Microdetermination of Pentafluorobenzyl Ester Derivatives of Organic Acids by Means of Electron Capture Gas Chromatography

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TRACES of organic acids (*1*) adversely affect the palatability of water. To aid in monitoring waterways, sensitive and rapid methods are necessary for measuring these compounds which constitute a small portion of the complex organic residue obtained by evaporation of the chloroform extract of the carbon adsorption (*2*) samples. Sufficient amount of activated carbon contained in a hollow cylinder effectively concentrates from metered water the numerous organic pollutants which are carried to the stream by runoff, in domestic sewage, or in industrial wastes. The adsorbed organic pollutants may be recovered from the activated carbon by means of extraction with chloroform. Evaporation of the extract leaves an organic residue. Details for determining the carbon chloroform extract of monitored surface waters are reported in the adopted, tentative method (*3*).

With the aid of the solubility separation procedure, the strong acid fraction (*4*) is isolated from the organic residue. Normally, the strong acid fraction should contain only the strong acids soluble in 5% sodium bicarbonate solution. Initial findings, however, suggest that this acid fraction isolated from the organic pollutants of surface waters is difficult to analyze for strong organic acids at 200 °C with a flame ionization detector, as large amounts of unknown substances interfere in the gas chromatographic measurements.

Conventional methods (*5*, *6*) of analyses require time consuming procedures to eliminate the interference present in the strong acid fraction of the organic pollutants from surface waters. Large volumes of water and laborious methods of extraction, removal of interferences by back-washing, re-extraction, and concentration are involved. Several workers (*7*, *8*) have reported the quantitative analyses by gas chromatography of a synthetic mixture of volatile fatty acids in aqueous solution. A method for the direct measurement of trace amounts of organic acids without preliminary cleanup of the organic extract from surface waters is not available.

The flame-ionization detector gas chromatographic analyses of the strong acids from the carbon chloroform extracts (*2*) revealed that the minimum measurable amount of a low molecular weight organic acid is about a tenth of a microgram. Even the detection of trace amounts of the lowest molecular weight acids is difficult when the flame ionization detector method is used. With F.I.D. gas chromatography, the analyses of trace amounts of acids appear limited to their measurements in uncontaminated samples.

Because of the interference in the strong acid fraction, a highly selective method is required. Pentafluorobenzyl ethers and thio ethers (*9*), derived from phenols and mercaptans, are shown to give excellent response to the electron capture detector and can be determined in presence of unreactive materials which are not in the group of organic compounds soluble in 5% sodium hydroxide solution. Unreactive materials are presumably solubilized at trace concentrations in the operation involving the solubility separation procedure (*4*). Pentafluorobenzyl derivatives provided selective measurements at trace concentrations of the resolved derivatives of phenols and mercaptans. Similarly, the conversion of acids to a derivative which can exhibit electron capture response seemed attractive for their measurement. At the nanogram range, the pentafluorobenzyl esters of these organic acids can be analyzed on the electron capture gas chromatograph. This selective property of the unusual derivatives ensures valuable measurements in the presence of large amounts of interfering substances, as only acids will react with the reagent under the described conditions. These esters, unprepared previously, are highly amenable to gas liquid chromatographic separation, and there is virtually no tailing observed in the chromatograms. The stability of the derivatives of low molecular weight acids in water is fair. With proper extraction procedures, very favorable recoveries are possible for these esters.

EXPERIMENTAL

Apparatus. The apparatus and its operating conditions were described previously (*9*). The gas chromatographic column, through previous conditioning with injections of aldrin, endrin, heptachlor, etc., and through several months' use for pesticide analyses, had yielded excellent separations and was highly amenable for the experimental work.

Preparation of Esters. The acids were converted to the derivative in similar manner to that (*10*) used for the preparation of the novel ethers (*9*). A weight of 0.004 mole of acid dissolved in 100 ml of acetone is treated with 1.044 grams (0.004 mole) of alkylhalide and with 0.522 gram (0.004 mole) of potassium carbonate. Alcohol and standardized alcoholic potassium hydroxide may be substituted for the acetone and potassium carbonate. Caution should be exercised in the handling of α-bromo-2,3,4,5,6-pentafluorotoluene, because it is a strong lachrymator. A 2% ethanolic solution of the reagent is handled in a well ventilated hood. After three hours of reflux, the solvent was removed at 50 °C and 50 mm of pressure, and the residue was dried at 50 °C and 0.5 mm of pressure. The residue was taken up with 500 ml of ether and the extract was washed with 10 ml of water saturated with ether. This extract was dried over 8 grams of anhydrous sodium sulfate. After the sodium sulfate and filter paper were washed with 50 ml of ether, the solvent was removed at 40 °C/50 mm pressure. Further drying at 0.5

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(1) M. B. Ettinger and F. M. Middleton, *J. Amer. Water Works Ass.*, **48**, 1265 (1956).
(2) H. Braus, F. M. Middleton, and G. Walton, ANAL. CHEM., **23**, 1160 (1951).
(3) "Standard Methods for the Examination of Water and Wastewater," 12th ed., Amer. Public Health Ass., Inc., New York, N. Y., 1965, pp 214–18.
(4) R. L. Shriner and R. C. Fuson, "Systematic Identification of Organic Compounds," 2nd ed., Wiley, New York, N. Y. 1940, pp 252–3.
(5) W. L. Lamar and D. F. Goerlitz, *J. Amer. Water Works Ass.*, **55**, 797 (1963).
(6) H. F. Mueller, T. E. Larson, and M. Ferratti, ANAL. CHEM., **32**, 687 (1960).
(7) R. A. Baker, *J. Gas Chromatogr.*, **4**, 418 (1966).
(8) V. Mahadevan and L. Stenroos, ANAL. CHEM., **39**, 1652 (1967).
(9) F. K. Kawahara, *ibid.*, **40**, 1009 (1968).
(10) L. Claisen, O. Eisleb, and F. Kramers, *Ann. Chem.*, **418**, 69 (1919).

Table I. Properties of Pentafluorobenzyl Esters

Pentafluorobenzyl esters	Yield, %	R. I. $n_D^{24°}$	°C, mp	Retention time, min	Relative retention (to benzoate)
Acetate	100	1.4068		1.26	0.34
Propionate	71	1.4295		1.42	0.38
Isobutyrate	99	1.4284		1.42	0.38
Butyrate	67	1.4302		1.57	0.43
n-Pentanoate	96	1.4292		1.78	0.48
n-Hexanoate	89	1.4340		2.12	0.57
n-Heptanoate	86	1.4358		2.54	0.68
n-Octanoate	84	1.4368		3.15	0.85
Benzoate	98		72–74	3.72	1.00
n-Decanoate	99	1.4405		5.02	1.35
n-Undecenoate	100	1.4497		6.38	1.72
n-Dodecanoate	100		23–24.5	8.34	2.24
n-Tetradecanoate	86		40–41	14.52	3.90
n-Hexadecanoate	84		46–48	25.58	6.87
n-Octadecanoate	92				12.3
Oleate	100	1.4555			
Linoleate	100	1.4664			
DL-Methionine	96				

mm of pressure left a residue on which the physical properties were determined. Table I records the yields, refractive indices, melting points, and retention times of the novel ester derivatives.

The micropreparation for recovery study of the derivatives was conducted as follows: a mixture of 0.8 mg each of butyric, hexanoic, benzoic, and *n*-dodecanoic acids was reacted as before, except that 250 mg (25-fold excess) of the fluoro organic and 50 mg (10-fold excess) of the alkaline reagent were used. After three hours of reflux, the mixture was taken up with 500 ml of ether and 20 ml of ethyl acetate. Solvents for the reaction mixture were not removed prior to extraction. The extract was contacted briefly with 10 ml of water and was dried over sodium sulfate. After the solvent was removed under gentle reflux using an all-glass distillation apparatus, the residue was dried at 40 °C and at 50 mm of pressure. The residue was dissolved in 100 ml of hexane containing 1% each of acetone and diethyl ether. One ml of this solution was further diluted to 100 ml with the same solvent mixture. From this dilution, 6-μl injections were introduced for the GLC analyses.

Table II. Comparative Responses of Pentafluorobenzyl Derivatives with Aldrin

Compound	Response per nanogram, (sq. in.)
Phenyl pentafluorobenzyl thio ether	0.68
Phenyl pentafluorobenzyl ether	0.78
Pentafluorobenzyl caproate	0.86
Aldrin	1.09
Pentafluorobenzyl benzoate	1.12

Table III. Analytical Results of Gas-Chromatographic Separation of Pentafluorobenzyl Esters (In Order of Emergence)

Pentafluorobenzyl ester	Added weight acid (mg)	Calcd. weight ester (mg)	Found weight ester (ng)	Recovery, %
n-Butyrate	0.8	2.46	2.16×10^6	88
n-Hexanoate	0.8	2.03	1.72×10^6	85
Benzoate	0.8	1.98	1.60×10^6	81
n-Dodecanoate	0.8	1.52	1.32×10^6	87

RESULTS AND DISCUSSION

Yield. Mild drying conditions reflected higher yields given in Table I, as the weights of acetate and isobutyrate which were dried at 45 °C and at 50 mm of pressure show excellent yields. On the other hand, when the propionate and the butyrate esters were dried at 50 °C and at 0.5 mm of pressure, the yields become poor. Consequently, these lower molecular weight esters can be volatilized under reduced pressure, as their lower yields reflect this property. The remaining esters were dried at 0.5 mm of pressure for varying periods. Losses may be incurred in the washing treatment for alkali removal if care is not exercised.

Separation of Esters. A single mixture containing the first 14 pentafluorobenzyl esters of organic acids designated in Table I was resolved into 13 components. Equal weight of all esters was used, with the exception of the fourteenth component which contained three times the normal amount. The injection of 4 μl contained 0.32 nanogram of the novel esters.

Particular attention was given to the gas chromatographic operation at 200 °C with a gas flow of 40 ml per minute for reasons of convenience. All components except the propionate and the isobutyrate could be resolved as indicated by their respective retention times. Peak 4 represents both of these esters. At 153 °C and at a flow rate of 26 ml per minute, the acetate could be resolved completely from the propionate and isobutyrate mixture which, in turn, may be separated completely from the butyrate. These compounds exhibit very little tailing.

The comparative responses to a few of these novel derivatives are listed in Table II.

These derivatives are compared with aldrin, as it is widely analyzed as a pollutant in water. The electron capture detector response to the benzoate of pentafluorobenzyl alcohol is comparable to that given to aldrin at the 1-nanogram level. However, the response to the ester of caproic acid is slightly smaller at the same level of concentration, due to its non-aromatic character or lack of pi-electron system in the acidic moiety of the ester. In the aromatic series of these pentafluorobenzyl derivatives, the detector response to the benzoate ester is greater than to the phenyl ether. The latter derivative, in turn, evokes a stronger response from the detector than does the thioether.

A synthetic mixture of four acids was chosen to represent the strong acid fraction of the carbon adsorption extract. Its GLC analysis is illustrated in Table III.

These recovery data are the result of 6-μl injections, which contained 1.3 to 0.8 nanograms each of butyrate, *n*-hexanoate, benzoate, and *n*-dodecanoate of pentafluorobenzyl esters. The very good recovery for these esters when excess reagents are introduced for the reaction indicate that the use of equivalent amounts of acids and reagents does not appear to be critical. Recovery values, presumably, may be increased with shorter contact time of traces of water for removal of the alkali during the extraction of the reaction mixture. For analysis of trace amounts of volatile organic acids, the recovery of their respective esters is favorable when the esters are retrieved without concentrating the original reaction mixture, with little water washing, and with mild drying conditions of the derivatives. The overall recovery process for the submicro scale includes reaction, extraction, concentration, and analyses of the ester derivatives.

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A Radioisotope Source–Target Assembly for X-Ray Spectrometry

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DEVELOPMENT of high-resolution semiconductor detectors has made them applicable for X-ray emission spectrometry when used in conjunction with radioisotope sources for exciting the X-rays (*1*). Semiconductor detectors with resolutions better than 0.5 keV (full width at half maximum) are now available commercially. L. Reiffel (*2*), J. S. Watt (*3*), and J. R. Rhodes (*4*), employing other detection systems, have described radioisotope source-target assemblies for nondispersive X-ray fluorescence analysis. A modification of these assemblies is described here which makes it compatible with semiconductor counters as detectors.

The source-target assemblies reported by Reiffel (*2*), Watt (*3*), and Rhodes (*4*), all employ a two-stage system of excitation. A radioactive source is used to excite the K X-rays of a selected target material, and these in turn excite X-rays from the specimen. The geometrical arrangement is such that the specimen sees primarily the X-rays of the target while the detector sees primarily the X-rays excited from the specimen and the backscattered target X-rays. In these systems, point radioactive sources were used along with scintillation or proportional counters as detectors.

The assembly described here is a modification made necessary by the fact that the lithium-drifted silicon and germanium detectors of high resolution are quite small in area. As shown in Figure 1, the radioisotope source has annular geometry as does the primary target. Some of the radioisotopes that can be used as sources are ^{241}Am, ^{109}Cd, ^{125}I, and ^{57}Co. Table I lists the half lives of these radioisotopes, their useful radiations, and the intensities of these radiations per disintegration. ^{241}Am is a particularly good source because its useful radiations cover a wide range of energies and its long half life provides a constant intensity source. To obtain high sensitivity in analysis, primary targets are chosen whose characteristic K X-ray energies are not too far above the absorption edges of the elements to be determined. Similarly, the radioisotope source should have photons not too much higher in energy than the absorption edge of the target.

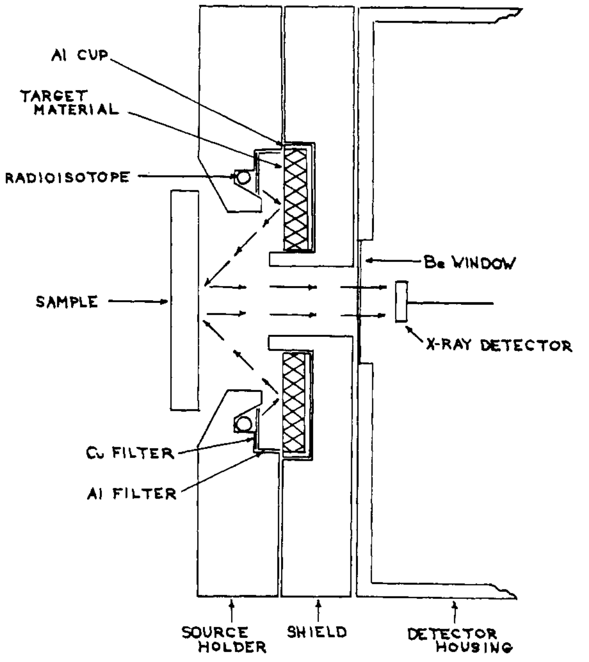

Figure 1. Source–target assembly

^{241}Am annular sources have been made by absorbing a solution of AmCl$_3$ on Al$_2$O$_3$ powder and loading the powder in an aluminum tube which has been previously annealed. The tube has an od of $^1/_{16}$ inch and a wall thickness of 0.0005 inch. Once the tube, bent into annular shape, has been placed in the groove of a lead block source holder, a thin layer of epoxy resin is cast over the top of the tube. The purposes of the Al$_2$O$_3$ powder and the aluminum tube are to distribute the activity evenly and to contain the alpha radiation. When exciting K X-rays of targets with an atomic

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(1) H. R. Bowman, E. K. Hyde, S. G. Thompson, and R. C. Jared, *Science*, **151**, 562 (1966).
(2) L. Reiffel, *Nucleonics*, **13**, 22 (1955).
(3) J. S. Watt, *Int. J. Appl. Radiat. Isotopes*, **15**, 617 (1964).
(4) J. R. Rhodes, *Analyst* (London), **91**, 693 (1966).