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ARTICLE *in* ANALYTICAL CHEMISTRY · NOVEMBER 1986

Impact Factor: 5.64 · DOI: 10.1021/ac00126a023

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Determination of Acrylic Acid in Aqueous Samples by Electron Capture Gas Chromatography after Extraction with Tri-*n*-octylphosphine Oxide and Derivatization with Pentafluorobenzyl Bromide

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A method is described for the determination of acrylic acid at nanomolar concentrations in complex aqueous samples. This method involves extraction and preconcentration from the acidified aqueous sample using tri-*n*-octylphosphine oxide dissolved in methyl *tert*-butyl ether and subsequent derivatization with pentafluorobenzyl bromide using 18-crown-6 as catalyst. The pentafluorobenzyl ester of acrylic acid is then quantified by capillary gas chromatography with electron capture detection. The limit of detection is about 6 fmol, corresponding to a concentration of 3 nmol L^{-1} of the acid in a 100-mL sample. Acrylic acid has been measured in aqueous samples, including natural waters, to illustrate the performance of this procedure.

There are few studies on trace concentrations of low molecular weight free fatty acids in complex aqueous samples such as seawater, largely due to the analytical problems that are encountered in their determination. Since natural water samples such as seawater or sediment porewaters are complex matrices containing a multitude of compounds, the determination of organic substances present in trace (e.g., nanomolar) concentrations poses a serious analytical problem. The analytical scheme in such instances requires a selective preconcentration step prior to instrumental analysis. Methods that have been reported for the preconcentration of short-chain fatty acids in aqueous samples include vacuum distillation (*1*), solvent extraction (*2*, *3*), ion pair extraction (*4*, *5*), adsorption on polymeric, nonionic resins such as Amberlite (*6*), and use of ion exchange resins (*7*). However, these methods have been found to be limited in one way or another, e.g., their sensitivity, efficiency, convenience of use, or suitability for subsequent gas chromatographic analysis.

Acrylic acid (CH_2=$CHCOOH$) and dimethyl sulfide ($(CH_3)_2S$) are produced in equimolar quantities from the enzymatic cleavage of (dimethylsulfonio)propionate ($(CH_3)_2S^+CH_2CH_2COO^-$), which is present in most marine algae (*8–10*). Dimethyl sulfide (DMS) has been identified as the major biogenic sulfur compound emitted from the oceans into the atmosphere, and its marine chemistry has been subjected to detailed investigations due to its importance in the global sulfur cycle (*11*). On the other hand, acrylic acid has not been previously determined in seawater. Its concentration is expected to be on the order of less than 100 nM (nmol L^{-1}), comparable to the levels of DMS in seawater (*12*). Acrylic acid has been shown to have a broad-spectrum antibiotic effect (*1*, *13*), and its function against bacterial attack on algae has been proposed (*14*). It has also been postulated that acrylic acid may serve as a structural component of the mucilaginous substance produced by algae (*13*). However, so far there have been no studies on the distribution of acrylic acid in natural waters, mainly due to the lack of a suitable method for its determination.

We report here for the first time a capillary gas chromatographic method with electron capture detection for the trace-level determination of acrylic acid in complex aqueous samples after extraction/preconcentration, using tri-*n*-octylphosphine oxide (TOPO) dissolved in methyl *tert*-butyl ether (MTBE). In a recent study by MacGlashan et al. (*15*), TOPO dissolved in an organic solvent has been proposed as a suitable extractant for the recovery of phenols from dilute aqueous solutions. Previously, Helsel (*16*) reported the use of TOPO to extract acetic acid from aqueous wastes. The extracted fatty acids were analyzed by electron capture gas chromatography after crown-ether-catalyzed derivatization with pentafluorobenzyl bromide, based on the methods reported by Durst et al. (*17*) and Davis (*18*). We use the determination of acrylic acid in algal cultures, sediment porewaters, and seawater to illustrate the performance of our technique.

EXPERIMENTAL SECTION

Apparatus. Analyses were carried out in the splitless injection mode on a Hewlett-Packard 5840 gas chromatograph equipped with a ^{63}Ni electron capture detector and a DB-5 (30 m × 0.25 mm) fused-silica capillary column (J & W Scientific, Rancho Cordova, CA). Hydrogen was used as the carrier gas and argon–methane (95:5) as the make-up gas.

Reagent. All organic solvents including methyl *tert*-butyl ether (the diluent for TOPO) were distilled-in-glass, highest quality solvents obtained from Burdick and Jackson Laboratories (Muskegon, MI). Organic-free water was made by extracting double-glass-distilled water with hexane and methylene chloride. Pentafluorobenzyl bromide was obtained from Pierce Chemical Co. (Rockford, IL). TOPO and 18-crown-6 were obtained from Aldrich Chemical Co. (Milwaukee, WI). These chemicals were used without further purification. Potassium carbonate and anhydrous sodium sulfate were of reagent grade and extracted with hexane prior to use. Silica gel (Davidson 923) was obtained from Supelco, Inc. (Bellefonte, PA). Acrylic acid was obtained from Mallinckrodt, Inc. (St. Louis, MO), and other fatty acids from Applied Science Laboratories (Waltham, MA).

Procedure. *Extraction/Preconcentration Step.* An aqueous sample of 50–300 mL was placed in a separation funnel and extracted at its natural pH (8.0 ± 0.3) with hexane to remove most of the nonionic and hydrophobic substances. Subsequently it was

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0003-2700/86/0358-2684$01.50/0

acidified to pH 2.0 ± 0.5 with 6 N HCl and extracted three times with 0.5% TOPO in methyl *tert*-butyl ether. The ratio of total extractant volume to sample was about 1:3. The TOPO–MTBE extracts were combined and washed with small aliquots of acidified organic-free water. The combined MTBE extract was filtered through anhydrous sodium sulfate to remove traces of water, placed in a flat-bottomed flask, and rotary-evaporated at 30 °C to a volume of 12 ± 3 mL. This volume was then transferred to a screw-capped tube (100 mm × 16 mm) and evaporated by a stream of air, which was purified by passing through a molecular sieve trap and a liquid nitrogen trap. Evaporation leaves a residue of TOPO containing bound fatty acids, including acrylic acid, and other substances extracted from the aqueous sample.

Derivatization Step. Subsequent to the removal of all the MTBE, derivatization was carried out in the same screw-capped tubes. Benzene (1–2 mL), 2–5 μmol of pentafluorobenzyl bromide (PFBBr) dissolved in acetone, 10 μL of 18-crown-6 solution (0.01% in acetone), and a few grains of powdered potassium carbonate were added to the TOPO-bound fatty acids in the tube. The tube was tightly closed with Teflon-lined caps and incubated at 70 °C in a block heater for 2 h with occasional shaking. After the 2-h heating period, the tube was allowed to cool and the benzene solution was passed through a minicolumn of silica gel packed in a Pasteur pipet. This step removes all polar compounds, leaving the pentafluorobenzyl derivatives in benzene.

Gas Chromatography. The benzene solution containing the pentafluorobenzyl derivatives was diluted appropriately with cyclohexane and 0.5-μL volumes were injected for analysis. The following conditions were used: initial temperature, 70 °C; initial hold, 1.0 min; rate, 5 °C/min; final temperature, 275 °C; injector temperature, 225 °C; detector temperature, 325 °C; attenuation, 64; and chart speed, 0.5 cm/min.

Samples. Seawater samples were collected in acid-washed, methanol-rinsed plastic bottles and kept under cold storage until analysis within 48 h. Sediment porewater was obtained by centrifugation of thawed samples that had been kept deep-frozen. The marine phytoplankton species *Hymenomonas carterae* and *Skeletonema costatum* were cultured bacteria-free in an artificial seawater medium (*19*), and the cultures were filtered to obtain the samples. All samples, including the algal cultures, were filtered through Whatman GFC glass fiber filters that had been previously washed with 0.01 M HCl and organic-free water and subsequently combusted at 350 °C for 18 h.

Standards. Primary standards in the range of 0.1–0.2 mol L^{-1} were made in HPLC-grade acetone and organic-free, double-distilled water and kept refrigerated. Secondary standards in the range of 0.1–10 mmol L^{-1} were made in a similar manner. Working standards made in acetone were used for the determination of derivatization yields. Suitable working standards were made in 3.5% sodium chloride solution and organic-free seawater for the estimation of extraction yields with TOPO and for calibration.

RESULTS AND DISCUSSION

Pentafluorobenzyl Bromide Derivatization. Determination of fatty acids as pentafluorobenzyl esters by gas chromatography with electron capture detection was first reported by Kawahara (*20*). Since then, several studies have been reported, and in most of them ion-pair extraction has been used to transfer fatty acids from the aqueous to organic medium prior to derivatization. These steps were carried out as combined extractive alkylation (*21–23*) or as discrete steps (*24*). However, these methods have severe limitations when applied to short-chain fatty acids and did not result in appreciable recoveries (*24*). The use of a crown-ether catalyst for the preparation of phenacyl esters was first introduced by Durst et al. (*17*) and applied to the pentafluorobenzyl derivatization of fatty acids by Davis (*18*). These authors have shown derivatization to be nearly quantitative with more than 90% yield. The derivatization procedure used in the present study is based on these methods, and indirect estimates indicate similar high yields for the derivatization of pure acrylic acid standards made in acetone.

The pentafluorobenzyl ester of acrylic acid eluted as a separate peak slightly ahead (12 s) of the propionic acid ester

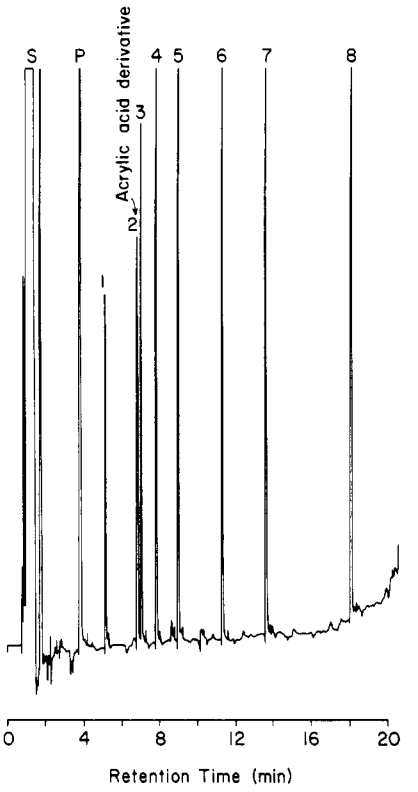

Figure 1. Chromatogram of pentafluorobenzyl esters of some short-chain fatty acids obtained by derivatization of standards made in acetone: S, solvent peaks; P, pentafluorobenzyl bromide; 1, acetic; 2, acrylic; 3, propionic; 4, isobutyric; 5, butyric; 6, pentanoic; 7, hexanoic; 8, octanoic.

Table I. Retention Data for the Pentafluorobenzyl Esters of Some Short-Chain Fatty Acids

fatty acid	mol wt	bp, °C	retention time, min
acetic acid	60.05	116–117	5.27 ± 0.03
acrylic acid	72.06	139	6.96 ± 0.03
propionic acid	74.08	141	7.17 ± 0.03
isobutyric acid (2-methylpropionic acid)	88.11	153–154	7.98 ± 0.03
butyric acid	88.11	162	9.13 ± 0.03
valeric acid (pentanoic acid)	102.13	185	11.43 ± 0.03
caproic acid (hexanoic acid)	116.16	202–203	13.73 ± 0.03
caprylic acid (octanoic acid)	144.21	273	18.24 ± 0.03
2,3-dibromopropionic acid	231.88	160	12.88 ± 0.03

(Figure 1). Since the order of elution basically depends on the molecular weight and boiling point of the compounds (Table I), this behavior of acrylic acid is as expected. Formic acid and glycolic acid could not be derivatized using the method described here.

Extraction with Tri-*n*-octylphosphine Oxide. Acrylic acid is a polar and hydrophilic substance, properties commonly shared by the short-chain fatty acids. These properties make the extraction of these compounds difficult by nonpolar, hydrophobic organic solvents. Attempts to extract acrylic acid from aqueous solutions using macroreticular, nonionic resins such as Amberlite XAD-2 did not prove successful (*6*). Studies on reversed-phase enrichment using C_{18} Sep-PAK cartridges (Waters Associates, Milford, MA) indicated very low recoveries for short-chain fatty acids, whereas long-chain fatty acids gave nearly quantitative yields (*25*).

A suitable extractant for a polar molecule from an aqueous medium must be expected to have considerable polarity along with extremely low solubility in water. TOPO presents a unique solution to this problem because its solubility in water

Table II. Acrylic Acid Concentrations in Different Aqueous Samples

sample	concn, nmol L^{-1}
culture of *Hymenomonas carterae*	1700 ± 300[a]
culture of *Skeletonema costatum*	17[b]
sediment porewater 1 (surface down to 3 cm, (Gulf of Mexico, 27°4.8′N, 93°12′W	54[b]
sediment porewater 2 (surface down to 3 cm, Gulf of Mexico, 27°10′N, 96°6′W)	56 ± 5[a]
seawater (Gulf of Mexico, boat basin, Pelican Island, 29°20′N, 94°48′W)	5.3 ± 0.6[a]

[a] Two or more determinations. [b] Single determination.

is extremely low (below 4 ppm) in spite of its polarity resulting from the phosphoryl group (*26*). Recent studies have shown that the extraction efficiency of TOPO dissolved in an organic diluent with some electron-donating ability was 1–2 orders of magnitude larger than that of the organic diluent itself (*15*).

It has been shown that TOPO effectively extracts only the nonionized species of fatty acids or phenols (*27*). Therefore, it is necessary to bring the pH of the aqueous samples containing fatty acids to 2–3 pH units below the pK_a value of the acids to be analyzed. Since acrylic acid has a pK_a value of 4.5, the pH of the aqueous samples was brought to 2.0 ± 0.5 prior to extraction with TOPO. The mechanism of TOPO extraction has been suggested to involve hydrogen bonding between the phosphoryl oxygen of TOPO and the acidic hydrogen atoms of the nonionized fatty acids or phenols (*28*).

It has been shown that the selection of the organic diluent makes little difference in the extraction efficiency as long as it has some basic (electron-donating) character and does not preferentially associate with the phosphoryl group. The diluent of choice in the present study, methyl *tert*-butyl ether, satisfies this requirement in addition to being a non-peroxide-forming solvent (*29*). Although MacGlashan et al. (*15*) used TOPO at concentrations of 15–25%, we have used a lower concentration of 0.5%, as higher amounts were found to interfere in the derivatization step. In the concentration range of 10–15 nmol L^{-1} of acrylic acid, with 0.5% TOPO in MTBE and an extractant-to-sample volume ratio of about 1:3, we have obtained a 40–50% recovery. The use of higher concentrations of TOPO, as used in previous studies, may result in improved recoveries: purification of TOPO may be necessary prior to such use.

Analytical Parameters. The analytical precision, estimated by the coefficient of variation as determined by repeated analyses of a sample solution, is about 15% at 10–50 nmol L^{-1} of acrylic acid. Due to the lack of certified standards, the accuracy of the method cannot be assessed rigorously. Under the gas chromatographic conditions used in this method, the detection limit, defined as the amount of sample that produces a signal twice the noise level, corresponds to about 6 fmol of acrylic–PFBBr derivative per injection. For a natural water sample (sample volume, 100 mL), the concentration detection limit corresponds to 3 nmol L^{-1} on the basis of a 40% recovery in the preconcentration step, using 1 mL of benzene for derivatization, and a 10-fold dilution of the benzene solution prior to injection. The dilution of the benzene solution is required to minimize the effect due to the numerous small peaks that often interfere with resolution and produce base-line noise.

Chromatograms obtained from blanks indicate peaks corresponding to significant amounts of acetic and isobutyric acids. The origins of these acids were not investigated in the present study.

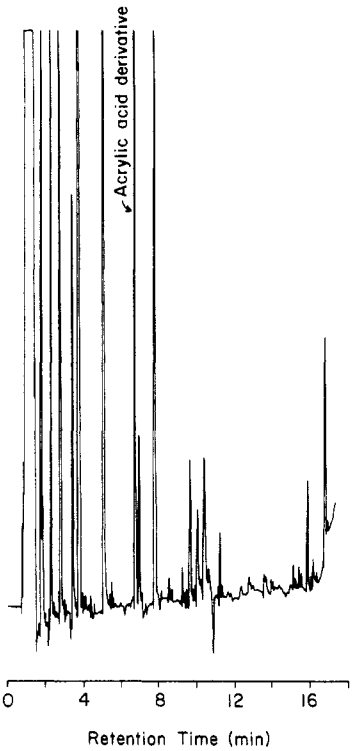

Figure 2. Chromatogram of pentafluorobenzyl derivatives obtained from *Hymenomonas carterae* culture.

Results from Environmental Samples. The performance of the method is shown by the results obtained with some aqueous samples (Table II). Relatively high levels of acrylic acid (1700 nmol L^{-1}) have been found in a culture of *Hymenomonas carterae*. Figure 2 shows the chromatogram obtained with the filtered culture medium. Dimethyl sulfide and (dimethylsulfonio)propionate have been shown to be produced in large amounts by this alga (*30*), and the present observation of acrylic acid excretion in high levels lends support for the hypothesis that both dimethyl sulfide and acrylic acid are coproduced by the enzymatic cleavage of the precursor, (dimethylsulfonio)propionate. Further studies are required to establish the quantitative relationships between dimethyl sulfide and acrylic acid production.

Figure 3 shows the chromatogram obtained with a porewater sample from the surface layer of a deep-sea sediment. It indicates the presence of acrylic acid at a concentration of about 50 nmol L^{-1}, along with many other acids. On the basis of the presence of both acrylic acid as shown here and dimethyl sulfide as documented in a previous study (*31*), it may be suggested that (dimethylsulfonio)propionate cleavage takes place in the deep-sea sediment.

The presence of acrylic acid in the sediment may explain the occurrence of thiols, particularly 3-mercaptopropionic acid, which has been shown to occur in high levels in some anoxic sediments (*32*). Laboratory studies carried out by Mopper and Taylor (*32*) have shown the abiotic formation of 3-mercaptopropionic acid from acrylic acid and hydrogen sulfide. The present observation of the occurrence of acrylic acid lends support for this hypothesis, and this abiotic pathway may well be the mechanism for the formation of 3-mercaptopropionic acid in anoxic sediments.

The analysis of a seawater sample from the Gulf of Mexico gives a concentration of about 5 nmol L^{-1}. Since acrylic acid and dimethyl sulfide are known to be produced in equimolar quantities, the concentration level of acrylic acid in seawater is expected to be on the same order of magnitude as dimethyl sulfide. The acrylic acid level of the Gulf of Mexico water, in fact, shows excellent agreement with the average concen-

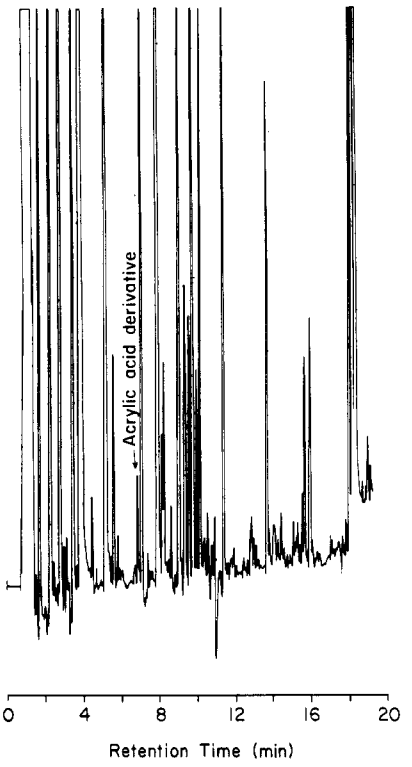

Figure 3. Chromatogram of pentafluorobenzyl derivatives obtained form sediment porewater sample 1.

tration of dimethyl sulfide in seawater, which is about 3 nmol L^{-1} (*12*).

Although the present study was limited to the determination of acrylic acid, we have observed, as shown in the chromatograms given in Figures 2 and 3, that phytoplankton excretions and natural water samples contain many other acids that can be determined as pentafluorobenzyl derivatives. So far the studies on these important lipid constituents have been limited mainly due to the lack of a suitable method for trace-level determinations. The present method may prove useful for future studies concerned with short-chain fatty acids.

ACKNOWLEDGMENT

We thank Elliot Atlas, Texas A&M University, for use of the gas chromatograph, assistance, and helpful discussions during the study. Assistance given by M. C. Kennicutt II and Terry L. Wade of Texas A&M University is acknowledged.

Registry No. Acrylic acid, 79-10-7; tri-*n*-octylphosphine oxide, 78-50-2; pentafluorobenzyl bromide, 1765-40-8; 18-crown-6, 17455-13-9.

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RECEIVED for review March 25, 1986. Accepted July 1, 1986. This work was supported in part by National Science Foundation Grants OCE-8315733 to Florida State University and OCE-8301538 to Texas A&M University.