

# Organic Acids in Naturally Colored Surface Waters

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GEOLOGICAL SURVEY WATER-SUPPLY PAPER 1817-A



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By WILLIAM L. LAMAR and DONALD F. GOERLITZ

ORGANIC SUBSTANCES IN WATER

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GEOLOGICAL SURVEY WATER-SUPPLY PAPER 1817-A

*An analytical study of the organic acids  
in naturally colored surface waters*



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### ORGANIC ACIDS IN NATURALLY COLORED SURFACE WATERS

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#### ABSTRACT

Most of the organic matter in naturally colored surface waters consists of a mixture of carboxylic acids or salts of these acids. Many of the acids color the water yellow to brown; however, not all of the acids are colored. These acids range from simple to complex, but predominantly they are nonvolatile polymeric carboxylic acids.

The organic acids were recovered from the water by two techniques: continuous liquid-liquid extraction with n-butanol and vacuum evaporation at 50°C. (centigrade). The isolated acids were studied by techniques of gas, paper, and column chromatography and infrared spectroscopy.

About 10 percent of the acids recovered were volatile or could be made volatile for gas chromatographic analysis. Approximately 30 of these carboxylic acids were isolated, and 13 of them were individually identified. The predominant part of the total acids could not be made volatile for gas chromatographic analysis. Infrared examination of many column chromatographic fractions indicated that these nonvolatile substances are primarily polymeric hydroxy carboxylic acids having aromatic and olefinic unsaturation. The evidence suggests that some of these acids result from polymerization in aqueous solution. Elemental analysis of the sodium fusion products disclosed the absence of nitrogen, sulfur, and halogens.

#### INTRODUCTION

In 1786, Achard extracted a brown substance from soil and peat using alkali solutions. Since that time, colored organic substances have been extracted from soil, peat, coal, water, and decaying plants by many investigators. The early literature abounds with ideologic confusion. No less than 45 substances or fractions have been "discovered" and generically named. The literature contains many names, such as humus, ulmin, humus acid, humic acid, crenic acid, apocrenic acid, ulmic acid, torfic acid, fumic acid, hymatomelanic acid, fulvic acid, and humolimnic acid. Waksman (1938, p. 22-64) provided a significant historical summary of the studies and concepts of these natural organic substances.

The names most frequently used for the organic acids occurring in naturally colored water are humic, humatomelanic, and fulvic. This generic nomenclature is based on solubilities in water, alkali, acid, and alcohol. Because of the lack of specific separations and identifications, these generic names have continued in common use.

Organic acids are the most predominant organic material in naturally colored waters. They are produced by biological and oxidative processes and perhaps by condensation reactions, and the result is a complex mixture of many acids both colored and colorless. The mixture of these substances ranges from the well-defined simple acids to complex polymeric nonvolatile ones which apparently consist of several or more building units. In this mixture the polymeric nonvolatile acids predominate, and these substances, after more than 150 years of study, remain the most difficult to separate and identify.

### PREVIOUS RESEARCH

In isolating organic acids from naturally colored water, a mixture of many acids is obtained. It is difficult to isolate a fraction which contains only the nonvolatile colored acids and to separate the complex nonvolatile acids into individual components. Usually, studies of the colored organic acids in water relate to a mixture of many acids in which the colored nonvolatile acids predominate. Many values have been reported for the molecular weight of the colored organic acids because these values usually represent an undetermined mixture of many acids—colored, colorless, simple, and complex.

Mueller, Larson, and Lennarz (1958) studied the organic acids in surface water and sewage. The acids were isolated by continuous extraction with ether, separated by column chromatography, and characterized by measuring the characteristic effluent volumes obtained by titration of the fractions. In a later paper, Mueller, Larson, and Ferretti (1960) used both column and paper chromatography for the analysis of the organic acids in water. The fractions separated by column chromatography were tentatively identified by their elution volumes, and the presence of some of the acids was confirmed by paper chromatography.

The colored organic acids exert a holding action for a number of metallic ions. This holding action has been referred to as adsorption, bonding, complexion, and chelation. In water chemistry it has been particularly observed that many naturally colored waters carry up to a part per million of iron or more in apparent solution, and this iron is not readily precipitated by aeration. In other aerated waters whose pH is above about 5, ferric iron can be in excess of 0.01 ppm only as a suspension of oxide or hydroxide (Hem and Cropper, 1959).

The fouling of anion-exchange resins by the colored organic substances in water has been discussed by many authors. Frisch and Kunin (1960) conducted a number of experiments with the eluted foulant recovered from commercial anion-exchangers that were used to treat surface water. They called the fouling material humic acids and referred to them as large organic acids commonly found in surface waters. They eluted the organic foulant from the resin with an 8 percent sodium chloride solution, whereas Rice, Simon, and Rice (1962) used concentrated hydrochloric acid for the same purpose. In both papers the fouling material was identified as a carboxylic acid by infrared analysis. Rice, Simon, and Rice concluded that a part of the organic material eluted from fouled anion-exchange resins must be unsaturated aliphatic polycarboxylic acids.

Ruchti (1962) reported on the fractionation of low-molecular-weight substances extracted from the B horizon of podzol soils. He noted that the ether-soluble part condensed in aqueous solution and was oxidized on exposure to air. The observed condensation in aqueous solution could be significant to the complex structure of the colored organic acids occurring in water.

Lamar and Goerlitz (1963) conducted studies of the organic acids in natural waters and developed procedures for the isolation and identification of a number of organic acids that are volatile or could be made volatile for gas chromatographic analysis. The present paper includes studies of the volatile and nonvolatile organic acids in naturally colored surface waters.

Shapiro (1957, 1964) used vacuum evaporation, freeze-out, and extraction to concentrate the organic acids from large volumes of colored water (up to 900 liters) collected primarily from Linsley Pond near New Haven, Conn. He used a number of techniques in the study of the iron-holding characteristics and other properties of these acids. Black and Christman (1963a) studied the organic matter as it occurs in colored waters. On the basis of angular-light-scattering data and ultraviolet spectroscopy they concluded that the organic substances responsible for color in water exist primarily in colloidal suspension. This observation on the raw water is in contrast to Shapiro's (1957, 1964) findings of molecular solution for the colored organic matter after it was concentrated and extracted. The different conditions in these experiments may account for this disagreement. In another paper Black and Christman (1963b) described isolation techniques for fractions of organic matter which they called fulvic acid, hymatome-lanic acid, and humic acid. The fulvic acid fraction comprised over 80 percent of the acids so isolated. They concluded from solubility

relationships, chemical spot tests, and infrared spectra that the fulvic acids are aromatic polyhydroxy methoxy carboxylic acids.

Christman (1964) studied the colored solids extracted from garden soil, Douglas-fir bark, and water. He compared the degradation products and reported evidence of the aromatic and phenolic nature of the color molecules by identification of 3-methoxy-4-hydroxybenzaldehyde in oxidation mixtures of the color solids.

Hoak (1962) described problems in the recovery and identification of organic substances in water. Several extraction techniques were used, and it was reported that continuous liquid-liquid extraction in a Scheibel 18-stage countercurrent column appeared to offer advantages over adsorption on carbon. Examinations were made by colorimetric tests, ultraviolet and infrared spectroscopy, and paper and gas chromatography. Phenol, guaiacol, m-cresol, and 3,5-xylenol were identified by paper chromatography in extracts from river waters and oak-leaf fermentations; several other phenolic compounds and a few steam-volatile fatty-acids were tentatively identified.

Packham (1964) provided a discussion of the nature of organic color. Macroporous resins were utilized to isolate the complex organic acids. The fractions, which he called humic, humatomelanic, and fulvic acids were obtained by solubility techniques. It appears that the differences in these fractions may be more related to the process used and to the physicochemical state of the solution because solubility techniques have not provided separations than can be relied upon.

### LABORATORY STUDIES

The organic acids were removed from the water by two techniques: Continuous liquid-liquid extraction and vacuum evaporation. A 23.5 liter sample of water was used in both of these processes. The organic acids were isolated by selective extraction and examined by techniques of gas, paper, and column chromatography and infrared analysis.

### WATER SAMPLES

The water samples were collected from unpolluted streams in California, Hawaii, and Washington. If the sample contained particulate matter or turbidity that did not settle overnight, the water was filtered by suction through a Büchner funnel having a sintered-glass disc of medium porosity. The temperature of the waters when collected ranged from 46° to 72°F. The values for certain characteristics of these waters ranged as follows: Bicarbonate 2-270 ppm, carbonate none, specific conductance 27-509  $\mu$ mho (micromhos) at 25°C (centigrade), pH 5.2-7.7, and color 7-250 units (platinum-cobalt scale). The iron measured in selected samples ranged from 0.23 to 0.83 ppm.

## APPARATUS

*Extractor.*—A specially designed cylindrical extractor of 26-liter capacity was used to separate the organic matter from the water sample. The solvent is dispersed continuously in fine bubbles from four matched dispersion discs at the bottom of the extractor and travels through the water sample into a 1-liter boiling flask, where the extracted organic compounds are concentrated. The solvent is distilled from the concentrate to complete the cycle of continuous operation.

*Evaporator.*—A Buchler rotating vacuum evaporator was used for concentration of large volumes, and a Rinco rotating vacuum evaporator was used for the concentration of small volumes.

*Gas chromatograph.*—Gas chromatographic analyses were performed with the Perkin-Elmer, model 154-D, isothermal gas chromatograph having a thermistor thermal-conductivity detector. The analytical columns were stainless steel, 4 mm ID, 1.5- and 2-meters long.

*Recorder.*—A Leeds and Northrup Speedomax G strip chart recorder having 1- and 5-millivolt nominal spans.

*Gas chromatographic effluent collector.*—Because difficulty occurred in the collection of pure gas chromatographic samples for infrared analysis, a suitable collector as shown in figure 1 was designed for this purpose (Goerlitz and Lamar, 1964). The 18-gage stainless-steel admission tube (hypodermic tubing) is shown in the illustration in the vertical position, but it may be placed in the horizontal arm of the collector, depending upon the access to the detector vent. Each collector is easily handled, individually cooled, and conveniently sealed from atmospheric moisture and coolant-bath vapor. Importantly, the apparatus allows collection of each component very near the end of the analytical column in an individually complete assembly.

*Infrared spectrophotometer.*—Infrared analyses were made on a Perkin-Elmer model 21 spectrophotometer equipped with sodium chloride optics. A 4× beam condenser with microcells and micropellets was used where necessary.

## ISOLATION OF ORGANIC ACIDS

In the liquid-liquid extraction process, the water sample was adjusted to pH 2 and continuously extracted for 40 hours with n-butanol. The extract was made slightly basic with sodium hydroxide and concentrated to about 100 ml (milliliters) at 50°C on a rotary vacuum evaporator. The organic acids were removed from this concentrate by batch extraction with one 100-ml and two 50-ml amounts of aqueous 5 percent sodium bicarbonate. After acidification to pH 2, the acids were extracted back into n-butanol, titrated to pH 7.0 with sodium

hydroxide, and evaporated to dryness at 30°C on a small rotary vacuum evaporator.

In the evaporation process, the water sample was adjusted to pH 9 and then evaporated at 50°C to about 100 ml on a rotary vacuum evaporator. Batch extraction of this concentrate was performed with one 100-ml and two 50-ml amounts of 1:1 ethyl ether-ethanol to remove the neutral and basic components. The aqueous layer containing the organic acids was acidified to pH 2 with sulfuric acid and steam distilled. The steam distillate, containing steam volatile acids, was flushed with helium to remove carbon dioxide and titrated with sodium hydroxide to the phenolphthalein end-point. This neutralized dis-

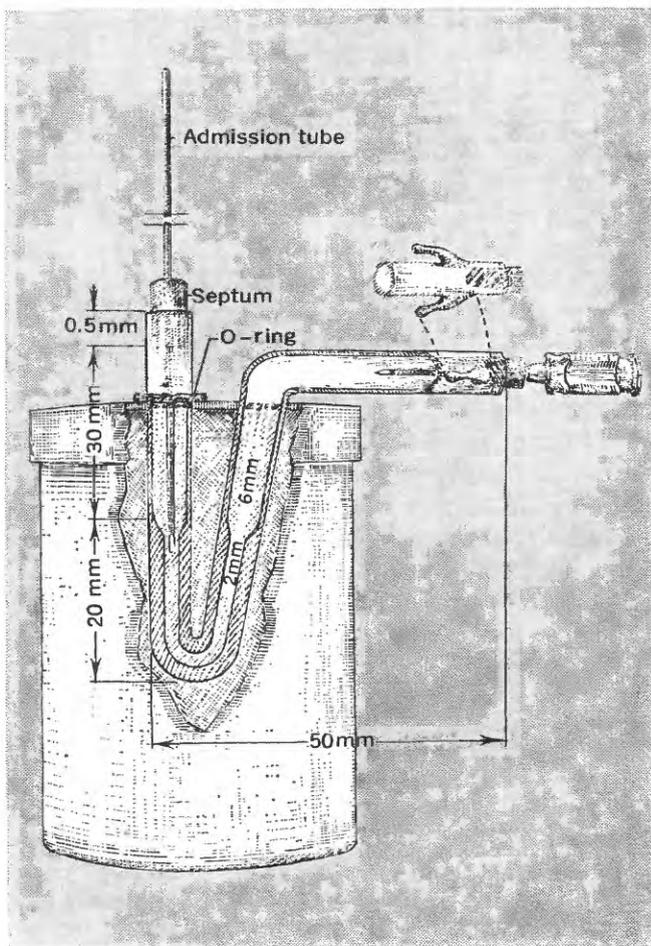


FIGURE 1.—Gas chromatographic effluent collector.

tillate was evaporated to dryness on a steam bath to remove water and nonacidic volatile substances. The acids were released from their corresponding salts by the method of Gehrke and Lamkin (1961). The organic acids remaining in the steam distillation residue were extracted with *n*-butanol and evaporated to dryness at 30°C on a small rotary vacuum evaporator.

#### GAS CHROMATOGRAPHY

The organic acids, isolated by the evaporation technique, were separated into steam-volatile acids and higher boiling acids, whereas the acids from the extraction process were not. Because the evaporation technique was performed at pH 9, all the organic acids were retained in the concentrate. The most volatile acids were not expected in the extraction process, owing to the relatively high boiling point of the solvent. The acids were analyzed by gas chromatography; those that were not volatile or sufficiently stable for the gas chromatographic analysis were converted to their methyl ester derivatives by the procedure of Radin, Hajra, and Akahori (1960).

The steam-volatile acids in ether solution were chromatographed on a 2-meter column packed with 80–100 mesh Chromosorb W which had previously been coated with a mixture of 9:1 Dow Corning 550 silicone oil and stearic acid. This coating (liquid phase) was 15 percent of the weight of the uncoated material (solid phase). The chromatographic analysis was made at a temperature of 140°C with a carrier gas-flow rate of 30 ml per minute.

The steam distillates yielded three steam-volatile acids. These components were identified as acetic, propionic, and butyric acids by direct comparison of retention volumes of the samples with standard retention volumes obtained by chromatographing solutions of known steam-volatile acids. There was indication of minute quantities of other volatile acids, such as formic and higher boiling homologues.

The methyl ester derivatives of the higher boiling acids, in methylene chloride solution, were gas chromatographed on a 2-meter polar column at 140°C with a carrier gas-flow rate of 69 ml per minute, and on a 1.5-meter nonpolar column at 160°C with a carrier gas-flow rate of 32.5 ml per minute. The polar column (3:1 solid-liquid by weight) was prepared by coating 80–100 mesh silanized Chromosorb W (solid support) with diethylene glycol succinate polyester, purified by the method of Corse and Teranishi (1960). The nonpolar column (4:1 solid-liquid by weight) was prepared by coating the same solid support with Apiezon L grease.

To characterize the methyl ester derivatives, the samples and standards were run on the polar and nonpolar columns. Retention volumes

were recorded for each component in the samples and standards. A graph, as shown in the figure 2, was prepared by plotting log retention volume against the carbon number of the esterified fatty-acid homologues used as standards. The curves are used to obtain reproducible values for the carboxylic acids which were chromatographed on both polar and nonpolar columns. These values have been designated as effective chain lengths by Miwa, Mikolajczak, Earle, and Wolff (1960), and they are independent of column temperature, column dimensions, and carrier gas-flow rate.

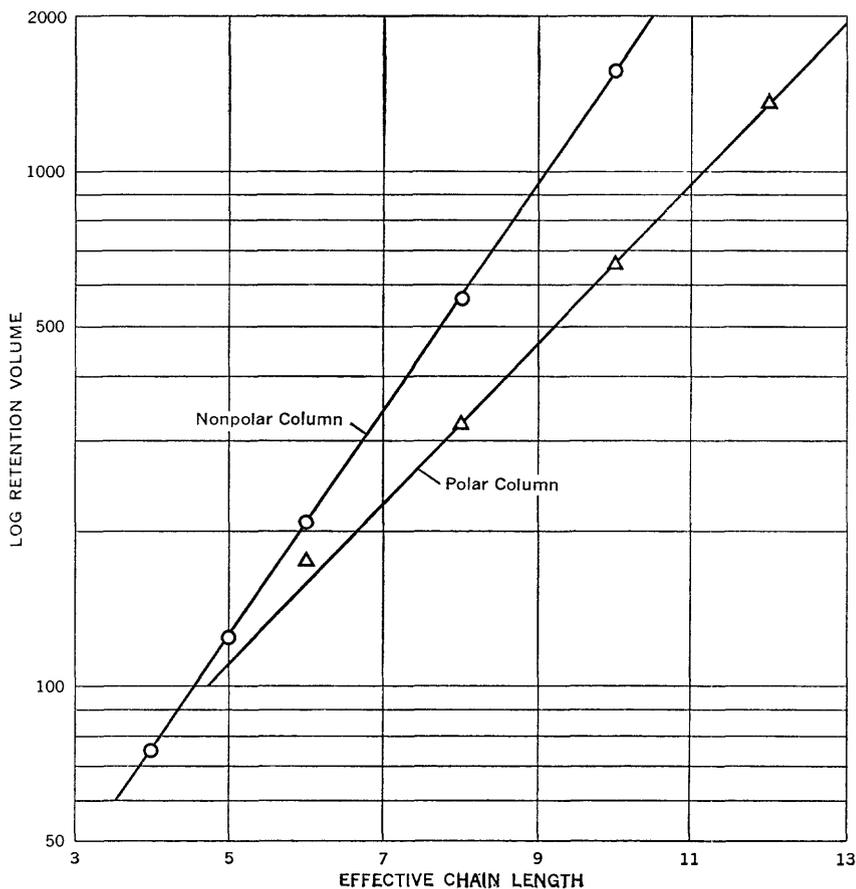


FIGURE 2.—Effective chain lengths of carboxylic acid methyl esters.

TABLE 1.—*Effective chain lengths for methyl esters of carboxylic acids*

Reference acid	Effective chain length				
	Reference	Water sample			
		1	2	3	4
<b>Polar Column</b>					
Butyric.....	4.8	4.8			
Valeric.....	5.4	5.5	5.5	5.5	5.5
Crotonic.....	5.9				
Caproic.....	6.2	6.2	6.3	6.3	
Unknown.....					6.4
Do.....		7.5			
Pyruvic.....	7.8				
Unknown.....		7.9	7.9	7.9	7.9
Caprylic.....	8.0				
Lactic.....	8.3	8.3		8.3	
Unknown.....		8.6			
Do.....		9.2	9.2	9.2	
Oxalic.....	9.8	9.8	9.8	9.8	9.8
Capric.....	10.0				
Malonic.....	10.9	10.9	10.9	10.9	10.9
Unknown.....		11.2			
Fumaric.....	11.6				
Succinic.....	11.8	11.8	11.8	11.8	11.8
Lauric.....	12.0				
Unknown.....		12.3			12.3
Maleic.....	12.8	12.8			
Unknown.....		13.0		13.0	
<b>Nonpolar Column</b>					
Unknown.....		3.7			
Butyric.....	4.0				
Pyruvic.....	4.0				
Lactic.....	4.3	4.3	4.3		
Crotonic.....	4.6				
Oxalic.....	4.7				
Valeric.....	5.0	5.0	5.0	5.0	5.0
Malonic.....	5.5				5.5
Caproic.....	6.0	6.0	6.0	6.0	6.0
Maleic.....	6.6	6.6		6.6	
Succinic.....	6.7	6.7			6.7
Fumaric.....	6.9				6.9
Unknown.....		7.3	7.3	7.3	7.3
Do.....		7.9			
Caprylic.....	8.0				
Unknown.....		8.3	8.3	8.3	8.3
Do.....		8.7			
Adipic.....	8.9			8.9	8.9

Table 1 gives the effective chain lengths of the esterified carboxylic acids—reference acids and acids isolated from the water samples. These substances are reported in the order of their emergence from the polar column and from the nonpolar column. Identifications of the

carboxylic acids in the extracts were made by comparison of their effective chain lengths with those of reference acids.

Primarily, the effective chain lengths are nearly the same for the less polar aliphatic saturated methyl esters on both types of columns used in this study but are usually quite different for more polar methyl esters. If there is little difference in the peak areas of a complex mixture, the emergence of a particular component is difficult to follow on both columns. Greater confidence can be placed in the identification of the components of a mixture if each component is collected individually and then rechromatographed on another type of column.

The gas chromatographic analysis of the methyl esters revealed a number of carboxylic acids of which 10 were identified; namely, adipic, butyric, caproic, fumaric, lactic, maleic, malonic, oxalic, succinic, and valeric. Butyric acid, identified in the steam-volatile fraction from the evaporative process, was also found in the esterified fraction from the extractive process. Butanol was used as a solvent in the liquid-liquid extraction, but it is significant that it was not used in the evaporative process. Thus, the butyric acid did not result from oxidation of the butanol solvent.

During the course of this investigation it was found that the yield of the methyl ester derivatives decreased in the presence of increasing amounts of inorganic salts. The results of an experiment to improve the yield indicated that a good yield of the ester derivatives, in the presence of appreciable inorganic salts, can be obtained by esterifying the organic acids with sulfuric acid catalyzed n-butanol. In addition, gas chromatographic analysis of the butyl ester derivatives revealed phthalic acid which had not been previously identified by gas chromatography of the methyl esters.

In all, the gas chromatographic analyses revealed approximately 30 carboxylic acids which were volatile or could be made volatile for this purpose. Thirteen of these acids were identified. Confirmatory results were obtained by several methods of analysis that included isolation of the acids by both liquid-liquid extraction and evaporative processes and by gas chromatographic analysis on both polar and non-polar columns. The presence of phthalic and succinic acids was additionally confirmed by infrared analysis. All the gas chromatographically volatile carboxylic acids were present in small amounts. The highest individual concentration measured was about 60 parts per billion for valeric acid.

The preponderant part of the isolated organic acids was nonvolatile and could not be made volatile for gas chromatographic analysis. These nonvolatile acids, commonly referred to as humic acids, or by other generic terminology, were examined by paper and column chromatography and infrared spectroscopy.

### PAPER CHROMATOGRAPHY

Paper chromatograms were prepared by spotting 1–3  $\mu$ l (microliter) amounts of 5 percent solutions of the isolated organic acids on Whatman No. 1 chromatographic paper. Reference compounds, phenol, pyrogallol, and m-digallic acid were spotted alongside and the paper was allowed to dry. The chromatograms were developed using the ascending technique with four parts n-butanol, one part glacial acetic acid, and five parts water. These chromatograms were air dried, sprayed with 2 percent ferric chloride, and then dried in an oven at 100°C. Observations before and after spraying indicated that although individual spots were vaguely discernible, there was an overall smear from the starting line to the solvent front. The lack of distinct separations was characteristic of the total acids from all water samples examined, whereas well-defined rounded spots were obtained from the reference compounds. Although no real separations were observed, the paper chromatograms augmented the conclusion that the nonvolatile acids were polymeric.

### COLUMN CHROMATOGRAPHY

Repeated column chromatographic analyses indicated that the non-volatile colored acids in natural water are polymeric, condensed molecules. The majority of the column chromatographic separations were made on 1-centimeter-diameter columns containing 7 grams of 100 mesh silicic acid coated with 4.9 ml of 0.5*N* sulfuric acid. A 50–100-mg (milligram) amount of the organic acids was chromatographed each time. The elution was performed with chloroform and increasing amounts of n-butanol in a graded series of solvent mixtures, from 0 to 45 percent n-butanol. Each solvent mixture was equilibrated with 0.5*N* sulfuric acid prior to addition to the column. The solvent flow rate through the column was 3–5 ml per minute, and each fraction was collected with a 5-ml volumetric siphon. Additional separations of the components of the organic acids were attempted on powdered cellulose using a graded series of eluents, namely, hexane, carbon tetrachloride, benzene, ether, acetone, chloroform, methanol, glacial acetic acid, and water.

From time to time during the elution, the column was observed in long-wave ultraviolet light. A true chromatographic separation of the colored acids was not visually observed to pass through the column but rather with each new solvent front a surge of yellow-brown solution was seen. Selected fractions from each solvent mixture were washed with a small amount of distilled water and evaporated to dryness, either on a steam bath or with the aid of a rotary evaporator. Some of the chromatographic fractions of the acids were taken into

solution with carbon tetrachloride or carbon disulfide for infrared analysis, whereas others were pressed into potassium bromide pellets for this analysis.

#### INFRARED SPECTROSCOPY

A number of infrared spectroscopic examinations were made on the total acids but the most extensive examinations were performed on the chromatographic fractions. Figure 3A shows the infrared spectrum of the total organic acid fraction isolated from an unpolluted stream in California. Spectra of the total acids isolated from the other natural water samples were always nearly identical. Similar spectra were obtained by Shapiro (1957) and Black and Christman (1963b) for the colored acids they had isolated from water and also by Frisch and Kunin (1960) for the deionized foulant that was eluted from an ion-exchange resin, previously used in the treatment of surface water. In figure 3A, the band at  $3.1\mu$  (microns) represents a bonded hydroxyl and carboxyl absorption. Normally, the free OH absorbs at  $2.75\mu$  and the bonded OH maximum absorption is shifted to longer wavelengths over a broader range ( $2.7$ – $3.2\mu$ ). The carboxyl OH absorbs over the broad range from  $3.0$ – $3.5\mu$ . Absorption due to NH is ruled out because nitrogen was absent in the elemental analysis.

In figure 3A the carbon-hydrogen stretch absorption at  $3.36\mu$  is probably due to asymmetric methyl and asymmetric methylene groups. The shoulder at  $3.45\mu$  is due to symmetric methyl and methylene absorption. The band at  $6.2\mu$  is indicative of unsaturation. The most intense band, shown at  $5.78\mu$ , is in the region for carbonyl absorption. Ester carbonyl occurs at  $5.76\mu$  whereas carboxylic acid carbonyl absorption is shifted up in wavelength and is usually found at  $5.86\mu$ . For carboxylic acid salts, the absorption wavelength moves even higher to  $6.1$ – $6.4\mu$ . An electron withdrawing group, influencing the acid carbonyl would shift the absorption band down in wavelength as may be the situation here. The rather broad and strong band at  $8.3$ – $8.4\mu$  is possibly due to carbon-oxygen stretch such as with esters or carboxyl groups.

Spectra of column chromatographic fractions of the acids isolated from highly colored water indicated very little difference from fraction to fraction except to reveal some increased band intensities in the  $11.5$ – $14.5\mu$  range. Spectra of the acids of chromatographic fractions from lightly colored water, however, were varied in the initial fractions; a typical example is shown in figure 3B. The spectra of later fractions returned to the characteristic spectrum shown in figure 3A.

The spectrum shown in figure 3B confirmed the presence of phthalic acid which was also identified in the gas chromatographic analysis.

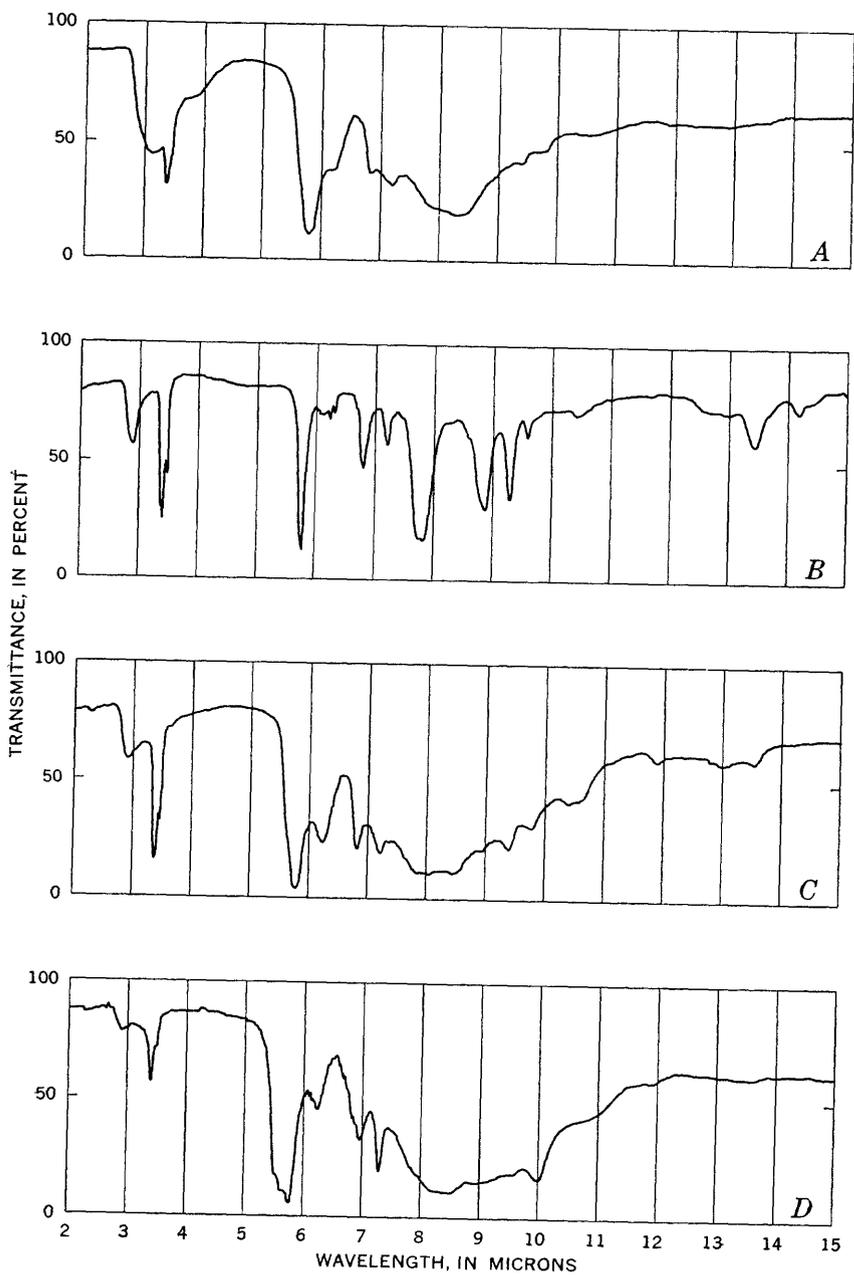


FIGURE 3.—Infrared spectra of organic acids. *A*, Total organic acids; *B*, chromatographic fraction; *C*, esterified acids; *D*, acetylated acids.

Because the acids were eluted from a sulfuric acid-coated silicic acid column with chloroform-butanol, the resulting spectrum, figure 3*B*, is the spectrum of dibutyl phthalate. The hydroxyl band in this spectrum may be due to the possible elution of silicic acid with this fraction. Black and Christman (1963b, p. 912) in their paper on fulvic acids showed a spectrum similar to figure 3*B* which they called fulvic acid oxidation products. The rechromatographing of a number of fractions (combined eluates from a solvent system) yielded a large number of partially separated polymers or other compounds.

Esterification, using sulfuric acid catalyzed butanol, decreased the OH absorption band and made it sharper. A spectrum of the esterified organic acids is shown in figure 3*C*. The OH absorption observed after esterification indicates that in addition to carboxylic acid groups there are alcoholic and probably phenolic hydroxy groups present. Because the esterification had no noticeable effect on the band at  $6.2\mu$ , unsaturation is strongly suspected.

Reaction of acetic anhydride with the total organic acid fraction almost completely eliminated the hydroxy absorption. This elimination can be observed in figure 3*D*, which is a spectrum of the acetylation reaction products. This spectrum indicates that most of the hydroxy groups are free to react. Again, the band at  $6.2\mu$  remained unchanged. Bands characteristic of anhydrides are evident.

### DISCUSSION AND SUMMARY

The organic acids in naturally colored waters result from biologic and oxidative processes. Most of the organic matter in these waters consists of a complex mixture of carboxylic acids or the salts of the acids which impart a yellow to brown color to the water. The colored acids have an indicator effect—the intensity and shade of color increases with pH. Although some of the acids isolated are not colored, the mixture of the acids is dark brown to black. The mixture of acids in solution in various solvents fluoresces under ultraviolet light, whereas the acids in the solid state were not observed to do so.

The colored organic acids generally appear to be stable and remain in apparent solution for considerable periods of time. Four samples retested for color after prolonged storage (approximately 1–2 yr) indicated that considerable reduction in color had occurred. This loss in color was accompanied by the formation of a flocculent brown organic precipitate. Active samples may show considerable variation in short periods of time. For example, the color of the water of one sample was 7, 17, and 26 units on successive days after collection. At 6 and 28 days after collection, the color of this water had decreased to 17 and 4 units, and this decrease was accompanied by the formation of a flocculent brown organic precipitate.

The observed increase in color after collection was confined to some of the lightly colored waters. Processing these waters before the color increased made the elution of more distinct column chromatographic fractions most apparent. The spectrum, shown in figure 3B, was obtained from a lightly colored water that noticeably darkened after collection but was processed promptly before the color increased. This sample was collected after a period of surface runoff following an extended period of drought. As these observations were not evident with the more mature and darkly colored waters, it seems probable that some of the complex nonvolatile acids result from polymerization in aqueous solution.

The organic acids in naturally colored waters reflect the characteristics of a composite in which polymeric nonvolatile carboxylic acids predominate. The polymeric acids in water behave like negatively charged colloids. In the raw water they can be coagulated with alum in the acid range. The optimum coagulating pH may be as low as 5 for alum and even lower for ferric coagulants. These substances extracted, dried, and taken into solution with dilute NaOH were similarly coagulated with alum.

The usual mineral analysis of naturally colored waters frequently shows an excess of cations over anions. This imbalance is small in terms of equivalents, usually being less than 0.2 equivalent per million even for the highly colored waters. The nature of this imbalance can be interpreted as indirect evidence that these colored polymeric substances have a high average molecular weight or exist in water as a colloidal sol that has complexed or adsorbed cations.

The various techniques for separating the colored acids in water by differences in solubilities are not reliably reproducible. If the organic acids in a water sample are concentrated by extraction, evaporation, or freezing, the concentrate will contain variable amounts of inorganic material. These inorganic substances tend to provide an adsorbing media for the organic acids—apparently altering their solubilities. For example, a water sample concentrated twentyfold by vacuum evaporation contained filterable solids consisting of 30 percent organic and 70 percent inorganic compounds. Acidification of the soluble part of the concentrate to pH 2 with hydrochloric acid precipitated material which consisted of 60 percent organic and 40 percent inorganic compounds. The colored organic matter in both precipitates redissolved in dilute sodium hydroxide but would not again precipitate by adjustment to pH 2.

Although the concentration of the individual acids recovered and then identified by chromatography can be determined readily, it is difficult to determine accurately the total quantity of the organic acids present in the water. Loss occurs in extraction, freeze concentration,

and where the acids are sorbed on a carbon filter or ion-exchange materials. The contamination with inorganic salts is particularly extensive in the evaporative and freezing processes. Experiments with commercial electro dialyzers for desalting the concentrates were not successful. With the nonionic membrane circulating electrolyte desalter, uncontrollable foaming occurred whereas with ion-exchange membrane type, loss due to precipitation at the anode occurred. Extraction of the organic acids from the concentrate with n-butanol was found to be an acceptable method for removing the inorganic material but some loss occurred. In the gas chromatographic studies, it was found that esterifying with sulfuric acid catalyzed n-butanol provided salt-free esters.

The isolated organic acids were found to be soluble in polar solvents such as acetone and the alcohols, slightly soluble in ether, and insoluble in hexane, methylene chloride, and benzene. The solid acids were determined to be noncrystalline by X-ray diffraction. Even after careful preparation of the sodium salts and analysis by X-ray camera technique, no crystallinity was observed. The solid acids softened at 110°C and decomposed over a broad temperature range. Elemental analysis of the sodium fusion products showed the absence of nitrogen, sulfur, and the halogens.

Most of the gas chromatographic analyses were made with an isothermal gas chromatograph having a thermistor thermal-conductivity detector. Some of the later work was performed with a dual-column temperature-programmed gas chromatograph having a flame-ionization detector. Generally 23.5 liters of water were used to provide a quantity of the organic acids sufficient for repeated examinations. Smaller samples, however, may be used, particularly for gas chromatographic analysis with the more sensitive temperature-programmed flame-ionization instrument.

About 10 percent of the mixed acids isolated from the water yielded carboxylic acids which were volatile or could be made volatile for gas chromatographic purposes. The gas chromatographic analysis indicated about 30 carboxylic acids of which 13 were identified. The individual concentrations of the gas chromatographically volatile acids ranged from trace quantities to about 600 parts per billion.

The predominant part of the organic acids are nonvolatile and could not be made volatile for gas chromatographic purposes. Infrared analysis of many column chromatographic fractions indicated that these nonvolatile substances are primarily polymeric hydroxy carboxylic acids with aromatic and olefinic unsaturation.

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