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Determination of resin and fatty acids in sediments near pulp mill locations

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ABSTRACT

A gas chromatographic method for the determination of resin and fatty acids in sediments is described. In this procedure, the sediment sample was air-dried and soxhlet-extracted with a mixture of acetone—methanol (88:12, v/v) in the presence of hydrochloric acid. The acids extracted were converted into their pentafluorobenzyl esters and were then cleaned up on a deactivated silica gel column. Final analysis was performed on either a DB-17 or a DB-5 capillary column with electron-capture detection. Quantitative recovery was obtained from fortified sediments for all acids except palustric, neoabietic and levopimaric acids. The detection limit of all acids in this method was 0.1 μg/g based on 1 g of sample. This procedure has been successfully applied to the monitoring of resin and fatty acids in sediment samples collected in the vicinity of several Canadian pulp mill locations.

INTRODUCTION

Pulp and paper mill effluents contain a large number of environmental contaminants. During the past twenty years, extensive research has been carried out in the identification of those toxic components present in various pulp mill discharges [1–5]. In general, diterpene resin acids (Fig. 1), fatty acids, diterpene alcohols and other neutral lignin degradation products can be found in nearly all effluents [3,4]. In addition, chlorinated phenols, guaiacols, catechols, vanillins and resin acids as well as other volatile chlorinated acids and aliphatics are also present in chlorobleaching kraft mill effluents [6–8]. Resin acids, which are commonly found in the debarker and woodroom effluents from pulp mills, have been identified as the major contributor to the toxicity in effluents to fish [9–11]. The levels of these acids are generally higher if there is no secondary waste treatment on the effluents. In some cases, the effluents are acutely toxic to fish if undiluted. Therefore, chemical analysis for resin and fatty acids (RFAs) has been one of the core components in the Canadian pulp mill water quality monitoring program.

Several methods have been developed and used in the determination of RFAs in effluents [12,13]. They involved extraction of the acids either by solvent or by a ion-exchange column then followed by the flame ionization detection (FID) of the methyl or ethyl derivative of the acids. Very little information regarding the levels of

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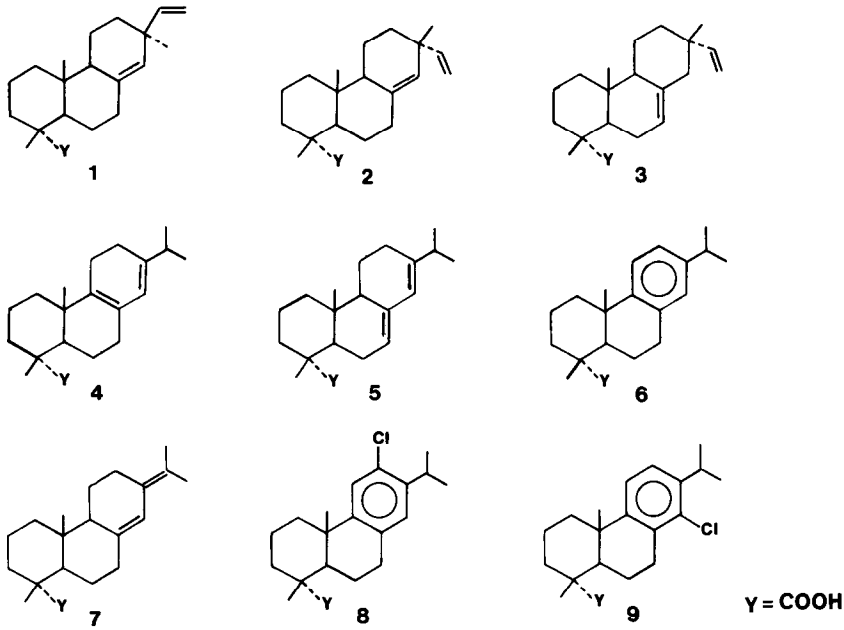

Fig. 1. Molecular structures for selected resin acids. Acids: 1 = pimaric; 2 = sandaracopimaric; 3 = isopimaric; 4 = palustric; 5 = abietic; 6 = dehydroabietic; 7 = neoabietic; 8 = 12-chlorodehydroabietic; 9 = 14-chlorodehydroabietic. Dichlorodehydroabietic acid has chlorine substitutions at both 12 and 14 positions.

RFAs in sediments at pulp mill locations is available, and few methods have been reported in the literature for the determination of RFAs in such samples. In one paper describing the distribution of dehydroabietic acid in sediments adjacent to a kraft pulp and paper mill, the resin acid was soxhlet-extracted by acetone, methylated and analyzed by gas chromatography (GC)–FID with a packed column [14].

In the past two years, we have been working on new and improved methods for the analysis of RFAs in various matrices. Our approach involves the conversion of the acids into their pentafluorobenzyl (PFB) esters. With these esters, an electron-capture detection (ECD) instead of FID is used for analysis. In addition, negative-ion chemical ionization mass spectrometry (NICI-MS) can be applied to the quantitation and positive identification of RFAs. This new approach has been succesfully applied to the determination of RFAs in many final effluents [15]. Based on the same derivatization and detection technique, a method for the quantitative analysis of RFAs in sediment samples is presented. Factors affecting the extraction recoveries of RFA in sediments are also discussed.

EXPERIMENTAL

Reagents and chemicals

All solvents used were of distilled-in-glass grade supplied by Burdick and Jackson (Muskegon, MI, USA).

Resin acids were purchased from Helix-Biotech Scientific (Vancouver, Canada)

and used without further purification. All fatty acids and pentafluorobenzyl bromide (PFBBr) were acquired from either Aldrich (Milwaukee, WI, USA) or Sigma (St. Louis, MO, USA).

Stock solutions of individual RFAs at 1000 μg/ml were prepared in acetone and kept at −20°C in crimped top vials. Spiking solutions of the mixed RFAs at 50 μg/ml in acetone were kept at 4°C in the dark. Because of the gradual decomposition of neoabietic acid, a new stock solution of this acid and fresh RFA mixtures were made up once every two months.

A PFBBr solution was prepared by dissolving 1 g of the reagent in 20 ml of acetone with a water content of 0.2% or less by volume and kept at −20°C until use. A 30% (w/v) potassium carbonate solution was made by dissolving 3 g of the anhydrous base in 10 ml of water. Anydrous sodium sulfate was heated at 600°C overnight before use.

Sampling of sediment samples

Grab river sediments were collected and excess water was decanted off. The samples were kept in 500-ml wide-mouth, brown, screw-capped bottles with aluminium foil liners and frozen at −20°C in the dark until analysis.

Fortified sediment samples for the recovery runs were prepared by spiking known amounts of RFAs to a wet sediment which was known to have negligible amounts of native resin acids and low levels of fatty acids. The sediment was then thoroughly mixed, air-dried and extracted as described below.

Extraction, derivatization and clean-up

The wet sediment was homogenized before a 5.00-g subsample was air-dried in a fumehood at room temperature. Another sample was taken for moisture content determination. An aliquot of 25 μg of tricosanoic acid in 100 μl of acetone was evenly applied to the sediment, and the sample was quantitatively transferred to the top of a 5-cm Celite 545 layer in a glass thimble with a coarse fritted disc. The sediment was then soxhlet-extracted for 7 h with 300 ml of methanol–acetone (12:88, v/v) in the presence of 100 μl of concentrated hydrochloric acid. The sample extract was evaporated to dryness with a rotary evaporator using a 40°C bath and redissolved in three 2-ml aliquots of acetone. This acetone extract was passed through a column made up of 3 cm of Celite in a disposable Pasteur pipet with a glasswool plug for the removal of particulate matters. The extract was then adjusted to a volume of 10.0 ml in a calibrated centrifuge tube.

A 2-ml volume of the filtered acetone extract was transferred to a centrifuge tube and evaporated to 1 ml. A 100-μl volume of 30% K_2CO_3 and 250 μl of PFBBr solution were added and the mixture was heated at 60°C for 2 h. After reaction, the acetone was evaporated and the residue redissolved in hexane. The derivatized products were then cleaned up by passing the above hexane solution through a 5.00-g 5% deactivated silica gel column, as described for the effluent samples [15]. The final volume was adjusted to 2.0 ml in isooctane. Depending on the concentrations of RFAs in the sample, further dilution or concentration of this extract could be required.

Chromatographic analyses

Sediment extracts were analyzed with a Hewlett-Packard 5880A gas chromatograph equipped with an electron-capture detector and a 30 m × 0.25 mm I.D. fused-silica DB-17 or a DB-5 capillary column (J&W Scientific). The chromatographic conditions used were as follows: the initial temperature of the column oven was held at 70°C for 0.75 min and then programmed to 160°C at 30°C/min. It was further raised to 290°C at 2°C/min. Injector and detector temperatures were 250 and 300°C, respectively. Helium was used as carrier gas and the column head pressure was 105 kPa. Samples of 2 μl were injected in the splitless mode with a valve time of 0.75 min. Known amounts of RFAs were directly derivatized and cleaned up as described above alongside the sediment samples and used as external standards for the quantitation of the acids.

Confirmation of peak identity was achieved by electron-impact (EI) MS in the full-scan mode [15]. For samples of low RFA concentrations, selected-ion monitoring (SIM) using NICI-MS as described before [15] can be applied. In this case, the characteristic $[M-181]^-$ ions monitored were *m/z* 199 for lauric acid, *m/z* 227 for myristic acid, *m/z* 255 for palmitic acid, *m/z* 283 for stearic acid, *m/z* 281 for oleic acid, *m/z* 279 for linoleic acid, *m/z* 277 for linolenic acid, *m/z* 311 for eicosanoic acid, *m/z* 339 for behenic acid, *m/z* 299 for dehydroabietic acid, *m/z* 333 for chlorodehydroabietic acids, *m/z* 367 for dichlorodehydroabietic acid and *m/z* 301 for the other resin acids.

RESULTS AND DISCUSSION

Comparison of the PFB and methyl esters of RFAs

In the past, nearly all information regarding the levels of RFAs in pulp mill effluents and related samples was obtained by GC analysis of their methyl esters using a flame ionization detector. Although this technique is applicable in many cases, it lacks the sensitivity required for the monitoring of RFAs in final effluents and other environmental samples collected further downstream from the mills. This problem can be solved by the conversion of the RFAs into their PFB ester derivatives [15] so that the more sensitive electron-capture detector can be used in the final analysis. Aside from an over 100-fold enhancement in sensitivity, the PFB esters of RFAs have the following advantages over the corresponding methyl ester derivatives. (1) Formation of methyl esters requires diazomethane, a toxic, carcinogenic, explosive and unstable reagent which has to be generated each time before use. For the PFB esters, a less hazardous and readily available reagent, PFBBr, which does not have the other undesirable properties, is used. (2) Methyl esters of some resin acids are unstable in hydrocarbon and chlorinated solvents for an extended period of time [13]. In contrast, the PFB esters are stable for months in hydrocarbon solvents so that the derivatized extract can be stored for a long time for further analysis or confirmation if necessary. (3) The presence of methyl esters of RFAs has been reported in pulp mill effluent samples [4]. Thus with the methyl ester method, the RFA results would have a high bias unless the native methyl esters are removed from the effluent extract before methylation of the free acids takes place. However, this partitioning step is unnecessary in the PFB ester procedure. (4) Since the characteristic $[M-181]^-$ ion is the base peak for the PFB ester of every RFA under the NICI condition, RFA can be quantitated and positively identified by this MS technique with a sensitivity similar to ECD [15].

Extraction of RFAs in sediments

At the early stages of method development, we investigated three techniques commonly used for the extraction of organics in sediments. Using a freeze-dried and homogenized sediment obtained from a pulp mill as a reference sample and acetone as the extraction solvent, the recoveries of RFAs obtained by soxhlet, polytron (a high-speed sample homogenizer) and ultrasonic extraction techniques were compared. In each case, the RFA concentrations obtained were summed. Since soxhlet extraction produced 10–30% higher recoveries for total acids than the other two techniques, this method was chosen for the subsequent experiments.

The choice of extraction solvent also played an important role in the recovery of RFAs. Although acetone–hexane mixtures and pure acetone are usually adequate for the extraction of most neutral pesticides and organics in sediments, an increase of 30–40% for the recovery of RFAs in the reference sample was obtained by using the azeotropic mixture of 12% methanol in acetone (v/v) instead of pure acetone. The use of higher methanol content or pure methanol did not further improve the recovery. On the other hand, the addition of 100 μl of hydrochloric acid to the solvent prior to extraction had an even greater (*ca.* 200–300%) improvement on the recovery of all RFAs in the reference sample except for palustric and neoabietic acids. Further increase in the amount of hydrochloric acid gave even lower recovery of palustric and neoabietic acids and no improvement of the other resin acids. Using the results for five representative acids, namely palmitic, oleic, isopimaric, dehydroabietic and palustric acids, the effect of various solvent systems on the recovery of RFAs in sediments is depicted in Fig. 2.

In light of the reported isomerization of palustric and neoabietic acids under acidic conditions, it is not surprising to find that an extraction procedure using an acidified solvent mixture is not ideal for the recovery to those unstable resin acids. Since the use of non-acidified solvent systems produced much lower recoveries for all other acids, soxhlet extraction using 12% methanol in acetone with 100 μl of concentrated hydrochloric acid was chosen as a compromise for the optimal recovery of most RFAs in sediments.

Chromatographic separations of RFA-PFB esters

The DB-5 and DB-17 capillary columns were both used in the GC analysis of sediment extracts since the different elution patterns of the RFA esters on these columns allow tentative identification of peaks in the absence of a mass spectrometer. In general, the more polar DB-17 column had slightly better overall resolution of the esters. For instance, it was observed that the PFB esters of dehydroabietic acid and *o*-methylpodocarpic acid, a surrogate used by some workers for pulp and paper mill samples, as well as those of 12-chlorodehydroabietic acid and erucic acid (*cis*-13-22:1), could not be resolved on the DB-5 column.

Method performance and interference

Using fortified subsamples of a lake sediment containing non-detectable amounts or low levels of native RFAs, the precision and accuracy of our method were determined by the recovery of the acids in replicate runs. For sediments fortified at 10 and 1 μg/g, the recoveries were between 85 and 105%, with relative standard deviations ranging from 3 to 8% in nearly all cases (Table I). It should be noted that the

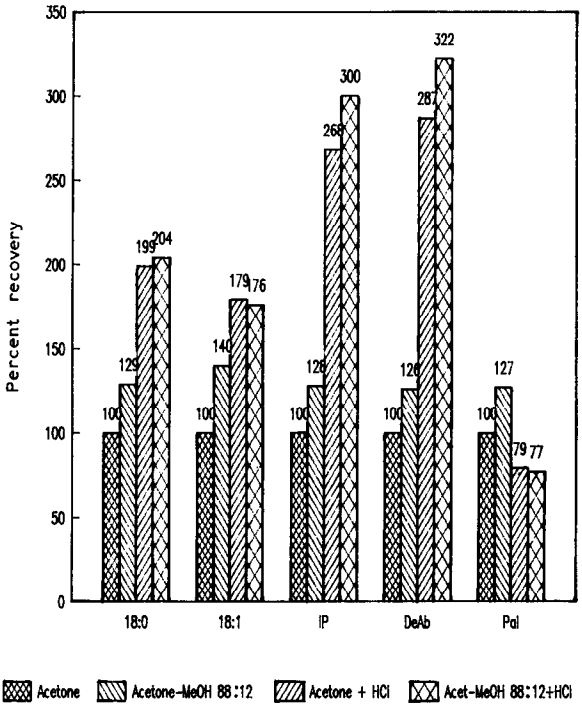

Fig. 2. Effect of methanol and acid on the recovery of resin and fatty acids. Legend: 18:0 = stearic acid; 18:1 = oleic acid; IP = isopimaric acid; DeAb = dehydroabietic acid; Pal = palustric acid; MeOH = methanol; Acet = acetone.

TABLE I

RECOVERIES OF REPLICATE DETERMINATION OF RESIN AND FATTY ACIDS IN FORTIFIED SEDIMENT SAMPLES

RFA	Recovery (mean ± S.D., $n=6$) (%)	
	10 μg/g	1 μg/g
Lauric (12:0)	97 ± 2.9	86 ± 6.7
Myristic (14:0)	103 ± 3.9	100 ± 8.8
Palmitic (16:0)	97 ± 4.0[a]	90 ± 7.9[a]
Stearic (18:0)	93 ± 5.3[a]	86 ± 7.5[a]
Oleic (18:1)	98 ± 5.7	88 ± 8.0
Linoleic (18:2)	95 ± 6.4	76 ± 8.1
Linolenic (18:3)	94 ± 5.7	77 ± 6.4
Arachidic (20:2)	91 ± 4.8[a]	86 ± 10[a]
Behenic (22:0)	96 ± 7.9	92 ± 12[a]
Pimaric	93 ± 6.2	101 ± 5.1
Sandaracopimaric	96 ± 7.9	105 ± 5.7
Isopimaric	93 ± 5.1	103 ± 5.3
Abietic	98 ± 5.2	90 ± 5.1
Dehydroabietic	101 ± 7.1	103 ± 6.0
14-Chlorodehydroabietic	95 ± 7.5	103 ± 5.7
12-Chlorodehydroabietic	96 ± 6.7	106 ± 5.7
12,14-Dichlorodehydroabietic	91 ± 7.0	102 ± 6.1

[a] After correction for sample blanks.

data presented in this table were obtained in the absence of levopimaric, palustric and neoabietic acids. The recoveries of palustric and neoabietic were low (between 15 and 25%), if they were also spiked to the sediment. At the same time, their recoveries could not be improved even if polytron extraction with a non-acidified solvent was performed at room temperature. Therefore, this method is only semi-quantitative for palustric and neoabietic acids and would have a high bias for abietic acid due to the conversion of palustric and neoabietic acids into the former. The present method was not applicable to levopimaric acid since it was never recovered from any fortified or naturally contaminated samples. The method detection limit, estimated to be 0.05 μg/g for the RFAs based on 1 g of sample, is sensitive enough for purposes such as environmental monitoring and toxicity evaluation.

Anhydrous sodium sulfate is often used for the removal of water in organic extracts of wet sediments. However, we found that a large number of fatty acids was present at high ng/g levels in analytical-reagent-grade anhydrous sodium sulfate from several suppliers (Fisher, BDH and Baker). The use of this adsorbent must therefore be avoided before the derivatization step of our procedure, and it should be substituted by air-drying of the sediment prior to extraction. Also, a method blank for the fatty acids should be run for each set of samples if the levels are lower than 1 μg/g.

Application to sediment samples

The present method has been applied to dozens of sediment samples collected in the vicinity of pulp and paper mill sites. The results of a few examples are given in Table II. Qualitatively, all RFAs except levopimaric acid were found in all or most sediment samples collected downstream of paper mills. Sample 1 was a river sediment collected from a site approximately 2 km upstream of a paper mill located in Ontario. Other than low levels of some native fatty acids and a trace amount of dehydroabietic acid, no other resin acids were found in this sample. Sample 2, collected from a site about 2 km downstream of the same paper mill, was quite heavily contaminated with RFAs, and the total amount of fatty and resin acids on dry weight basis were 58 and 211 μg/g, respectively. RFAs were still readily detected at a site about 5 km downstream of the same paper mill (sample 3). The presence of chlorinated dehydroabietic acids in samples 2 and 3 is consistent with the fact that this paper mill employs chlorobleaching of the pulps and that chloro- and dichlorodehydroabietic acids are formed by the chlorination of dehydroabietic acid under pulp bleaching conditions [16]. A GC–ECD profile of sample 2 is shown in Fig. 3. Sample 4 was a sludge sample collected from another pulp mill. This sample contained nearly 1 mg/g total resin acids and the concentration of dehydroabietic acid was over 400 μg/g.

Other acidic components in pulp mill sediments

Although this method was tested with the RFAs listed in Table I, the procedure is applicable to other fatty and resin acids occurring in pulp mill samples. Indeed, a large number of saturated and unsaturated fatty acids from C_6 to C_{24} were found in the sediment samples tested. Among them, the predominant ones were those saturated fatty acids from C_{16} to C_{24} with an even number of carbon atoms with the exception of *anteiso*-heptadecanoic (14-methylhexadecanoic) acid. Straight-chain fatty acids with an odd number of carbons such as pentadecylic (15:0), margaric (17:0) and tricosanoic (23:0) were also present in all samples, albeit at much lower concen-

TABLE II

LEVELS OF RESIN AND FATTY ACIDS IN SEDIMENTS COLLECTED FROM PULP MILL LOCATIONS

RFA	Concentration (μg/g dry weight)			
	Sample 1	Sample 2	Sample 3	Sample 4
Lauric (12:0)	0.77	1.44	1.27	6.83
Myristic (14:0)	0.85	2.87	1.53	11.72
Palmitic (16:0)	2.70	13.95	6.90	41.90
Stearic (18:0)	1.19	5.82	3.68	23.32
Oleic (18:1)	0.59	7.73	3.83	40.82
Linoleic (18:2)	<0.05	7.76	3.95	21.68
Linolenic (18:3)	<0.05	<0.05	<0.05	<0.05
Arachidic (20:0)	0.38	5.00	2.66	15.32
Behenic (22:0)	1.25	13.12	9.50	71.14
Pimaric	<0.05	11.62	6.84	72.02
Sandaracopimaric	<0.05	5.14	3.21	28.91
Isopimaric	<0.05	27.46	12.09	112.35
Palustric	<0.05	4.60	<0.05	60.73
Abietic	<0.05	12.38	5.18	87.47
Dehydroabietic	0.76	121.2	36.60	405.51
Neoabietic	<0.05	<0.05	<0.05	7.91
14-Chlorodehydroabietic	<0.05	9.45	5.74	10.65
12-Chlorodehydroabietic	<0.05	20.03	14.94	21.08
12,14-Dichlorodehydroabietic	<0.05	3.70	3.20	68.48
Total fatty acids	6.46	44.58	23.80	232.72
Total resin acids	0.76	210.98	87.78	875.09

trations. The major unsaturated fatty acids found were entirely straight-chained with an even carbon number and in the *cis* form, namely myristoleic (14:1), palmitoleic (16:1), eicosenoic (20:1), erucic (22:1) and nervonic (24:1) acids in addition to oleic (18:1) and linoleic (18:2) acids. Chlorinated stearic acids were not found in the sediments analysed in this study. A resin acid metabolite, 7-isopimarenoic (dihydroisopimaric) acid, resulting from the anaerobic reduction of isopimaric acid [17], was also found in a few sediments. Several other smaller peaks of m/z 301 and 303 observed in the NICI mass spectra of the derivatized extracts were presumably derived from other resin acids. Because of the lack of authentic standards in our laboratory, they could not be identified.

CONCLUSIONS

As demonstrated above, the analytical method described here is applicable to the determination of the major RFAs in sediments at the μg/g level. Presumably due to their stability and low water solubility, many RFAs could readily accumulate in river sediments. Indeed, high levels of the toxic RFAs were found in many sediment

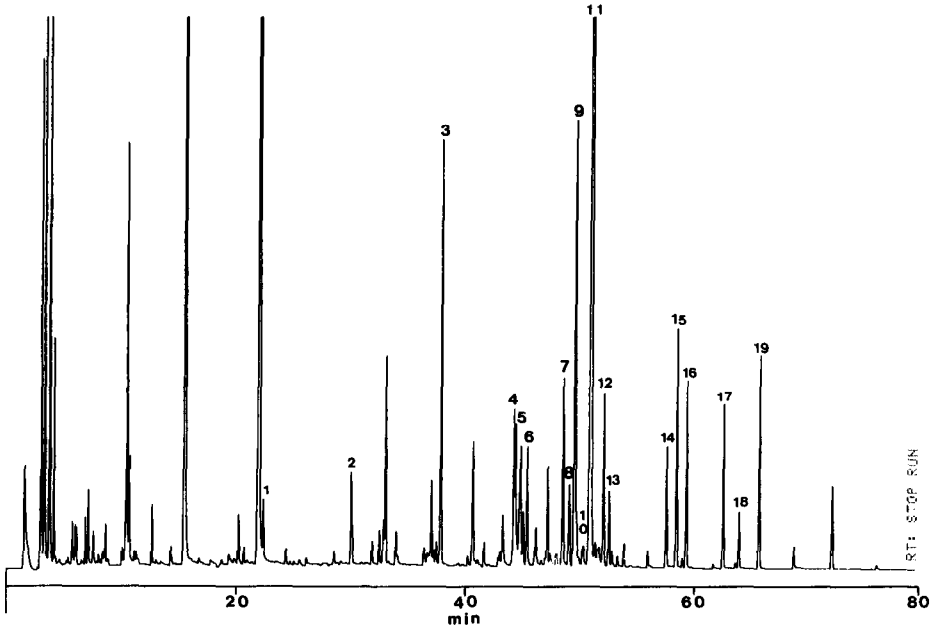

Fig. 3. GC–ECD profile of the PFB esters of resin and fatty acids in sediment sample 2 as chromatographed on a 30-m DB-5 column. Acids: 1 = lauric; 2 = myristic; 3 = palmitic; 4 = linoleic; 5 = oleic; 6 = stearic; 7 = pimaric; 8 = sandaracopimaric; 9 = isopimaric; 10 = palustric; 11 = dehydroabietic; 12 = abietic; 13 = arachidic; 14 = 14-chlorodehydroabietic; 15 = 12-chlorodehydroabietic; 16 = behenic; 17 = tricosanoic; 18 = dichlorodehydroabietic; 19 = lignoceric.

samples collected downstream of pulp and paper mills. Therefore, regular monitoring of RFAs in such locations is a high priority for the protection of fish and the aquatic environment.

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