UNIVERSITY OF WASHINGTON
DEPARTMENT OF
OCEANOGRAPHY

Technical Reports

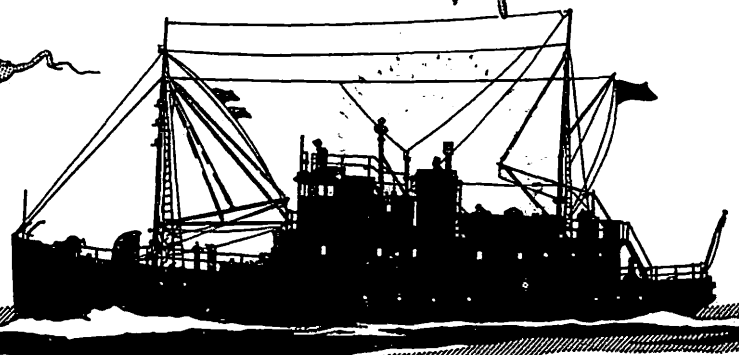
Nos. 174, 175, 176,

177, 178, and 179

A COMPILATION OF ARTICLES REPORTING RESEARCH SPONSORED BY THE OFFICE OF NAVAL RESEARCH

Office of Naval Research
Contracts Nonr-477(10)
and Nonr-477(37)
Project NR 083 012

Reference M66-78
December 1966



SEATTLE, WASHINGTON 98105

UNIVERSITY OF WASHINGTON
DEPARTMENT OF OCEANOGRAPHY
Seattle, Washington 98105

Technical Reports

Nos. 174, 175, 176,

177, 178, and 179

A COMPILATION OF ARTICLES REPORTING RESEARCH SPONSORED BY THE OFFICE OF NAVAL RESEARCH

Office of Naval Research
Contracts Nonr-477(10)
and Nonr-477(37)
Project NR 083 012

Reference M66-78
December 1966

RICHARD H. FLEMING
Chairman

ARTICLES REPORTING RESEARCH SPONSORED BY THE OFFICE OF NAVAL RESEARCH

Technical Report No. 174

IDENTIFICATION AND DETERMINATION OF ORGANIC ACIDS IN SEA WATER BY PARTITION CHROMATOGRAPHY, by Tadashiro Koyama and Thomas G. Thompson. The Journal of the Oceanographical Society of Japan, 20(5):209-220. 1964.

Technical Report No. 175

QUANTITIES OF ZOOPLANKTON AND PROPAGATION OF CALANUS FINMARCHICUS AT PERMANENT STATIONS ON THE NORWEGIAN COAST AND AT SPITSBERGEN, 1959-1962, by Ulf Lie. Fiskeridirektoratets Skrifter, Havundersøkelser, 13(8):5-19. 1965.

Technical Report No. 176

MAGNETIC AND PETROLOGIC STUDIES OF SEDIMENT FOUND ABOVE BASALT IN EXPERIMENTAL MOHOLE CORE EM7, by M. D. Fuller, C. G. A. Harrison, and Y. R. Nayudu. Bulletin, American Association of Petroleum Geologists, 50(3):566-573. 1966.

Technical Report No. 177

AN INSTRUMENT SYSTEM TO MEASURE BOUNDARY-LAYER CONDITIONS AT THE SEA FLOOR, by Richard W. Sternberg and Joe S. Creager. Marine Geology, 3(6): 475-482. 1965.

Technical Report No. 178

SOME CONSEQUENCES OF THE DECOMPOSITION OF ORGANIC MATTER IN LAKE NITINAT, AN ANOXIC FJORD, by Francis A. Richards, Joel D. Cline, William W. Broenkow, and Larry P. Atkinson. Limnology and Oceanography, 10(Suppl.): R185-R201. 1965.

Technical Report No. 179

LOW-FREQUENCY SEA LEVEL OSCILLATIONS ALONG THE PACIFIC COAST OF NORTH AMERICA, by Gunnar I. Roden. Journal of Geophysical Research, 71(20): 4755-4776. 1966.

The Journal of the Oceanographical Society of Japan
Vol. 20. No. 5, December 1964

Identification and Determination of Organic Acids in Sea Water by Partition Chromatography*

Tadashiro KOYAMA** and Thomas G. THOMPSON***

Abstract: Sea water was examined for the identification and determination of organic acids. Samples were frozen at −7°C at the time of collection, and subsequently melted and filtered through Millipore (registered trademark, Millipore Filter Corp., Bedford, Mass.) filters in the laboratory. From 4 to 8 liters of the filtered samples were condensed to about one-fifth of their volume by vacuum distillation, and organic acids were removed by continuous chloroform or ether extraction. Acetic (0.07 to 2.8 mg/liter), formic (0.03 to 1.0 mg/liter), lactic (0.00 to 0.13 mg/liter), and glycolic (0.00 to 1.4 mg/liter) acid were identified and determined by partition chromatgraphy on a silica gel column in samples from the northeast Pacific and inshore waters of the State of Washington. Offshore waters contained smaller concentrations of these acids, as did the deeper waters from the inshore stations. The evidence indicates that the major part of these acids are breakdown products of organic compounds of high molecular weight.

1. Introduction

Since the work of PÜTTER (1909), much effort has been spent on the problem of dissolved organic compounds in sea water, and several papers dealing with the separation of organic acids from sea water have been published. WANGERSKY (1952) isolated two kinds of organic crystals from sea water; one had the general absorption spectrum of dehydro-ascorbic acid, the other gave some indication of being a rhamnoside which occurs in amounts up to 0.1 g/liter in inshore waters of the Gulf of Mexico. COLLIER (1953) described the significance of organic compounds in sea water, using as examples carbohydrates, tyrosine, tryptophane, rhamnoside, ascorbic acid, and unidentified compounds isolated from sea water. CREAC'H (1955*a* and *b*) reported that in littoral Atlantic waters the concentration of citric acid ranged from 0.025 to 0.145 mg/liter and that of malic acid from 0.028 to 0.277 mg/liter; he studied the fate of citric and malic acids in a synthetic sea water under irradiation with 3100Å light and proved that acetone, acetaldehyde, acetic acid, formaldehyde, formic acid, and carbon dioxide were produced. He also determined the amounts of acetone, acetaldehyde, citric, malic, acetic, and formic acids in a single sample of littoral sea water. SHABAROVA (1956) reported on the intermediate decomposition products of the organic matter of marine organisms under laboratory conditions and found butyric, acetic, and formic acids in the products by means of biochemical assays. VALLENTYNE (1957) described the molecular nature of organic matter in lakes, oceans, sewage, and soil, and referred to many important papers on the subject.

Recently a number of papers have dealt with the separation and identification of organic acids by partition chromatography. ISHERWOOD (1946) reported a method for separating similar organic acids on a silica gel column by eluting the acids with n-butyl alcohol-chloroform mixtures. Similar procedures have recently been published by HOUSTON and HAMILTON (1952); BULEN, VARNER, and BURRELL (1952); ROBERTS and MARTIN (1954); and MUELLER, LARSON, and LENNARZ (1958). RESNIK, LEE, and POWELL (1955) used tertiary-butyl alcohol instead of n-butyl alcohol to make the end-point of the titration sharper and also to prevent the possibility of ester forma-

* Received Sept. 14, 1964
Contribution No. 317 of the University of Washington, Department of Oceanography
** Present address: Water Research Laboratory, Faculty of Science, Nagoya University
*** Department of Oceanography, University of Washington Seattle, Washington, U.S.A.

tion, which is likely to occur on the column.

The purpose of this investigation was to develop a method for the determination of organic acids in sea water, and to apply this method to determine the organic acids and their variation in the inshore of the State of Washington and in the northeast Pacific Ocean. However, it should be realized that in sea water there are some dissolved organic compounds from which various kinds of organic acids such as acetic, formic, lactic, and glycolic acids are easily produced during the ether and chloroform extraction processes. The technique described in the present paper is a modification of the method of BULEN, VARNER, and BURRELL (1952).

2. Preparation of Samples

1) Reagents

Chloroform. Purify chloroform just before use by shaking first with sodium hydroxide and then with distilled water; dry with calcium chloride and distill.

Ether. Purify ether just before use by shaking with alkaline Potassium permanganate solution, acidic ferrous sulfate solution, sodium hydroxide, and distilled water successively; dry with calcium chloride and distill.

0.2 N sodium hydroxide.

6 N hydrochloric acid.

Phenol red. Prepare phenol red indicator by dissolving 100 mg of the solid in 1.4 m*l* of 0.2 N sodium hydroxide. Add 50 m*l* of distilled water and make up to 500 m*l*.

2) Apparatus

Millipore filter apparatus.

Vacuum distillation apparatus (Fig. 1).

Continuous chloroform extractor (Fig. 2). A 500 m*l* Wehrli extractor was modified so that the chloroform, which dropped through the sea water sample, was transferred into a dilute sodium hydroxide solution in tube (A) as small particles, and then was transferred to the Erlenmeyer flask (B) to collect even the organic acids of low boiling point. The sea water sample in this extractor was cooled to about 15°C by running water.

Continuous ether extractor (Fig. 3). A 2-liter Kutscher-Stendel extractor was used for continuous ether extraction.

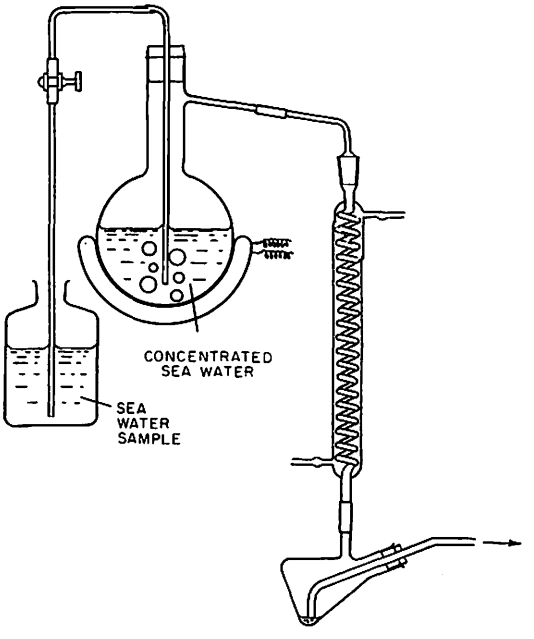


Fig. 1. Vacuum distillation apparatus for concentration of seawater samples.

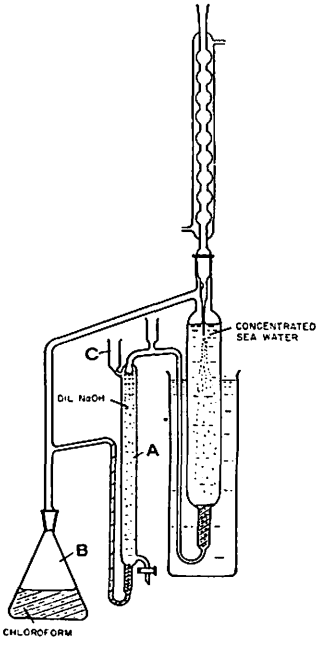


Fig. 2 Continuous extractor (chloroform).

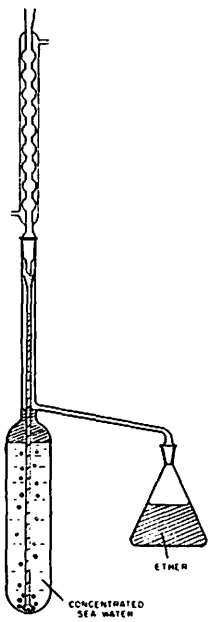


Fig. 3. Continuous extractor (ether).

3) Experimental

Twenty-liter samples of sea water were frozen at −7°C at the time of collection, then melted in the laboratory and filtered through Millipore filters. Using a vacuum distillation apparatus, 6 to 8 liters of the filtered sea water were concentrated to about 1.5 liters at temperatures below 60°C, adjusted to pH 3 with 6 N hydrochloric acid, then transferred with approximately 500 m*l* of distilled water into the continuous ether extractor. Four liters of the same water sample were concentrated to about 400 m*l*, then adjusted to pH 3, and transferred with about 100 m*l* of distilled water into the continuous chloroform extractor. Fifteen m*l* of sodium hydroxide (0.2 N) and a few drops of phenol red indicator were poured into tube (A) through funnel (C); and finally, distilled water was added to fill the tube (Fig. 2).

The chloroform and ether extractions were carried out at rates of 20 and 50 m*l* per minute, respectively, for a period of three to five weeks. At one week intervals, the ether, chloroform, and dilute sodium hydroxide were replaced by new reagents and the weekly yield of organic acids was determined. The chloroform and dilute alkali were transferred into a separatory funnel and shaken: the aqueous phase was separated from the chloroform, and the chloroform again shaken with a mixture of 5 m*l* of 0.2 N sodium hydroxide and 100 m*l* of distilled water, in successive portions. Finally, the entire aqueous phase containing the acid salts was evaporated to dryness. When, during the chloroform extraction process, the alkali was neutralized, 5 m*l* of 0.2 N sodium hydroxide solution were added to tube (A) (Fig. 2). The weekly yield from the ether extraction was transferred to a separatory funnel and shaken with a mixture of 10 m*l* of 0.2 N sodium hydroxide and 300 m*l* of distilled water, in successive portions. Finally, this aqueous phase containing the acid salts was also evaporated to dryness.

3. Chromatographic Technique

1) Reagents

Silica gel. Prepare silica gel from Mallinckrodt's silicic acid by treatment with hydrochloric acid. Remove chloride ion and fine particles by repeated suspension in distilled water and decantation of the slower settling particles. Filter the remaining fraction through a Büchner funnel and wash with water until chloride ion is completely removed; dry at 100°C for 48 hours and store in a closed container.

Chloroform. Purify chloroform as for the extraction.

Eluting solvents. Prepare eluting solvents from redistilled reagent grade chloroform (A. C. S. specifications) and redistilled C. P. tertiary-butyl alcohol to contain 4, 8, 14, 22, 26, 32% (v/v) of the alcohol in chloroform. Equilibrate each of the first three solvent mixtures with one-tenth of its volume of 0.5 N sulfuric acid and each of the other solvent mixtures with one-tenth of its volume of distilled water by shaking the two phases in a separatory funnel and passing the solvent layer through a dry filter paper with a piece of absorbent cotton on the bottom to remove suspended water droplets.

Phenol red indicator. Prepare phenol red indicator as for the extraction.

Methyl orange indicator. Prepare methyl orange indicator by dissolving the solid in water

to make 0.01% aqueous solution.

Standard 0.01 N sodium hydroxide Solution. Prepare with the addition of a small amount of barium chloride to free from carbonate.

0.5 N and 6 N sulfuric acid.

2) Apparatus

Chromatographic tube (Fig. 4). This consists of two parts. The upper part is a solvent

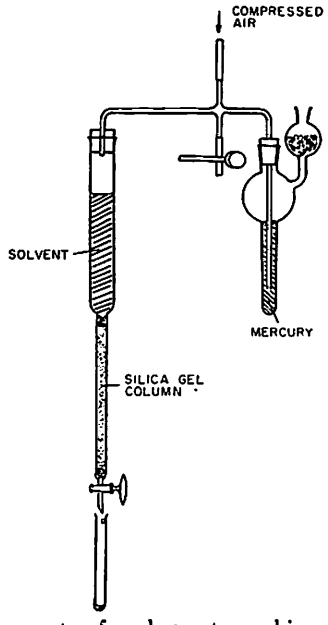


Fig. 4. Apparatus for chromatographic separation.

reservoir 15 cm long with an inside diameter of 30 mm; the lower part is a tube 30 cm long with an inside diameter of 12 mm. A stopcock is attached to the bottom. The pressure regulating device was used successfully to maintain the flow-rate of the solvent.

Automatic fraction collector.

Microburet. 2 m*l* capacity.

3) Experimental

The chromatographic procedure was carried out on a mixture of six organic acids: acetic, formic, lactic, glycolic, malic, and citric. The preliminary separation column was prepared, using seven grams of the silica gel triturated with 4.7 m*l* of 0.5 N sulfuric acid. The resulting freeflowing mixture was slurried in 30 m*l* of chloroform, previously equilibrated with water, and added to the chromatographic tube. A glass-wool plug placed at the base of the tube supported the column. The remaining silica gel was completely transferred to the tube with 30 m*l* of chloroform in successive portions. A gas pressure of 2 to 3 cm of mercury, applied to the top of the tube, hastened the drainage of excess chloroform. Care was exercised not to let the solvent level fall below the top of the column.

A mixture containing 0.03 to 0.06 millimoles of each of the organic acids was made alkaline with 5 m*l* of 0.2 N sodium hydroxide and evaporated to dryness. A drop of methyl orange indicator was added to the residue, which was subsequently placed in a freezer. While cooling, the residue was neutralized with 6 N sulfuric acid, a slight excess of the acid was then added, and finally the solution was diluted to 0.5 m*l* with 0.5 N sulfuric acid. The solution was thoroughly mixed with one gram of silica gel, chloroform was added, and the mixture transferred quantitatively to the top of the column. A glass-wool plug was forced down on the surface in order to prevent disturbance of the column during the addition of eluting solvents.

Development of the six organic acids was carried out by adding a series of tertiary-butyl alcohol-chloroform mixtures under suitable pressure. The selected schedule was 80 m*l* of 4%, 60 m*l*, of 8%, 60 m*l* of 14%, 100 m*l* of 22%, 100 m*l* of 26%, and 100 m*l* of 32% alcohol in chloroform. A pressure equivalent to 2 to 10 cm of mercury applied to the reservoir provided a flow-rate of approximately 2 m*l* per minute. Each solvent was added as soon as the previous solvent entered the upper glass-wool plug. Effluent fractions of about 3.5 m*l* were received in test tubes by an automatic fraction collector; 3 m*l* of water and one drop of phenol red indicator were added to each fraction and the acid was titrated with 0.01 N sodium hydroxide. The uppermost chromatogram (Fig. 5) shows the relative position of the acids and the relationship between the titration values and the fraction numbers.

In addition to organic acids, boric acid can be extracted from sea water by ether. A mix-

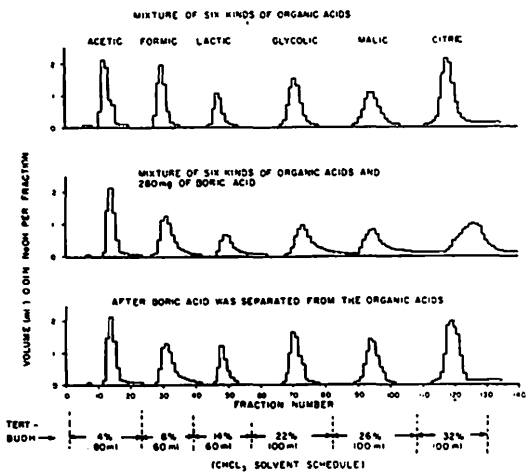


Fig. 5. Interference of boric acid in separation of organic acids.

ture of the organic acids and 280 mg of boric acid, corresponding to 10 liters of sea water, produced a chromatogram (center of Fig. 5) indicating that the peaks of acetic and formic acids were still sharp whereas those of the other acids were less pronounced. To determine accurately the presence of acids other than acetic and formic, it was necessary to separate them from boric acid by the following process: after the acetic and formic acids were eluted, the other acids were eluted with 100 m*l* of 26% and 100 m*l* of 32% tertiary-butyl alcohol-chloroform mixtures. The latter effluent fractions were combined in a separatory funnel, and the organic acids were extracted by shaking with dilute sodium hydroxide. The aqueous phase was then evaporated to dryness and the organic acids determined by repeating the chromatographic separation. Acetic, formic, lactic, glycolic, malic, and citric acids in the presence of 280 mg of boric acid were separated with recoveries of 95% to 100%. The lowest chromatogram in Fig. 5 illustrates the results thus obtained.

4. Confirmation

The organic acids were removed from an 8-liter sample of Millipore filtered sea water which had been concentrated to 2 liters by four weeks of continuous ether extraction, following the adjustment of the pH to 3. Known concentrations (0.006 to 0.06 millimoles) of acetic, formic, lactic, glycolic, malic, and citric acids were added to the concentrated water. After one week of ether extraction, 80% of the first four acid were recovered, and 95% to 100% were recovered in three weeks; however, not more than 5% of the malic and citric acids were recovered even after three weeks (Fig. 6). Four liters of sea water were similarly concentrated to 500 m*l*, and 0.006 to 0.06 millimoles of acetic, formic, lactic, and glycolic acids were added to the concentrations; continuous chloroform extraction for four weeks yielded a recovery of

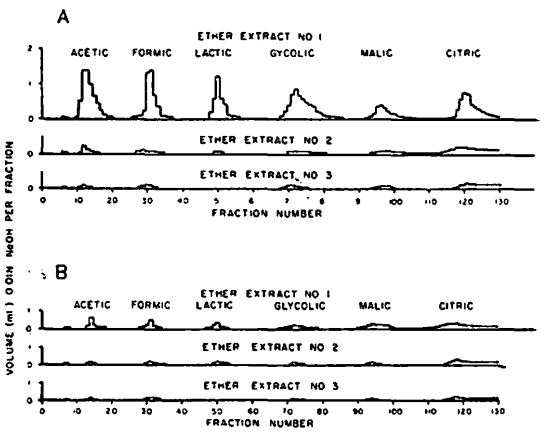


Fig. 6. Extraction efficiency: Recovery of organic acids added to a concentrated sea water free of organic acids (ether extract).
A: 0.03 mmole OF LACTIC ACID AND 0.06 mmole OF THE OTHER ORGANIC ACIDS WERE ADDED TO 2 L OF CONCENTRATED SEA WATER FREE OF ORGANIC ACIDS.
B: 0.006 mmole OF EACH OF THESE SIX ORGANIC ACIDS WERE ADDED TO 2 L OF CONCENTRATED SEA WATER FREE OF ORGANIC ACIDS.

85% to 100% of these acids (Fig. 7).

Six liters of filtered sea water were concentrated to 2 liters by vacuum distillation. The organic acids in this water were determined after extraction by ether for four weeks. 1.80 mg of acetic acid and 1.38 mg of formic acid were added to 6 liters of the same sample and then concentrated to 2 liters using the vacuum distillation apparatus. The organic acids in

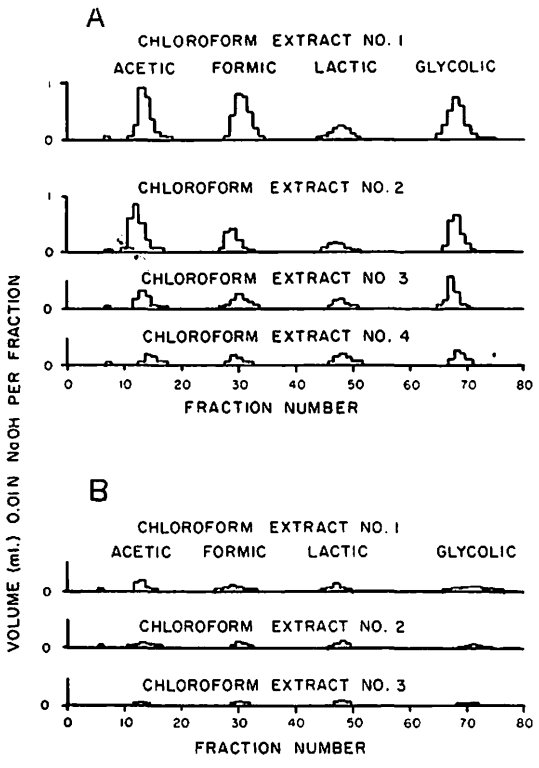


Fig. 7. Extraction efficiency: Recovery of organic acids added to concentrated sea water free of organic acids (chloroform extract).
A: 0.03 mmole OF LACTIC ACID AND 0.06 mmole OF EACH OF THE OTHER ORGANIC ACIDS WERE ADDED TO 2 L OF A CONCENTRATED SEA WATER FREE OF ORGANIC ACIDS.
B: 0.006 mmole OF EACH OF THESE SIX ORGANIC ACIDS WERE ADDED TO 2 L OF CONCENTRATED SEA WATER FREE OF ORGANIC ACIDS.

this sample were determined after extraction by the same method for the same period. The amounts of acetic and formic acids present in the first sample were 2.51 and 0.37 mg, respectively; those in the second sample were 4.37 and 1.56 mg, respectively. Fig. 8 shows the chromatograms thus obtained.

5. Application

The residues containing organic acid salts obtained from concentrated sea water samples by ether or chloroform extraction were acidified with sulfuric acid, mixed with silica gel, and

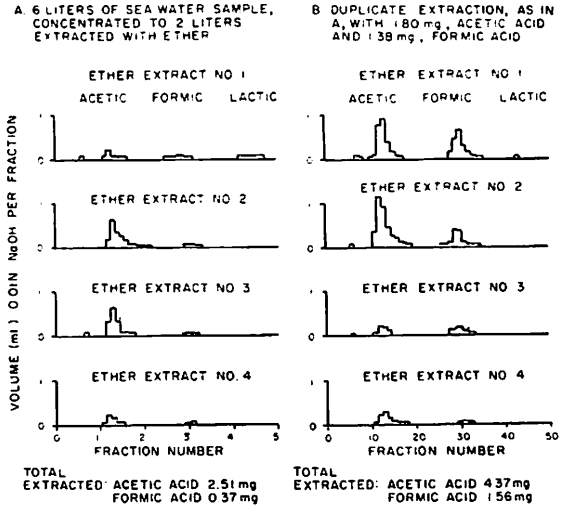


Fig. 8. Recovery of organic acids from seawater.

chromatographed according to the previous procedure. Usually more than 0.5 m*l* of 6 N sulfuric acid was necessary to acidify the residue from chloroform extraction. In such a case the amount of silica gel had to be increased at the rate of 1 g for 0.5 m*l* of sulfuric acid.

Five surface samples from the northeast Pacific and 16 samples from inshore waters of the State of Washington were analyzed. Fig. 9 shows the typical chromatograms of the organic acids for two of these samples, and a reference chromatogram. A comparison of these two chromatograms with the reference chromatogram shows several peaks corresponding to acetic, formic lactic, and glycolic acids. However, the sharp peaks corresponding to glycolic acid which were found in the chloroform extracts were not found in the ether extracts.

The peaks corresponding to malic and citric acids were not found in any of the sea water samples examined.

To confirm the identify of each of the four separated organic acids, the procedures that follow were carried out. Tests were made according to directions in FEIGL (1956).

Acetic acid. Collect titrated fractions for the peak corresponding to acetic acid in a separatory funnel. Separate the aqueous phase containing the acid salts and then evaporate it in the micro-distillation apparatus shown in Fig.

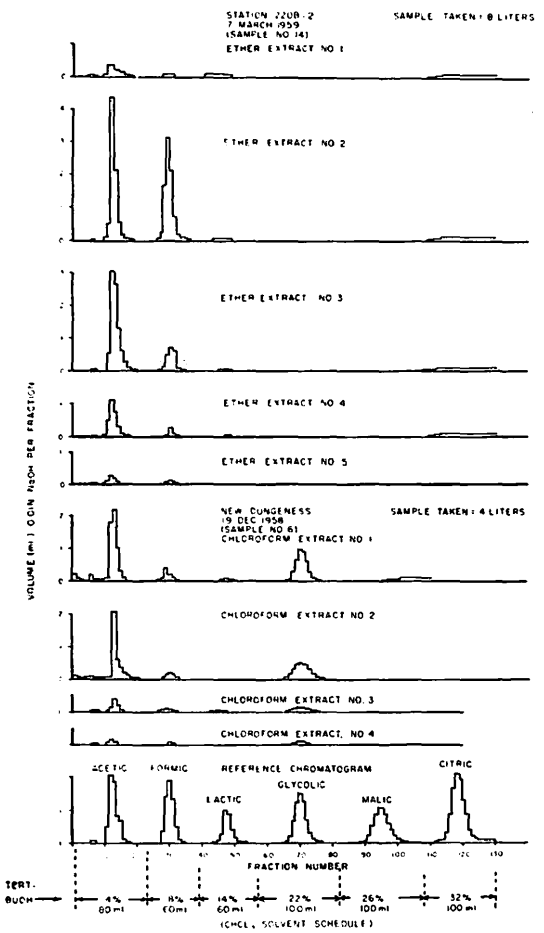


Fig. 9. Separation of organic acids from seawater.

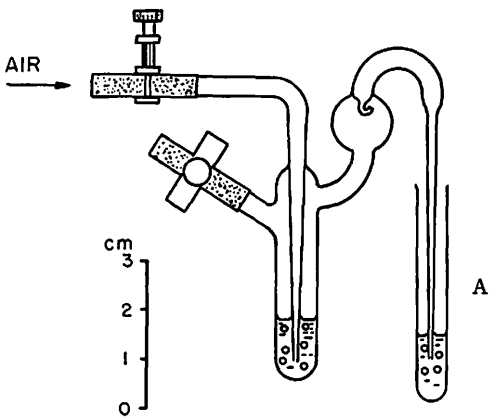


Fig. 10. Microdistillation apparatus.

10. Add 0.2 m*l* of concentrated phosphoric acid to the residue and warm under aeration. Introduce the air, containing the volatile acid, from the outlet into the micro test-tube (A) containing a definite amount of standard sodium hydroxyde solution. Remove an aliquot and back titrate with a standard hydrochloric acid. Test with lanthanum and iodine, according to FEIGL (1956, p. 342).

Formic acid. Collect titrated fractions for the peak corresponding to formic acid in a separatory funnel. Separate and evaporate the aqueous phase in the micro-distillation flask and continue procedure as for acetic acid. Test with mercuric chloride and test by conversion to formaldehyde according to FEIGL (1956, pp. 341 and 340).

Lactic acid. Collect fractions for the peak corresponding to lactic acid in a separatory funnel; separate the aqueous phase and evaporate it in a 50 m*l* beaker. To remove the large amounts of phenol red indicator remaining in the residue, acidify with sulfuric acid, mix with silica gel, and then chromatograph with 80 m*l* of 8% and 60 m*l* of 14% tertiary-butyl alcohol-chloroform mixtures. Separate the indicator from the organic acid with the first solvent mixture and elute the separated acid with the second mixture. Extract the organic acid in the eluate by shaking with a diluted sodium hydroxide solution. Separate the aqueous phase and concentrate to a small volume. Use this concentrated solution, and test with p-hydroxydiphenyl and sulfuric acid, and with o-hydroxydiphenyl and sulfuric acid (fluorescence test) according to FEIGL (1956, pp. 348 and 349).

Glycolic acid. Collect fractions for the peak corresponding to glycolic acid in a separatory funnel, separate the aqueous phase, and evaporate it in a 50 m*l* beaker. Acidify the residue with sulfuric acid, mix it with silica gel, and then chromatograph with 80 m*l* of 14% and 100 m*l* of 22% of tertiary-butyl alcohol-chloroform mixtures. Separate the indicator in the residue from the organic acid with the first solvent mixture, and elute the organic acid with the second mixture. By shaking with a diluted sodium hydroxide solution, extract the organic acid in the eluate. Use this concentrated solution and test with 2, 7-dihydroxynaphthalene and sulfuric acid, and with chromo-

Table 1. Organic acids in the ether and chloroform extracts from sea water.

Sample No.	Station* and Location	Date	Depth in meters	Extract	Organic acid	Ext. 1**	Ext. 2	Ext. 3	Ext. 4	Ext. 5	Total
	NORTHEAST PACIFIC					mg/l	mg/l	mg/l	mg/l	mg/l	mg/l
1	BB 199-9	7-11-58	0	Ether	Ace.	0.03_7	0.03_6	0.01_5			0.09
	47°54′ N				For.	0.02_3	0.00_6	0.00_5			0.03
	146°22′ W				Lac.	0.04_7	0.00_6	0.00_0			0.05
					Gly.	0.00_0	0.00_0				0.00
				Chloroform	Ace.	0.06_8	0.02_9	0.00_9			0.10
					For.	0.04_8	0.01_8	0.00_5			0.07
					Lac.	0.04_5	0.00_8	0.00_0			0.05
					Gly.	0.03_8	0.01_4	0.00_7			0.06
2	BB 199-11	7-13-58	0	Ether	Ace.	0.05_2	0.06_5	0.09_5	0.00_9		0.22
	44°00′ N				For.	0.04_7	0.04_9	0.01_3	0.00_2		0.11
	146°40′ W				Lac.	0.02_7	0.00_7	0.00_0			0.03
					Gly.	0.00_0					0.00
3	BB 199-14	7-17-58	0	Chloroform	Ace.	0.25_0	0.00_9	0.01_1			0.27
	38°14′ N				For.	0.09_3	0.01_6	0.02_0			0.13
	140°28′ W				Lac.	0.04_3	0.01_8	0.01_8			0.08
					Gly.	0.11_5	0.10_5	0.02_3			0.14
4	BB 202-7	9-27-58	0	Ether	Ace.	0.02_9	0.03_3	0.29_4	0.04_0	0.03_5	0.43
	50°01.2′ N				For.	0.02_1	0.04_3	0.04_7	0.02_1	0.00_5	0.14
	139°49.3′ W				Lac.	0.02_6	0.02_3	0.00_5			0.06
					Gly.	0.02_0	0.00_0				0.02
				Chloroform	Ace.	0.27_0	0.02_8	0.02_4			0.32
					For.	0.30_0	0.04_0	0.03_5			0.38
					Lac.	0.03_4	0.02_7	0.01_8			0.08
					Gly.	0.66_2	0.08_4	0.03_4			0.78
5	BB 202-10	9-29-58	0	Ether	Ace.	0.05_9	0.01_3	0.27_0	0.25_8	0.11_0	0.71
	47°58.1′ N				For.	0.02_1	0.02_7	0.06_0	0.14_5	0.06_2	0.32
	133°08.0′ W				Lac.	0.04_4	0.00_0	0.00_4	0.00_0	0.00_0	0.06
					Gly.	0.00_0	0.02_9	0.00_0			0.03
				Chloroform	Ace.	0.38_5	0.14_2	0.22_2	0.07_1		0.82
					For.	0.10_0	0.05_9	0.05_7	0.02_1		0.24
					Lac.	0.05_0	0.01_8	0.01_1	0.00_0		0.08
					Gly.	0.30_0	0.33_8	0.31_2	0.08_6		1.04

Sample No.	Station* and Location	Date	Depth in meters	Extract	Organic acid	Ext. 1**	Ext. 2	Ext. 3	Ext. 4	Ext. 5	Total
	INSHORE WATERS					mg/l	mg/l	mg/l	mg/l	mg/l	mg/l
6	BB 209										
	New Dungeness	12-19-58	0	Ether	Ace.	0.26_0	0.20_3	0.48_9	0.08_0		1.03
	48°15.78′ N				For.	0.38_4	0.04_3	0.10_0	0.00_5		0.53
	123°04.73′ W				Lac.	0.05_6	0.01_1	0.00_4	0.00_0		0.07
					Gly.	0.03_0	0.00_0				0.03
				Chloroform	Ace.	0.82_2	0.47_7	0.13_7	0.03_7		1.47
					For.	0.10_8	0.06_6	0.03_8	0.00_7		0.22
					Lac.	0.10_1	0.00_0	0.03_2	0.00_0		0.13
					Gly.	0.70_0	0.54_2	0.12_0	0.03_8		1.40
7	BB 209										
	New Dungeness	12-19-58	120	Ether	Ace.	0.32_1	0.17_9	0.01_1	0.00_2		0.51
	48°15.78′ N				For.	0.16_6	0.07_7	0.01_2	0.00_0		0.26
	123°04.73′ W				Lac.	0.04_9	0.016	0.00_9			0.07
					Gly.	0.00_0	0.00_0				0.00
				Chloroform	Ace.	0.33_8	0.48_2	0.01_8	0.01_7		0.86
					For.	0.10_2	0.20_1	0.03_0	0.00_6		0.34
					Lac.	0.03_4	0.05_4	0.01_8	0.00_0		0.11
					Gly.	0.18_6	0.66_8	0.16_0	0.05_7		1.07
8	BB 209	12-20-58	0	Ether	Ace.	0.07_3	0.29_0	0.20_0	0.10_6	0.05_0	0.72
	Pt. Pillar				For.	0.03_0	0.01_7	0.07_2	0.01_7	0.00_5	0.14
	48°18.40′ N				Lac.	0.06_7	0.01_7	0.01_8	0.00_0		0.10
	123°05.1′ W				Gly.	0.04_0	0.00_3	0.00_0			0.04
9	BB 209	12-20-58	150	Ether	Ace.	0.12_0	0.04_5	0.01_7			0.19
	Pt. Pillar				For.	0.03_3	0.01_6	0.00_6			0.06
					Lac.	0.11_5	0.01_1	0.00_4			0.13
					Gly.	0.00_0	0.00_0				0.00
				Chloroform	Ace.	0.17_6	0.049	0.02_9			0.25
					For.	0.04_6	0.05_4	0.03_3			0.13
					Lac.	0.04_9	0.04_4	0.02_9			0.12
					Gly.	0.16_5	0.06_0	0.01_7			0.24
10	BB 182	4-12-58	0	Ether	Ace.	0.05_1	0.16_8	0.15_3	0.04_6		0.42
	Protection Is.				For.	0.02_3	0.02_3	0.01_2	0.00_0		0.06
	48°12′ N				Lac.	0.01_8	0.00_0				0.02
	122°54′ W				Gly.	0.00_0	0.00_0				0.00
			50	Ether	Ace.	0.03_4	0.00_7	0.025	0.00_5		0.07
					For.	0.02_7	0.00_4	0.01_6	0.00_2		0.05
					Lac.	0.04_7	0.00_0				0.05
					Gly.	0.00_0					0.00

Sample No.	Station* and Location	Date	Depth in meters	Extract	Organic acid	Ext. 1**	Ext. 2	Ext. 3	Ext. 4	Ext. 5	Total
	INSHORE WATERS					mg/l	mg/l	mg/l	mg/l	mg/l	mg/l
12	BB 196-4	5-23-58	0	Ether	Ace.	0.03_5	1.11	0.02_5			1.17
	48°40.29′ N				For.	0.02_4	0.14_6	0.01_2			0.18
	122°54.00′.W				Lac.	0.01_9	0.00_9	0.00_0			0.03
					Gly.	0.00_0	0.00_0				0.00
13	〃 〃		20		Ace.	0.02_9	0.29_3	0.24_7	0.09_3		0.66
					For.	0.00_1	0.03_4	0.02_9	0.02_1		0.09
					Lac.	0.00_1	0.00_0				0.00
					Gly.	0.00_0					0.00
14	BB 220B-2	3-7-59	0	Ether	Ace.	0.09_5	0.74_0	0.63_4	0.21_2	0.04_1	1.72
	48°35.45′ N				For.	0.00_7	0.47_0	0.11_2	0.02_3	0.00_9	0.62
	122°51.0′ W				Lac.	0.03_0	0.01_0	0.00_7	0.00_3	0.00_0	0.05
					Gly.	0.00_0	0.00_0				0.00
15	220B-2	3-7-59	5	Ether	Ace.	0.11_2	0.38_8	1.45_1	0.30_5		2.25
					For.	0.05_2	0.13_0	0.75_0	0.10_0		1.03
					Lac.	0.06_7	0.02_7	0.01_4	0.00_0		0.11
					Gly.	0.04_8	0.00_0	0.00_0			0.05
16	BB 196-1	5-23-58	0	Ether	Ace.	0.70_9	2.02	0.08_3	0.01_9		2.83
	48°35.50′ N				For.	0.35_0	0.36_0	0.02_6	0.01_6		0.75
	122°50.75′ W				Lac.	0.06_6	0.00_0				0.07
					Gly.	0.04_0	0.02_0	0.02_0	0.00_0		0.08
17			30	Ether	Ace.	0.01_9	0.15_9	0.25_3	0.08_2		0.51
					For.	0.00_5	0.03_5	0.02_7	0.01_4		0.08
					Lac.	0.03_5	0.00_0				0.04
					Gly.	0.00_0	0.00_0				0.00
18	BB 182	1-21-58	0	Ether	Ace.	0.63_3	0.07_2	0.02_4			0.73
	Pt. Jefferson				For.	0.21_1	0.28_4	0.04_1			0.54
	47°44′ N				Lac.	0.02_8	0.02_3	0.00_0			0.05
	122°25′ W				Gly.	0.00_6	0.00_0				0.01
19			50-60	Ether	Ace.	0.05_4	0.25_0	0.03_9			0.34
					For.	0.05_1	0.06_6	0.02_7			0.14
					Lac.	0.01_7	0.00_8				0.03
					Gly.	0.00_0	0.00_0				0.00
20	〃 〃		100-110	Ether	Ace.	0.06_2	0.04_8	0.01_4			0.12
					For.	0.12_7	0.06_2	0.01_7			0.21
					Lac.	0.04_5	0.00_4				0.05
					Gly.	0.00_0					0.00
21	BB 209	12-23-58		Chloroform	Ace.	1.20	0.22_8	0.04_1			1.47
	Pt. Brown				For.	0.41_8	0.06_3	0.02_0			0.50
	47°19.2′ N				Lac.	0.00_9	0.01_8	0.00^0			0.03
	121°08.0′ W				Gly.	1.10	0.19_0	0.03_0			1.32

* BB—M. V. BROWN BEAR. First three digits represent cruise number. Digits following hyphen represent station number.

** Ext. 1, 2, 3. 4, and 5 are the extracts which were obtained during the first, second, third, fourth and fifth weeks respectively.

tropic acid and sulfuric acid according to FEIGL (1956, pp. 346–348).

Table 1 shows the amounts of the organic acids found in the samples.

6. Discussion

Table 2 shows the range of values and average values of the organic acids. This table indicates that (1) the amounts of acetic, formic, and glycolic acids, determined by the present study, are less concentrated in the offshore surface waters than in the inshore waters; (2) in the inshore water, the amounts of acetic and formic acids are greater in the surface waters than in the bottom waters.

Table 2. Ranges of values and average values of the organic acids in sea water examined.

Northeast Pacific surface waters.

		Ranges (mg/l)	Average values (mg/l)
Acetic		0. 09–0. 82	0. 37
Formic		0. 03–0. 38	0. 18
Lactic		0. 03–0. 08	0. 06
Glycolic	Ether	0. 00–0. 03	0–01
	Chloroform	0. 06–1. 04	0. 51

Inshore surface waters

		Ranges (mg/l)	Average values (mg/l)
Acetic		0. 72–2. 83	1. 83
Formic		0. 06–1. 03	0. 46
Lactic		0. 02–0. 11	0. 07
Glycolic	Ether	0. 00–0. 08	0. 03
	Chloroform	1. 32–1. 40	1. 36

Inshore bottom waters

		Ranges (mg/l)	Average values (mg/l)
Acetic		0. 07–0. 86	0. 39
Formic		0. 05–0. 34	0. 15
Lactic		0. 00–0. 13	0. 07
Glycolic	Ether	0. 00–0. 00	0. 00
	Chloroform	0. 24–1. 07	0. 66

Fig. 11 shows the distribution of acetic, formic, and glycolic acids in the northeast Pacific. There the quantity of acids decreases with the distance from shore. Analyses of sea water samples from the northeast Pacific and the inshore waters of the State of Washington indicated the following:

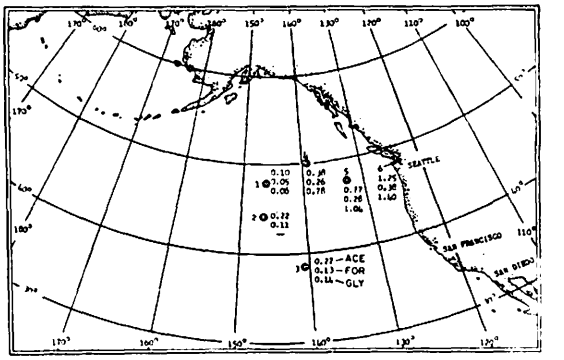


Fig. 11. Distribution of acetic, formic, and glycolic acids in surface water of the northeast Pacific Ocean.

(a) Acetic, formic, lactic, and glycolic acids were obtained in the ether and chloroform extracts. The chloroform extracts yielded concentrations as high as 1.4 mg/liter of glycolic acid, but the ether extracts did not yield concentrations higher than 0.08 mg/liter.

(b) Concentrations of acetic acid of the offshore surface waters varied from 0.09 to 0.82, of formic from 0.03 to 0.38, of lactic from 0.03 to 0.08, and of glycolic acid from 0.0 to 1.0 mg/liter. These concentrations decrease with the distance from shore. Five surface samples were analyzed.

(c) Concentrations of acetic acid of the inshore surface waters of the State of Washington varied from 0.72 to 2.8, of formic from 0.06 to 1.0, of lactic from 0.02 to 0.11, and of glycolic acid from 0.0 to 1.4 mg/liter. Eight samples were analyzed.

(d) Concentrations of acetic acid of the inshore bottom waters varied from 0.07 to 0.86, of formic from 0.05 to 0.34, of lactic from 0.0 to 0.13, and of glycolic acid from 0.0 to 1.1 mg/liter. Eight samples were analyzed.

Malic and citric acids were not found in any

of the sea water samples examined; therefore, from the accuracy of the present method, it may be deduced that the amounts of malic and citric acids in the samples may have been smaller than 0.05 mg/liter.

The following results support the theory that the greater part of the four acids are breakdown products of organic compounds of large molecular weight, formed during the extraction process:

(1) The chloroform extracts yield concentrations of 1.4 mg/liter of glycolic acid, but the ether extracts yield concentrations of less than 0.08 mg/liter.

(2) In many cases, the amounts of acetic and formic acids which were extracted by ether for the first week were smaller than 0.1 mg/liter and thus smaller than amounts of these acids extracted for the second or third weeks.

This indicates that the amounts of acetic and formic acids which will be in a free state in sea water may usually be smaller than 0.1 mg/liter.

(3) It usually took four or five weeks to extract the greater part of these four acids. If in a free state in sea water, 80 to 85% would be extracted by ether in the first week.

Acetic, formic, and lactic acids determined in the ether extracts were similar to those determined in the ether extracts obtained from river waters by MUELLER, LARSON, and LENNARZ (1958).

Acknowledgment

The authors wish to acknowledge financial aid from the National Science Foundation under provision of contract G-4521. The authors are also grateful to Dr. S. A. EL WARDANI and Dr. R. A. BARKLEY for their many helpful suggestions during the present study. Water samples were obtained on cruises of the M. V. BROWN BEAR of the University of Washington, Department of Oceanography, on a program supported by the Office of Naval Research, Contract Nonr 477 (10), Project NR 083-012.

References

BULEN, W.A., J.E. VARNER and R.C. BURRELL (1952): Separation of organic acids from plant tissues, chromatographic technique. Anal. Chem., **24**, 187–190.

COLLIER, A. (1953): The significance of organic compounds in sea water. Trans. 18th North American Wildlife Conf. 463–472.

CREAC'H, P. (1955a): Sur la presence des acids citrique et malique dans les eaux marines littorales. Compt. rend. Acad. Sci., Paris, **240**, 2551–2553.

CREAC'H, P. (1955b): Quelques composants de la matiere organique de l'eau de mer littoral, Heloxydation dans le milieu marin. Compt. rend. Acad. Sci., Paris, **241**, 437–439.

FEIGL, F. (1956): Spot tests in organic analysis. Fifth Ed. Elsevier Publish. Co. N. Y. 616.

HOUSTON, F. G. and J. L. HAMILTON (1952): Rapid determination of organic acids in cured tobacco. Anal. Chem., **24**, 415–416.

ISHERWOOD, F. A. (1946): The determination and isolation of the organic acids in fruit. Bio-chem. Jour., **40**, 688–695.

MUELLER, H. F., T. E. LARSON and W. J. LENNARZ (1958): Chromatographic identification and determination of organic acids in water. Anal. Chem., **30**, 41–44.

PÜTTER, A. (1909): Die Ernährung der Wassertiere und der Stoffhaushalt der Gewässer. Gustav Fisher, Jena, 97–107.

RESNIK, F. E., L. A. LEE and W. A. POWELL (1955): Chromatography of organic acids in cured tobacco. Anal. Chem., **27**, 928–931.

ROBERTS, E. J. and L. F. MARTIN (1954): Identification and determination of nonnitrogenous organic acids of sugar cane by partition chromatography. Anal. Chem., **26**, 815–818.

SHABAROVA, N. T. (1956): Intermediate decomposition products of the organic matter of marine organisms (in laboratory condition). Biokhimiya (Transl.) **21**(3), 343–348.

VALLENTYNE, J. R. (1957): The molecular nature of organic matter in lakes and oceans, with lesser reference to sewage and terrestrial soils. Jour. Fish. Res. Bd. Canada, **14**(1), 33–82.

WANGERSKY, P. J. (1952): Isolation of ascorbic acid and rhamnosides from sea water. Science, **115**, 685.