1960) for Norwegian Sea coastal water (dissolved organic carbon 1.0 mg/l, dissolved organic nitrogen 0.1 mg/l), the ratio of inorganic salt: dissolved organic carbon is approximately 35,000 : 1 and the ratio of inorganic salt: dissolved organic nitrogen is approximately 350,000 : 1.

A few reports have recently appeared which describe the isolation and identification of various dissolved organic compounds in sea water. Jeffrey and Hood (1958) have evaluated and discussed a number of methods for the analysis of organic matter in sea water. Slowey *et al.* (1959) made use of ethyl acetate extraction, methylation and gas chromatography in a study of fatty acids in sea water.

Tatsumoto *et al.* (1961), using co-precipitation with ferric hydroxide as described by Jeffrey and Hood (1958) followed by paper and column chromatography, found 18 amino acids in four hydrolyzed samples of surface sea water. Park *et al.* (1962), using the same procedure, found 17 amino acids in deep-sea water samples and also found that the concentrations of amino acids in a hydrolyzed sample were about three times greater than in an identical unhydrolyzed sample. They did not state, however, whether the free amino acids were isolated and identified as such. Amino acids have also been reported from marine deposits by Erdman *et al.* (1956). In addition, Belser (1959) worked out a bioassay technique for organic micronutritients in sea water and found that isoleucine, glycine, tryptophan and threonine were present.

Study of trace organic compounds such as amino acids may well throw light on presently unexplained biological differences in sea water. This subject has been discussed by Lucas (1955) and Saunders (1957), but the lack of experimental evidence makes it difficult to draw any conclusion about the possible significance of these compounds in sea water.

The present report describes a study of the free amino acids in three water samples taken along the Norwegian coast.

MATERIALS AND METHODS

Sea water samples were collected at three localities along the Norwegian coast as shown in Table 1.

Table 1.

Sample No.	I	II	III
Place of collection	Nordnes, Bergen	Solsvik 60° 41' N 04° 50' E	Skrova 68° 07' N 14° 39' E
Date.....	2/5, 1961	8/8, 1961	5/2, 1962
Depth	135 m	50 m	50 m
Size of sample.....	7 l	13 l	25 l
Salinity	33.88 ‰	34.16 ‰	33.06 ‰

Sample I was taken from the sea water intake to the Institute of Marine Research, The Directorate of Fisheries, Bergen.

Samples II and III were collected by sucking the sea water through rubber tubing into a carboy by means of a suction pump. The samples were filtered through H. A. Millipore filters (0,45 μ) within six hours of collection, and were treated with a few crystals of thymol to prevent bacterial activity.

The samples were adjusted to pH 7 with hydrochloric acid and concentrated in a continuous Buchler vacuum evaporator at 40°C. The precipitated salts were filtered off at intervals and washed with water. The wash water was added to the filtrate and the process continued until approximately 40 to 80 ml of sea water concentrate was obtained. The samples were then diluted to 1 l and desalted by passing them through a column (4 $\times$ 71 cm) loaded with Dowex 50 W $\times$ 12 20/50 mesh cation exchange resin. The column was then

washed with water until the eluate was neutral and eluted by the method of Buchanan (1957) with 0.1 M aqueous piperidine. The eluate, approximately 10 l, was concentrated in the continuous vacuum evaporator at 40°C and finally reduced to near dryness over phosphorus pentoxide in a vacuum dessicator. The sample was then redissolved in a measured volume of 10 % aqueous isopropanol and was ready for chromatographic analysis.

The circular paper chromatography technique originated by Rutter (1948) and modified by Giri and Rao (1952) was used to separate and identify the amino acids. Whatman No. 1 paper (circles of 27—35 cm diameter) was used in cabinets with a volume of approximately 1.5 l and the chromatograms were run at room temperature (about 20°C). The solvent systems used were mainly those employed by Grov (1963) together with a few described by Smith (1960).

The samples and the standard amino acids were applied with micro pipettes along arcs 2 cm from the centre on a paper marked into four or more sectors. The width of the spot-lines were kept less than 0.4 cm. After application of the samples and the standards the solvent was evaporated with warm air (approximately 40°C) from a hairdryer. The separations took from 8 to 24 hours depending on the solvent system used. The chromatograms were dried in a stream of warm air, observed under U. V. light, and the solvent front and any visible zones were marked with a pencil. The chromatograms or its sectors were then sprayed with different colour reagents (Grov 1963; Smith 1960) for identification. To separate amino acids with low Rf-values the chromatogram was run two or three times using the same solvent system, allowing the paper to dry in between, or the solvent was allowed to evaporate from the rim of the paper which extended outside the cabinet.

RESULTS AND DISCUSSION

The results of the analyses of the three samples are shown in Table 2. A total of 19 amino acids were identified and the following amino acids were present in all samples: cystine, aspartic acid, glycine, glutamic acid, threonine, β-phenylalanine, leucine and isoleucine.

These results indicate that the amino acid patterns of the three samples were different. It is not known if these differences reflect variations due to the different times of the year and/or different locations as the limited data so far obtained do not justify any conclusions on these points. The analyses will clearly have to be continued and it will be of great interest to determine whether a change

Table 2.

Amino acids found in three different sea water samples. The presence of an amino acid is represented by +

	Sample I	Sample II	Sample III
Cystine	+	+	+
Lysine	+	—	—
Histidine	+	—	—
Arginine	+	—	+
Serine	—	—	+
Aspartic acid	+	+	+
Glycine	+	+	+
Hydroxyproline	+	—	+
Glutamic acid	+	+	+
Threonine	+	+	+
α-Alanine	+	—	+
Proline	+	—	+
Tyrosine	+	—	+
Tryptophan	—	—	+
Methionine	—	—	+
Valine	+	—	+
β-Phenylalanine	+	+	+
Isoleucine	+	+	+
Leucine	+	+	+

of amino acid pattern will be found when repeated samples are taken from one locality at different times of the year.

It is realized that the filtration of large volumes of sea water containing relatively large quantities of phytoplankton, including many fragile species, may cause erroneous results if these organisms are broken up. In such a case their body fluids may contribute to the amino acid pattern of the sample. It is not known to what extent, if any, this has affected the present results but the filtrations were carefully done so that there was always a layer of liquid over the filter disc.

It is possible that the potential differences due to charged groups in a strong cation exchanger might cause hydrolysis of any peptides or proteins present in the concentrate. Paulson *et al.* (1957) have, in fact, reported the use of ion exchange resins as a catalyst in protein hydrolysis. As the present study concerns the free amino acids in sea water, it was of considerable importance to discover if the methods used would bring about such a hydrolysis.

Three di-peptides (glycyl-L-proline, glycyl-L-glutamic acid and glycyl-DL-aspartic acid) and one tri-peptide (DL-leucyl-glycyl-DL-

phenylalanine) were chromatographed before and after being subjected to the total concentration and separation procedure using an artificial salt water solution made from salts and distilled water. There were no differences in the chromatographic patterns so it was concluded that hydrolysis of the peptides did not occur.

SUMMARY

A method of concentrating and desalting sea water using evaporation and ion exchange techniques is described. Circular paper chromatography has been used to separate and identify free amino acids in desalted concentrates of sea water from three different localities along the Norwegian coast. The following amino acids have so far been identified: cystine, lysine, histidine, arginine, serine, aspartic acid, glycine, hydroxyproline, glutamic acid, threonine, α-alanine, proline, tyrosine, tryptophan, methionine, valine, β-phenylalanine, isoleucine and leucine.

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