39

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FISHERIES AND MARINE SERVICE
SERVICE DES PÊCHES ET DES SCIENCES DE LA MER

TECHNICAL REPORT No.
RAPPORT TECHNIQUE N° 465

1974

Environment Canada
Fisheries and Marine Service

Environnement Canada
Service des pêches et des sciences de la mer

Department of the Environment
Fisheries and Marine Service
Research and Development Directorate

Ministère de l'Environnement
Service des Pêches et des Sciences de la mer
Direction de la Recherche et Développement

TECHNICAL REPORT No. 465

RAPPORT TECHNIQUE N^{O}. 465

(Numbers 1-456 in this series were issued as Technical Reports of the Fisheries Research Board of Canada. The series name was changed with report number 457).

(Les numéros 1-456 dans cette série furent utilisés comme Rapports Techniques de l'office des recherches sur les pêcheries du Canada Le nom de la série fut changé avec le rapport numéro 457)

Organochlorine compounds in kraft bleaching wastes. Identification of two chlorinated guaiacols.

by I.H. Rogers, and L.H. Keith

This is the thirteenth
Technical Report from the
Research and Development Directorate
Pacific Environment Institute
West Vancouver, B.C.

Ceci est le treizième
Rapport Technique de la Direction de la
Recherche et Développement
Institut de l'environnement du Pacifique
Vancouver-Ouest

1974

TABLE OF CONTENTS

"This report represents a cooperative project. Dr. I. H. Rogers, Environment Canada, Pacific Environment Institute, is the principal investigator and directed sampling, isolation of toxic materials, and toxicity studies. Dr. L. H. Keith, U.S. Environmental Protection Agency, Southeast Environmental Research Laboratory, directed the spectral analysis and the chlorinated guaiacol synthesis".

Rogers, I.H., and L.H. Keith. 1974. Organochlorine compounds in kraft bleaching wastes.Identification of two chlorinated guaiacols. Fish. Mar. Ser. Res. Dev. Tech. Rep. 465:

ABSTRACT

Gas chromatographic analysis of several fish-toxic bleachery waste samples from two British Columbia kraft pulp mills - one Interior and the other Coastal - consistently revealed the presence of a pair of organochlorine compounds. Analysis by gas chromatography-mass spectrometry (GC-MS) of the methyl ether derivatives identified these as trichloro - and tetrachloroveratrole (I_a or II and III_a). Chlorination of guaiacol *in vitro*, under conditions similar to those used in the mill, followed by methylation yielded a complex mixture of products which was separated by preparative GC. On the basis of GC retention time and GC-MS the trichloroveratrole isolated was shown identical with that in the effluent. On the basis of NMR evidence it was ascribed the structure 1, 2 - dimethoxy - 3, 4, 5 - trichlorobenzene (I_a). Ethylation of a sample of the original isolate, followed by further GC-MS analysis, indicated that the parent compounds were chlorinated guaiacols rather than catechols. They are probably derived from lignin residues present in the unbleached pulp via oxidative cleavage, and their contribution to the toxicity of bleach plant effluent is currently unknown.

RÉSUMÉ

L'analyse par chromatographie en phase gazeuse de plusieurs échantillons de rejets d'usines de blanchiment de deux fabriques de pâte kraft de le Colombie Britannique (l'une de l'intérieur, l'autre du littoral), qui étaient toxiques pour le poisson, y a révélé de façon sûre la présence d'une paire de composés organochlorés. L'analyse par chromatographie en phase gazeuse et par spectrographie de masse des dérivés d'éthers méthyliques a permis de les identifier comme étant le trichloro et le tétrachlorovératrole (I_a ou II et III_a). Une chloration en laboratoire, aux conditions identique à celles qui prévalaient dans la fabrique, suivie par une méthylation, a donné un mélange compliqué de produits qu'on a par la suite isolés par chromatographie en phase gazeuse. À partir de la durée de rétention par le chromatographe en phase gazeuse et des données de chromatographie en phase gazeuse et de spectrographie de masse, on a montré que le trichlorovératrole isolé était identique à celui de l'efflu-

ent. Au moyen de la r.m.n. on lui a attribué la structure du diméthoxy-1,2 trichloro-3,4,5 benzène (1_a). L'éthylation d'un échantillon du produit initialement isolé, suivie d'une analyse par chromatographie de masse, a montré que les composés mères étaient des guaiacols chlorés plutôt que des catéchols. Ces composés proviennent probablement des résidus de lignine présents dans la pâte non blanchie et clivés par oxydation; leur contribution à la toxicité de l'effluent de l'usine de blanchiment est encore inconnue.

INTRODUCTION

The largest volume industrial contribution of dissolved organic compounds to receiving waters in British Columbia is that emitted by the pulp and paper industry. Of 21 mills in the province, two use the sulphite process, one produces only mechanical pulp and the remainder are kraft mills, some of which also produce mechanical pulp. Fully bleached pulp is manufactured in part or in toto by 17 out of 18 kraft mills and any organochlorine compounds these mills release to the receiving waters may have an impact on the fishery resources, resulting either from toxicity to aquatic organisms or from tainting of the flesh of commercial fish stocks. Although fisheries biologists have studied the toxicity of kraft mill effluents and of the various sewers within the mill for several years, the chemical complexity and variability of these effluents has defied specific chemical characterization until recently. However, within the past three years progress has been made in the recovery of fish-toxic organic compounds from samples of fully bleached kraft mill effluent (BKME) using Amberlite XAD-2 and XAD-7 synthetic resins.[1] The toxic compounds identified to date by GC-MS analysis are wood extractives formed in the living tree and surviving the kraft pulping operation. They are resin acids and unsaturated fatty acids (Rogers 1973; Leach and Thakore 1973; Maenpaa et al 1971; Rogers and Mahood, MS, 1974) and a mixture of diterpene alcohols and aldehydes closely related structurally to the resin acids in the case of mills pulping lodgepole pine (*Pinus contorta* Dougl. var.

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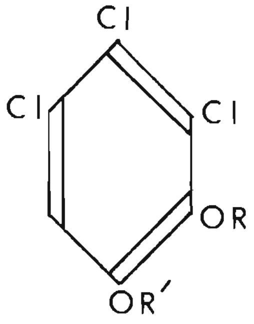
[1] manufactured by Rohm and Haas Inc.

latifolia Engelm) (Rogers and Mahood MS, 1974). The only organochlorine compound identified in the above studies was a trichloroveratrole found in the resin acid fraction of an untreated sample of BKME from the Weyerhaeuser Canada Ltd. mill at Kamloops, B.C. (Rogers 1973). This compound was present either as a trichloroguaiacol or as a trichlorocatechol in the original effluent and was then converted by diazomethane into its methyl ether derivative in the process of methylating the resin acid fraction for GC analysis. This derivative (a trichloroveratrole) may have had either structure I_a or II.



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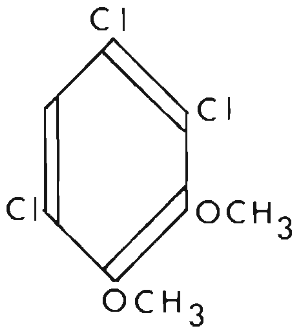
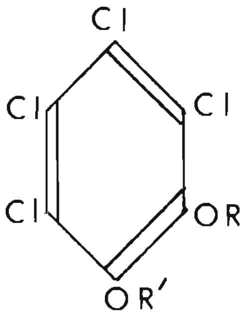
b $R = CH_3; R' = H$
c $R = H; R' = CH_3$



II



III a $R = R' = CH_3$
b $R = CH_3; R' = H$

There seem to be no reports on the toxicity of chlorinated guaiacols to salmonid species. However, the toxicity of dichoro - and tetrachlorocatechol to juvenile sockeye salmon (*Oncorhynchus nerka)* has been reported (Servizi et al.1968). This study was made on the theory that such compounds may be formed from lignin during bleaching with chlorine, as seemed likely from studies on model compounds (Dence and Sarkanen, 1960). Tetrachloro-o-benzoquinone has been identified indirectly in bleached kraft chlorination effluent and is toxic to Atlantic salmon (*Salmo salar)*

(Das *et al*. 1969).

The Rohm and Haas Company has developed a process for colour removal from bleachery wastes using Amberlite XAD-8 synthetic resin (Kennedy and Minor, 1972). We were interested in the efficiency of this process and used it to treat some samples of bleach plant effluent from a B.C. Interior mill (mill A). Subsequent analysis of the extract eluted from the synthetic resin showed the presence of two chlorinated compounds. We now describe the identification of these compounds and attempt to explain their mode of formation.

Glossary of Terms

GC-MS	Combination of a gas chromatograph interfaced with a mass spectrometer.
CRGC	Computer reconstructed gas chromatogram. A plot of summation ion current versus spectrum number over the period of time required to separate components of the extract.
LMRGC	Limited mass reconstructed gas chromatogram. The computer is commanded to scan the mass spectra obtained during real time analysis for certain ions. A plot of ion intensity versus spectrum number indicates which spectra contain these ions.
FID	Flame ionization detector for a gas chromatograph.
EC	Electron capture detector for a gas chromatograph.
SCOT	Support coated open tubular column for a gas chromatograph.
FT-IR	Fourier transform infrared spectroscopy.

EXPERIMENTAL

Static bioassays

Ten sockeye salmon fingerlings (presmolts) were placed in 30 litres of effluent from mill A in a glass aquarium. Oxygen content of the water was maintained at saturation throughout by means of an airstone and the water temperature kept at 45^{0}F. Fish density varied in the range 0.24 - 0.7 gm/l and mortality was recorded at 24 hour intervals until the end of the test at 96 hours. Controls were run in fresh Cultus Lake water with a hardness of 85 mg/l as $CaCO_3$. Effluent samples which had been processed through a bed of Amberlite XAD-8 synthetic resin were bioassayed under identical conditions.

Similar tests were performed in another laboratory on effluent from mill B. In this case five coho salmon (*Onchorhynchus kisutch)* underyearlings were used per test. Fish density was 0.45 gm/l and fresh tap water, hardness 12, was used in the control. Tests were run in glass aquaria at concentrations of 100%, 56%, 32%, 18% and 10% of full strength effluent. Plots of logarithmic concentration versus 50% mortality allowed an estimation of the 96 hr LC_{50} value for each effluent sample.

Recovery of toxic fractions

Samples from the caustic extraction and chlorination sewers of mill A were shipped to the laboratory. Chlorination (acid) effluent was titrated into an aliquot of caustic extraction effluent until pH 2.9 was reached because colour removal with Amberlite XAD-8 resin is most efficient in the pH range 2.5 to 3.0. From the volume ratio so determined, 5 Imperial gallons of caustic extraction effluent was blended with the appropriate volume of acid effluent. The sample was split into two equal volumes. One was neutralized with sodium hydroxide solution and used for bioassay purposes. The other was percolated through a bed of clean Amberlite XAD-8 resin (36 in X 1.725 in) at a rate of 70 ml/min. The eluate was resubmitted for bioassay and the resin bed regenerated by elution with methanol (3 1). The solvent was removed in a rotary evaporator and the black residue dissolved in water. After acidification with sulfuric acid to pH 2.0, the product was extracted three times with ether and an insoluble brown solid recovered by filtration. The ether-soluble portion of the extract was methylated with diazomethane for GC-MS analysis.

Samples from mill B comprised caustic extraction effluent only and were examined originally as part of another study. After acidification to pH<4 by the addition of sulphuric acid, 500 ml aliquots were extracted with ether (4 X 100 ml) in separatory funnels. The extracts were dried over anhydrous magnesium sulphate and the solvent removed as above. Bioassays were conducted on neutralized samples of caustic extraction effluent but not on the aliquots after ether extraction.

Additional samples from mill B were processed at a later date by adjusting the pH to 2.0 and passing them through Amberlite XAD-8 resin as previously described.

Analysis of extracts by gas chromatography

Toxic extracts were analysed before and after methylation with diazomethane on Hewlett Packard Model 5700 A instruments, one equipped with a flame ionization detector (FID) and the other with an electron capture detector (EC). For FID work a 6 ft X 1/8 in. stainless steel column packed with 3% OV-17 on 80-100 mesh Chromosorb W was used; the temperature was programmed from 150^{0} to 250^{0} at 4^{0}C/min. Nitrogen was used as carrier gas at 60 c.c./min. flow rate. In the EC mode a 3 ft X 1/4 in. glass column packed with 10% OV-1 on high performance Chromosorb W (80 - 100 mesh) was used isothermally at 250^{0}C. Carrier gas was argon/methane at 60 c.c./min flow rate.

Analysis of extracts by GC-MS

One methylated sample from each mill was analysed in a Finnegan 1015 quadrupole mass spectrometer interfaced with a modified Varian 1400 gas chromatograph. Separation was accomplished using a 50 ft SCOT column coated with OV-17 and temperature programmed from 150^{0} to 220^{0}C at 2^{0}/min. Data collection and output was controlled by a System 150 interface and a pdp 8/e computer having associated magnetic disc and magnetic tape units. Data output from the computer was via either a Houston plotter or a Tektronix R-4012 interactive graphics terminal with associated Digital Equipment Corporation pdp 8/m computer and Tektronix hard copy unit.

A further portion of the extract from mill A was ethylated with diazoethane and analysed by GC-MS as before.

Analysis of extracts by n.m.r.

Nuclear magnetic resonance spectra were recorded with a Varian HA-100 n.m.r. spectrometer, using a 2-4% solution of tetramethylsilane (TMS) as internal standard and carbon tetrachloride as solvent. Spectra were recorded in the frequency-sweep mode with a sweep time of 500 sec. and a sweep width of 1,000 Hz. Chemical shifts, expressed as τ values, were measured with a frequency counter and are accurate to $\pm$ 1.0 Hz.

Analysis of extracts by FT-IR

Fourier-transform infrared spectra were recorded with a Digilab FTS-14D/IR spectrometer equipped with the Digilab GC/IR accessory and controller. The instrument is connected with a Perkin-Elmer 990 gas chromatograph via a Wilks heated transfer line. The gas chromatograph is equipped with a flame ionization detector and a 14:1 splitter. The IR cell is a cylindrical cavity, 6 X 50 mm, bored through a heated metal block with KBr windows sealed to each end of the cavity.

Chlorination of guaiacol

Chlorine gas was slowly bubbled into a solution of guaiacol (3.9 g) in distilled water (800 ml) at room temperature in a 3-neck, 1-l flask equipped with stirring bar, a gas inlet and a gas outlet until a pH of 1.9 was reached. After 2 hours nitrogen was bubbled into the solution to expel unreacted chlorine. Half the product (400 ml) was heated in a water bath, while stirring, to approximately 70^oC and 50% NaOH solution added to raise the pH to 10.0 - 10.5. Dimethyl sulphate (90 ml) was added with vigorous stirring over a two-hour period. The methylated guaiacol was allowed to cool to 40^oC before adding 5 ml of conc. NH_4OH to destroy excess dimethyl sulphate. The sample was then extracted with chloroform (4 x 200 ml) and the extract was concentrated in a Kuderna-Danish apparatus to 8 ml.

A 3 ml-portion of the methylated sample was subjected to column chromatography on silica get (70 ml volume). Five 100 ml fractions were eluted: #1 and #2, benzene; #3, benzene - 1% methanol; #4 benzene - 3% methanol; #5, benzene - 5% methanol. All but one of the chlorinated monomers of the guaiacol methyl ethers (chloroveratroles) were in the first fraction. One isomer, subsequently identified as 3,6-dichloroveratrole, was isolated (with about 25% unidentified impurities) as fraction #3.

Another portion of the sample was chromatographed on a Perkin-Elmer-900 gas chromatograph with a 10:1 splitter using a 9 ft X 1/4 inch preparative glass column packed with 3% SE-30 on Gas Chrom Q. The first seven peaks, previously determined by GC-MS to be chlorinated monomers of veratrole, were separately collected in glass capillary tubes. Each capillary tube was fitted with a small plug of prewashed glass wool and was cooled with a piece of tissue paper soaked in methylene chloride and draped over the tube. Repeated injections provided enough sample for Fourier-transform infrared (FT-IR) spectra of all 7 compounds and nuclear magnetic resonance (n.m.r.) spectra of 5 of them. Each compound was washed from its capillary tube with 25 microliter aliquots of carbon tetrachloride, a solvent suitable for both infrared and n.m.r. analyses.

Analysis of ether-insoluble fractions

Sodium fusions were performed on the brown ether-insoluble portions of the Amberlite XAD-8 methanol extracts from both mills and standard tests performed for the presence of organochlorine species. Infrared spectra were recorded on a Perkin Elmer Model 467 spectrophotometer using nujol mulls prepared from these insoluble fractions.

RESULTS

Seven samples of effluent from mill A were processed and all were toxic to sockeye salmon. In all cases mortality was complete on the first day at 100% concentration. Because the effect of dilution on toxicity was not studied, LC_{50} values cannot be estimated. After passage through the Amberlite XAD-8 resin bed, four of the samples were non-toxic at 100% concentration and fish mortalities of 10%, 40% and 70% were observed in the other three cases. The EC gas chromatographic traces of the methylated ether extracts of all seven samples contained one large pair of peaks with matching retention times. Other small peaks, varying in number from three to nine, were also present. No significant peaks were present when the extracts were analysed prior to methylation. The pair of peaks was easily detectable also in the FID mode but appeared small in comparison with other components of the extract; this indicated they were chlorinated compounds,

Four samples of caustic extraction effluent from mill B also showed the identical pair of peaks in the EC mode with a number of minor components also present. In the FID charts, the chlorinated compounds were more prominent relative to other components than was the case with the mill A samples. All four samples were toxic and yielded 96 hr LC_{50} values of 10% or lower to coho salmon fingerlings. Bioassay data on these samples are given in Table 1.

Analysis of one of the extracts from mill A by GC-MS with computer spectral matching yielded the results summarized on the computer reconstructed gas chromatogram (CRGC) in Figure 1. A total of 20 compounds were observed of which seven were identified. In the other cases,spectra of unresolved components were obtained or the spectra were too weak for positive identification. The mass spectrum of each component was printed out by the computer and that corresponding to the organochlorine component with the shorter retention time is shown in Figure 2. The CRGC for a sample from mill B together with successful identifications is shown in Figure 3. In this case, 12 compounds out of a total of about 40 were identified.

An extract of mill A was ethylated with diazomethane and a shift to longer retention time noted for both organochlorine peaks in the EC mode. The output from GC-MS analysis of this sample is described in the following section.

A sample of guaiacol, which had been chlorinated and then methylated, was analysed by GC-MS. The CRGC of this reaction product is shown in Figure 4. Components were tentatively identified from their mass and infrared spectra as indicated. A search for the presence of free guaiacol in a sample of wet, unbleached pulp from mill A yielded negative results.

The brown insoluble fractions from both mills gave strongly positive organochlorine tests on sodium fusion analysis and strong absorption bands were observed at 725 cm^{-1} in the infrared spectra.

DISCUSSION

Bioassay results on the samples from the two mills studied are not directly comparable because two different salmonid species were used, fish density and water hardness values were different and the bioassay procedures were not standardized. Moreover, in the case of mill B, only the caustic extraction effluent was studied, whereas both caustic extraction and chlorination effluent from mill A were treated. All that can be said is that these bleachery wastes were of significant toxicity to two salmonid species. Also a marked toxicity reduction was noted to coincide with colour removal after passage through Amberlite XAD-8 resin.

The consistent presence of the two organochlorine compounds in toxic bleachery wastes from two kraft mills, so widely separated geographically, implies that these waste products may be of rather common occurrence in mills pulping softwoods and using the traditional chlorination and caustic extraction steps as the first two stages in the bleach sequence. We cannot say accurately what the concentration of these substances was in the original samples until the pure compounds themselves are available for the estimation of response factors. However, from the known sensitivity of the gas chromatograph in the FID mode, it appears likely that concentrations of 10 - 20 ppb were encountered.

From the mass spectra of the two organochlorine compounds as their methyl ethers, it was possible to deduce from the distribution of chlorine isotopes in the parent ion cluster that one compound contains three chlorine atoms per molecule and the other four. It was also deduced that the tetrachloro compound differs from the trichloro compound only by the substitution of an extra chlorine atom and that both compounds are aromatic. Moreover, the spectrum of the methyl ether of the trichloro compound (Figure 2) was identical with that of trichloroveratrole identified previously (Rogers, 1973) and the structural formula may be either I_a or II.

Selection of the correct structure was accomplished by chlorination of guaiacol at room temperature under conditions approaching as closely as possible those used in the mill. The result was the complex mixture of compounds shown in Figure 4. Besides unreacted guaiacol, two monochloro-, four dichloro- and one trichloro-derivative were present but the tetrachloro compound was apparently not formed. At least ten dimeric products were formed in the reaction.

Structural assignments were made to veratrole and to the seven

Chlorinated isomers of veratrole solely on the basis of spectroscopic evidence as follows:

Peak (a) was shown by its mass spectral fragmentation pattern and molecular ion at m/e 138 to be veratrole (IV). This was confirmed by comparison of its mass spectrum to that of a standard.

Peaks (b) and (c) had essentially identical mass spectra with molecular ions at m/e 172 and a chlorine isotope distribution pattern indicative of only one chlorine atom. Differentiation between the two structures of monochloroveratrole (V and VI) was made on the basis of Fourier-transform infrared (FT-IR) interpretation. Peak (c) (VI) exhibited an absorption band at 880 cm^{-1} indicative of lone hydrogen C-H out-of-plane deformation. Since only 4-chloroveratrole has a lone hydrogen this structure was assigned to peak (c) and, by elimination, peak (b) is 3-chloroveratrole (structure V).

Peaks (d), (e + f) and (g) all have essentially identical mass spectra with molecular ions at m/e 206 and a chlorine isotope distribution pattern indicative of two chlorine atoms.

The FT-IR spectrum of peak (g) contained an absorption band at 932 cm^{-1} that, although at higher frequency than usual, was indicative of lone hydrogens on the benzene ring. The n.m.r. spectrum showed only one signal for the magnetically equivalent methoxyl protons (τ 6.22) and only one sharp signal for the two aromatic protons (τ 3.19). Although both 4, 5-dichloroveratrole (X) and 3, 6-dichloroveratrole (IX) possess a plane of symmetry that would cause degeneracy of the methoxyl and aromatic proton signals, the relatively small downfield shift (2 Hz) of the methoxyl signal from its chemical shift in veratrole (τ 6.24) suggests that the chlorine atoms are remote from the methoxyl. This condition, together with the IR indication of lone hydrogens, is best met by the structure of 4, 5-dichloroveratrole (X).

The FT-IR spectrum of peak (d) also shows an absorption band indicative of lone hydrogens on the benzene ring (898 cm^{-1}). Because 3, 5-dichloroveratrole (VII) is the only other structure among the four dichloroveratrole isomers to contain lone hydrogens, this structure was assigned to peak (d). Not enough sample was obtained for n.m.r. analysis.

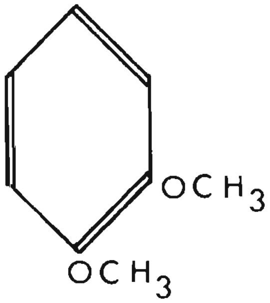
Peak (f), isolated from fraction #3 of the silica gel column, also showed a degeneracy of the methoxyl protons in its n.m.r. spectrum. The strongly deshielded singlet at τ 6.13 also indicated a plane of symmetry as well as <u>ortho</u> substituted chlorine atoms. The structure of 3, 6-dichloroveratrole (IX) meets these requirements.

Peak (e), the only remaining dichloro isomer of veratrole, must, by elimination, be 3, 4-dichloroveratrole (VIII).

The mass spectrum of peak (h) shows a molecular ion at m/e 240 and a chlorine isotope pattern characteristic of 3 chlorine atoms. Although there are two possible isomers of trichloroveratrole, only one (peak h) was found. The FT-IR spectrum of this compound revealed, of course, a lone hydrogen absorption band (930 cm^{-1}). The n.m.r. spectrum showed two signals for the methoxyl protons (τ 6.22 and 6.16). These chemical shifts are close to the predicted values (6.20 and 6.16), based on the additivity of substituent effects, for the chemical shifts of the two methoxyls in 3, 4, 5-trichloroveratrole (Ia). The predicted chemical shifts for the methoxyls in the other possible isomer, 3, 5, 6-trichloroveratrole (II), are 6.11 and 6.13. peak (h) was thus assigned structure Ia on the basis of the close correlation of the methoxyl chemical shifts.

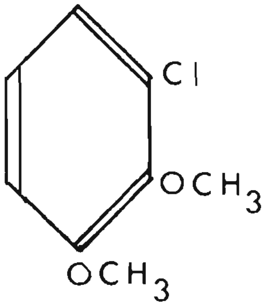
Peak (h), 3, 4, 5-trichloroveratrole, was shown by gas chromatographic retention times on a Carbowax 20M/TPA 50 ft. support coated open tubular (SCOT) column to be identical with the chlorinated trichloroveratrole in the pulp mill wastewater extracts (Peak 5, Figure 1, and Peak 2, Figure 3). As previously mentioned, the GC-MS spectra were also identical.

Individual structural assignments to the isomeric dimers were not attempted. Until assignments to the seven chlorinated isomers of veratrole are confirmed by comparison of at least two physical properties (e.g. mass, nuclear magnetic resonance or infrared spectrum, gas chromatographic retention time, etc.) of these isomers with the corresponding physical properties of their standards, the identifications are considered tentative.



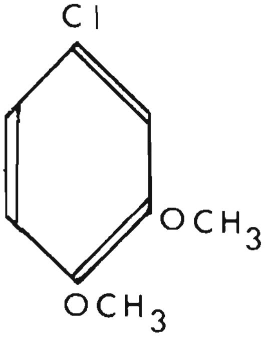
IV

Peak a



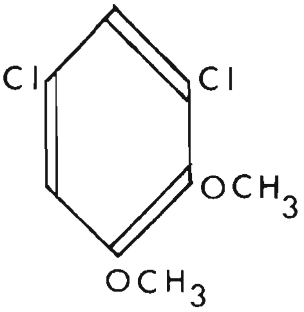
V

Peak b



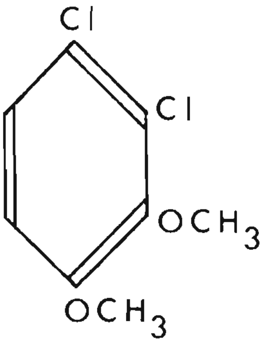
VI

Peak c



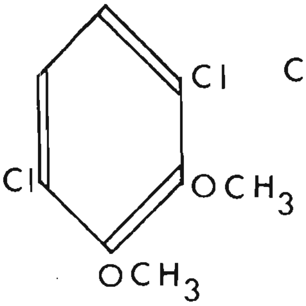
VII

Peak d



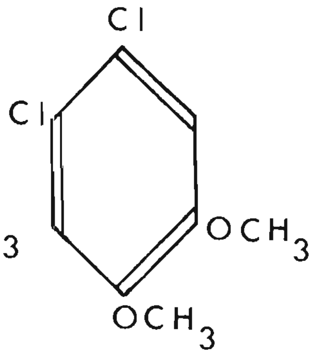
VIII

Peak e



IX

Peak f



X

Peak g

The question of whether these organochlorine compounds are present as chlorinated guaiacols or chlorinated catechols in the mill waste waters was resolved by ethylation of a sample of the original pulp mill extract. Analysis of the ethylated sample was most informative. The spectra of both chlorinated compounds indicated an increase in molecular weight of 14, relative to the methyl ester, corresponding to the addition of only one ethyl group. This means that the parent compound did not contain two hydroxyl groups (as in catechol), but one hydroxyl and one methoxyl group (guaiacol). Fragment ions corresponding to loss of a methyl group (M-15) from the methyl ethers were replaced by M-28 ions in the ethyl ethers. These correspond to loss of ethylene from the parent ions.

A more elegant demonstration of the above results was achieved when the computer searched through the total GC-MS scan of the ethylated sample for the ions m/e 254 (parent ion of methoxyethoxy trichlorobenzene) and m/e 290 (larger fragment of the parent ion envelope of methoxyethoxy tetrachlorobenzene). The resulting LMRGC is shown in Figure 5. Clearly both compounds were present. The computer then searched for ions at m/e 268 (diethoxytrichlorobenzene) and at m/e 302 (diethoxytetrachlorobenzene). This LMRGC is shown in Figure 6. Clearly the tetrachloro derivative was not present. The erratic plot for ions at m/e 268 indicated traces of this ion in other components of the extract. Thus spectra number 168 and 305 were identified as a fatty acid ethyl ester and a hydrocarbon, respectively. Spectrum number 176 is that of the tetrachloro compound already identified (cf Figure 5).

The shoulder observed at spectrum number 152 in Figure 5 indicates coexistence in the pulp mill extracts of traces of both structural isomers of trichloroguaiacol (Ib and Ic). This shoulder was also present in LMRGC's of m/e (M-C_2H_4) and m/e 211 (base peak). Moreover, the mass spectrum observed for the shoulder at number 152 was very similar to that of the more plentiful trichloro isomer at number 147.

On the basis of the evidence presented, we identify the trichloro compounds as trichloroguaiacol isomers Ib and Ic and the tetrachloro compound as tetrachloroguaiacol (III b). It was not possible to determine spectroscopically which trichloroguaiacol isomer was present in largest quantity.

Compound III b has been isolated previously (Dence and Sarkanen, 1960) from the nonaqueous chlorination of spruce lignin under forcing conditions (glacial acetic acid, 100-110^{o}C, 50 min.). Because evaporator condensate water is used to wash the brown stock, and frequently contains significant amounts of free guaiacol, we examined some of the wet brown stock from mill A but failed to find any trace of guaiacol.

Further doubt that the two chlorinated compounds are derived from this substance arises from the fact that direct chlorination of guaiacol, under conditions similar to those used in the bleach plant, failed to produce tetrachloroguaiacol. Moreover, the numerous dimeric compounds and the various monochloro- and dichloroguaiacols, produced in the chlorination experiment, were not observed in the extracts from the bleach plant wastes.

Compounds Ib, Ic and IIIb are believed to originate from oxidative cleavage of lignin still attached to the cellulose in the brown stock. This mechanism is in keeping with the work of Dence and Sarkanen (1960) who demonstrated cleavage of aromatic side chains and their replacement by chlorine atoms in work with model compounds.

Finally it is of interest to consider the question of toxicity of the bleachery effuent samples examined. Of the compounds identified in Figure 1, arising from mill A, none except the two chlorinated species are expected to be toxic to fish. The compounds identified in Figure 3, arising from mill B, also comprise mainly non-toxic saturated fatty acids but include traces of two toxic resin acids and the two chlorinated substances. It appears highly unlikely that these identified compounds in the concentrations present can explain the high toxicity of the effluents from either mill, and we believe that a considerable amount of toxicity is associated with clorinated lignin or other high molecular weight species present in the extracts. Such material would not emerge from the column in the gas chromatograph and is therefore not detected. The presence of organochlorine compounds in the brown ether-insoluble fractions from the methanol eluates of the XAD-8 resin bed was demonstrated by elemental analysis and infrared spectroscopy. Investigation of the chemical composition and molecular weight range of the species present awaits the delivery of a high pressure liquid chromatograph.

ACKNOWLEDGEMENTS

We thank Messrs. Harold Mahood and Ray Batchelor for technical assistance, Dr. J. Servizi and Mr. R. Gordon of the International Pacific Salmon Fisheries Commission for bioassays on mill A and Mr. R. Watts of the Environment Protection Service for those relating to mill B. We also thank Dr. Leo Azarraga, Mrs. Ann McCall, Messrs Mike Carter, Terry Floyd and Mrs. Ann Alford of the Southeast Environmental Research Laboratory for obtaining the GC-MS and FT-IR spectra. We acknowledge helpful discussions with Dr. R. Claeys and Mr. A. Caron of NCASI, Western Regional Centre, Corvallis, Oregon.

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Table 1; Mortality of coho salmon exposed to mill B effluent samples

Sample number	% concentration	% mortality Observation Time hr.				
		14	24	48	72	96
1	32	-	100	100	100	100
	18	20	80	100	100	100
	10	0	0	20	20	20
2	32	100	100	100	100	100
	18	-	100	100	100	100
	10	-	0	0	0	0
3	32	-	80	100	100	100
	18	-	0	60	80	80
	10	-	0	0	0	0
4	32	20	40	100	100	100
	10	0	0	20	20	20
	5.6	0	0	0	0	0

CAPTIONS FOR FIGURES

Figure 1. Compounds identified in mixed bleachery effluent from mill A. 1. methyl laurate. 2. methyl 2-methyldodecanoate. 3. methyl myristate. 4. methyl 2- methyltetradecanoate. 5. trichloroveratrole. 6. tetrachloroveratrole. 7,8. lignin degradation products. 9. methyl palmitate. 10. branched chain docosane. 11. normal docosane.

Figure 2. Mass spectrum of 3, 4, 5 - trichloroveratrole.

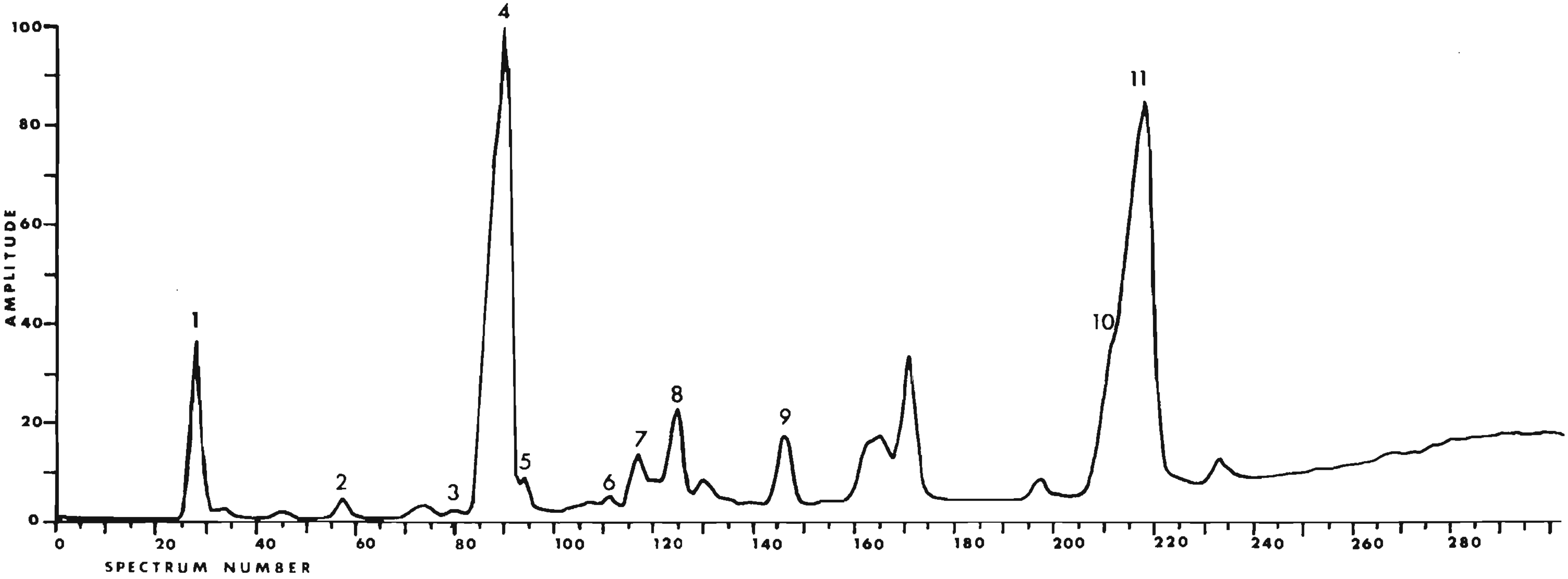
Figure 3. Compounds identified in caustic extraction effluent from mill B. 1. methyl myristate. 2. trichloroveratrole 3. methyl isopentadecanoate. 4. Tetrachloroveratrole. 5. methyl isopalmitate. 6. methyl palmitate. 7. methyl margarate. 8. methyl stearate. 9. methyl arachidate. 10. methyl dehydroabietate. 11. methyl behenate 12. methyl neoabietate.

Figure 4. Products from chlorination of guaiacol followed by methylation: (a) veratrole; (b) 3-chloroveratrole; (c) 4-chloroveratrole; (d) 3,5 - dichloroveratrole; (e) 3,4 - dichloroveratrole; (f) 3,6 - dichloroveratrole; (g) 4,5 - dichloroveratrole; (h) 3,4,5 - trichloroveratrole.

Figure 5. LMRGC's indicating the uptake by each compound of one ethyl ether group.

Figure 6. LMRGC's indicating absence of compounds containing two ethyl ether groups.

FIGURE 1



AMPLITUDE
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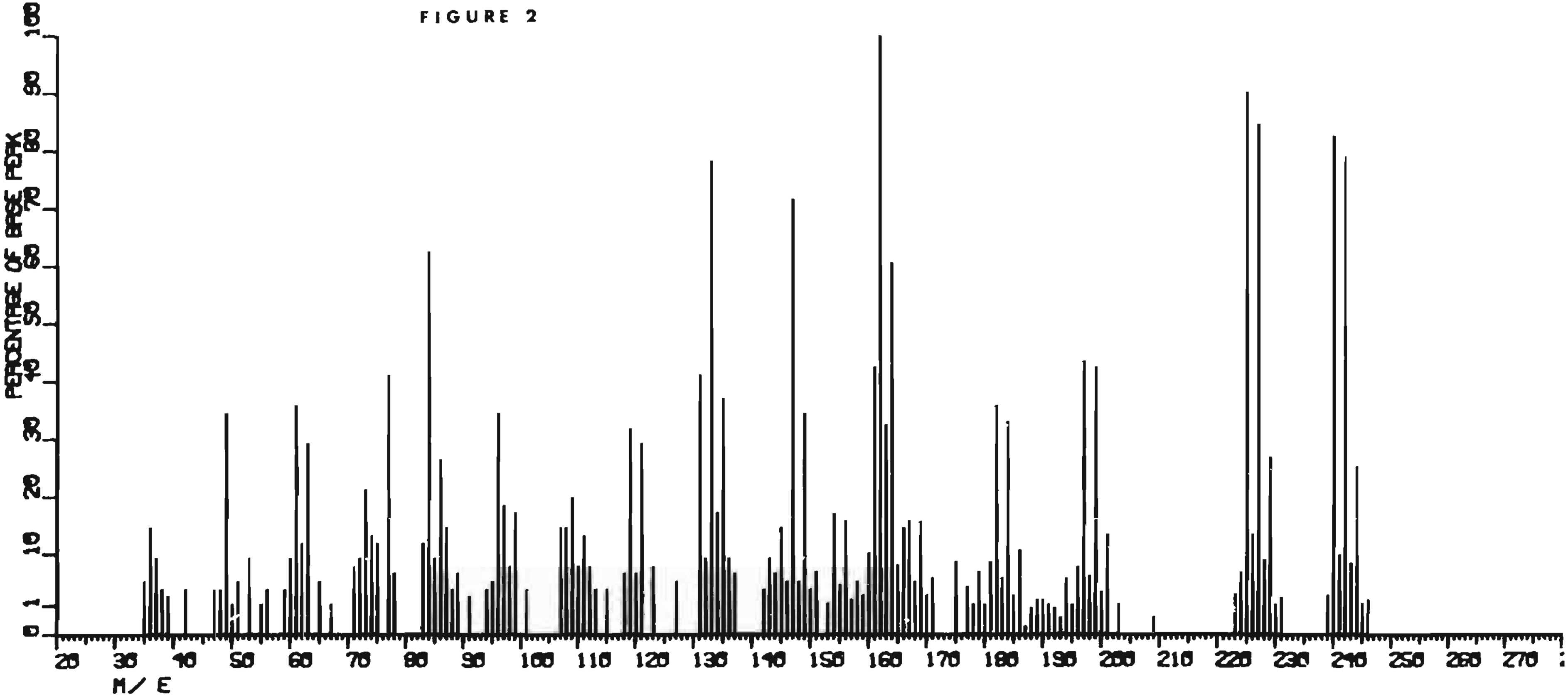
80
60
40
20
0
SPECTRUM NUMBER
0
20
40
60
80
100
120
140
160
180
200
220
240
260
280
1
2
3
4
5
6
7
8
9
10
11

FIGURE 2


PERCENTAGE OF BASE PEAK
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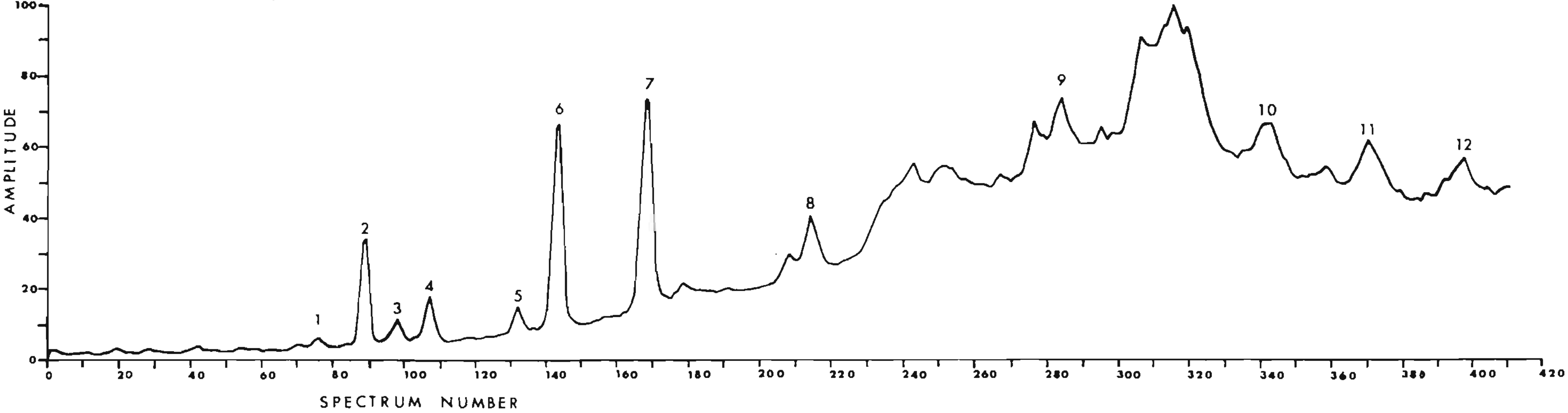
90
80
70
60
50
40
30
20
10
0
20
30
40
50
60
70
80
90
100
110
120
130
140
150
160
170
180
190
200
210
220
230
240
250
260
270
M/ E

FIGURE 3



AMPLITUDE
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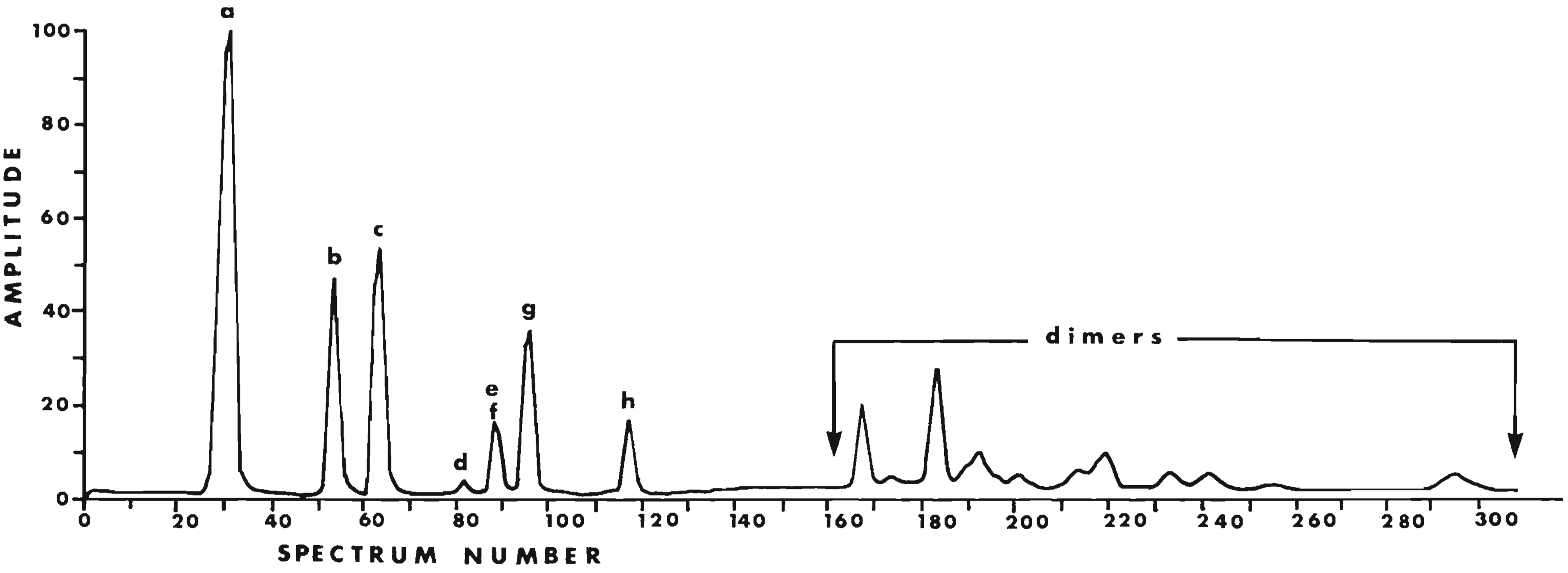
2
3
4
5
6
7
8
9
10
11
12
0
20
40
60
80
100
120
140
160
180
200
220
240
260
280
300
320
340
360
380
400
420
SPECTRUM NUMBER

FIGURE 4



a
b
c
d
e
f
g
h
dimers
AMPLITUDE
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60
80
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SPECTRUM NUMBER
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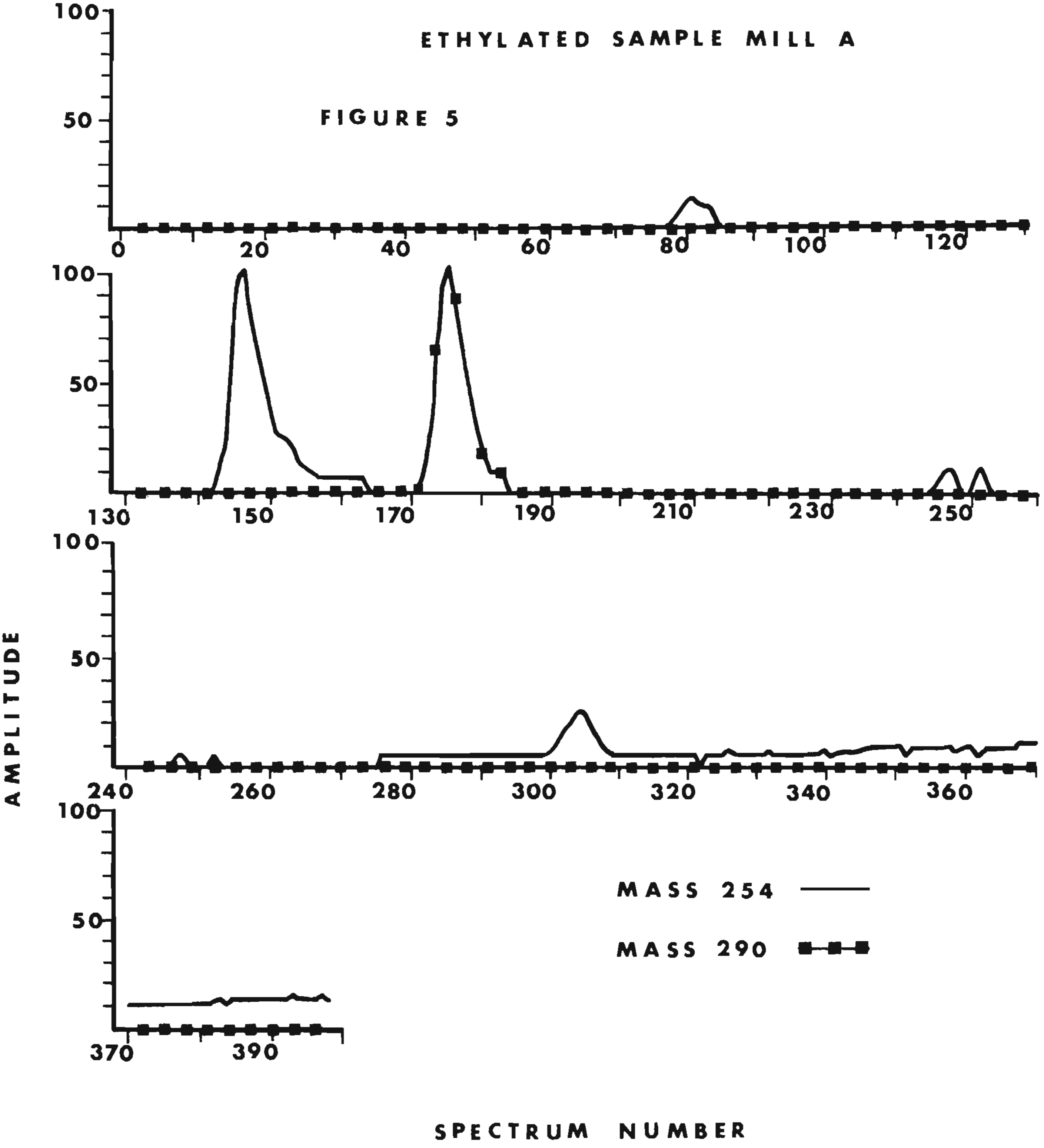

FIGURE 5

ETHYLATED SAMPLE MILL A

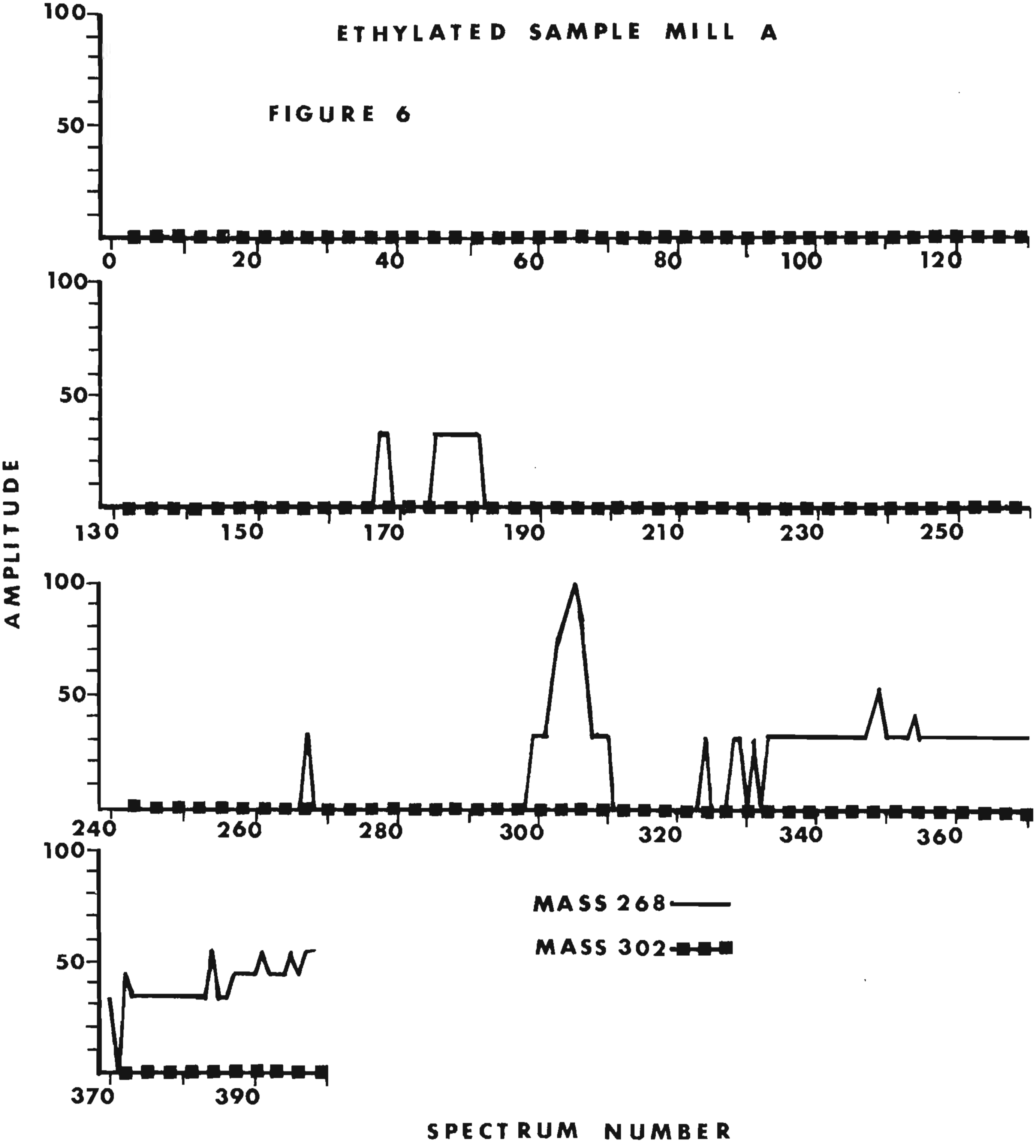

FIGURE 6

ETHYLATED SAMPLE MILL A