three MGs are given. Standard deviation and coefficient of variation (%) for C_{12}-, C_{14}- and C_{16}-MG on the C-8 column with a UV detector were 0.35-0.61 and 1.0-1.9%; 0.24-0.51 and 1.2-2.8%; and 0.47-0.67 and 1.4-1.9%, respectively. In the case of the C-18 column with an RI detector, the results were similar. Both HPLC methods gave reproducible results.

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Analysis for Trace Amounts of Geosmin in Water and Fish[1]

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Trace amounts of geosmin (*trans*-1,10-dimethyl-*trans*-9-decalol) were concentrated from relatively large volumes of water by vegetable oil extraction. After stirring the two phases for 30 min, the dispersed oil was allowed to separate. The oily layer was removed and centrifuged to break the emulsion and separate the two layers. The direct gas chromatographic technique was used to resolve the geosmin from other volatile components on a capillary gas chromatographic column. Volatiles were separated from the oil by securing an aliquot of the oil layer on volatile-free glass wool in the glass liner of the special gas chromatography inlet system. Geosmin was detected at the parts ber billion level with this simple and rapid technique. A technique also was developed for detecting geosmin in fish tissue; it involves steaming the fish to break up the tissue, centrifuging the residual oil phase, and detecting geosmin by capillary gas chromatography. The geosmin remains in the residual oil.

There have been many reports in the literature on the muddy or earthy odor of soil, and on the problem of muddy odor or flavor in surface waters and in fish taken from those waters (1). The major cause of this odor appears to be the production of geosmin (*trans*-1, 10-dimethyl-*trans*-9-decalol) by either Actinomycetes or blue-green algae or both, and methylisoborneol by Actinomycetes (2-9). These two compounds have been isolated and identified from surface water (7).

Yurkowski and Tabachek reported a study (1) that showed that all odor extracts from both muddy-flavored and non-muddy-flavored fish from the saline lakes of western Canada contained geosmin, suggesting that untainted fish (as determined by sensory panel) contained geosmin at levels below the taste threshold level. The results of their study also showed that, depending on the lake, the fish may be tainted throughout or for a short period of time during any portion of the growing season which lasts from early May to late October. Simple methods of analysis for geosmin content of fish, and of the water in which fish are raised, are therefore needed. Since geosmin has a very low organoleptic threshold level, estimated at 6 ppb in fish by Yurkowski and Tabachek (1), recovery of the odor-causing material from fish tissue or water is a primary consideration in any assay method. This problem is addressed in the present paper.

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EXPERIMENTAL PROCEDURES

Materials. Catfish filets were obtained at local seafood markets. Geosmin (98% pure) was purchased from Givaudan Corp., Clifton, New Jersey.

Gas chromatography. The gas chromatograph (GC) used was a Hewlett Packard (HP) 5790 series equipped with flame ionization detector (FID). Flow rates for hydrogen and air were 30 and 240 ml/min, respectively. Nitrogen flow rates were 1.2 ml/min through the column and 30 ml/min for the auxiliary make-up gas. A HP ultra performance capillary column was used (50 m × 0.31 mm ID column coated with 0.52 micron film of crosslinked 5% phenyl methyl silicone). An external closed inlet device (Scientific Instrument Service, River Ridge, Louisiana) designed from the inlet system previously described (10) was interfaced at the carrier gas arm of the insert Weldment assembly of the GC to facilitate direct gas chromatography. The inlet temperature of this device was set at 140 C, and the temperature of the six-port rotary valve was set at 180

C. The detector was set at 275 C. The column oven was lowered to −30 C with dry ice during an initial hold period of three min, and the six-port valve was positioned in the "inject'" mode to direct the carrier gas through the sample and purge the volatiles onto the head of the cold column. The oven temperature was programmed immediately from −30 to 30 C at 10 C/min, from 30 to 150 C at 2.5 C/min, and from 150 to 250 C at 5 C/min. Final hold was 250 C for 10 min.

A HP Lab Automation System 3357 was used for data acquisition and analysis. A HP 7221T Plotter was used to plot the GC curves.

Procedure for analysis for geosmin in water. Four g of good (low in total volatiles and containing none with peaks near that of geosmin) vegetable oil were added to 2 l of catfish-pond water containing geosmin in a two-l Erlenmeyer flask. A magnetic stirrer was used to thoroughly disperse the oily layer for 30 min. After standing, the oily layer was decanted and centrifuged. An aliquot of 300 mg of the clear oil was secured on glass wool in the liner of the external inlet system, which was interfaced at the carrier arm of the GC capillary injection port. The volatile components such as geosmin were swept onto the head of the cold capillary (−30 C) column by the flow of the carrier gas and the heat of the injection port (140 C). At the end of the desorption period (20 min), geosmin was resolved from other volatiles by temperature programming at the above listed rates.

Procedure for analysis for geosmin in fish. Two-hundred g of off-flavor catfish sliced finely with a knife were placed in a one-l Erlenmeyer flask with a 30-cm water condenser and a Tenax cartridge trap on top. The sliced catfish was heated at 100 C for one hr with a sandbath to break up the tissue and cause separation of the fish oil. The oil in the residue was separated from the catfish tissue by shaking with 400 ml of distilled water for 15 min. After decanting, the oil layer was centrifuged. An aliquot of the clear oil was analyzed by GC as described above for the oil extraction of geosmin from water.

RESULTS AND DISCUSSION

Many low-molecular-weight organic compounds are produced and liberated by bacteria and algae into surface waters (11). Of these, geosmin appears to be the principle compound associated with a muddy off-flavor in fish (1,12,13). Because geosmin, if present in the water, is readily absorbed by fish (1,13) and sometimes makes them unmarketable, a simple rapid method for determining geosmin in water is highly desirable. The methods of earlier investigators (1-3,8,11,13) are

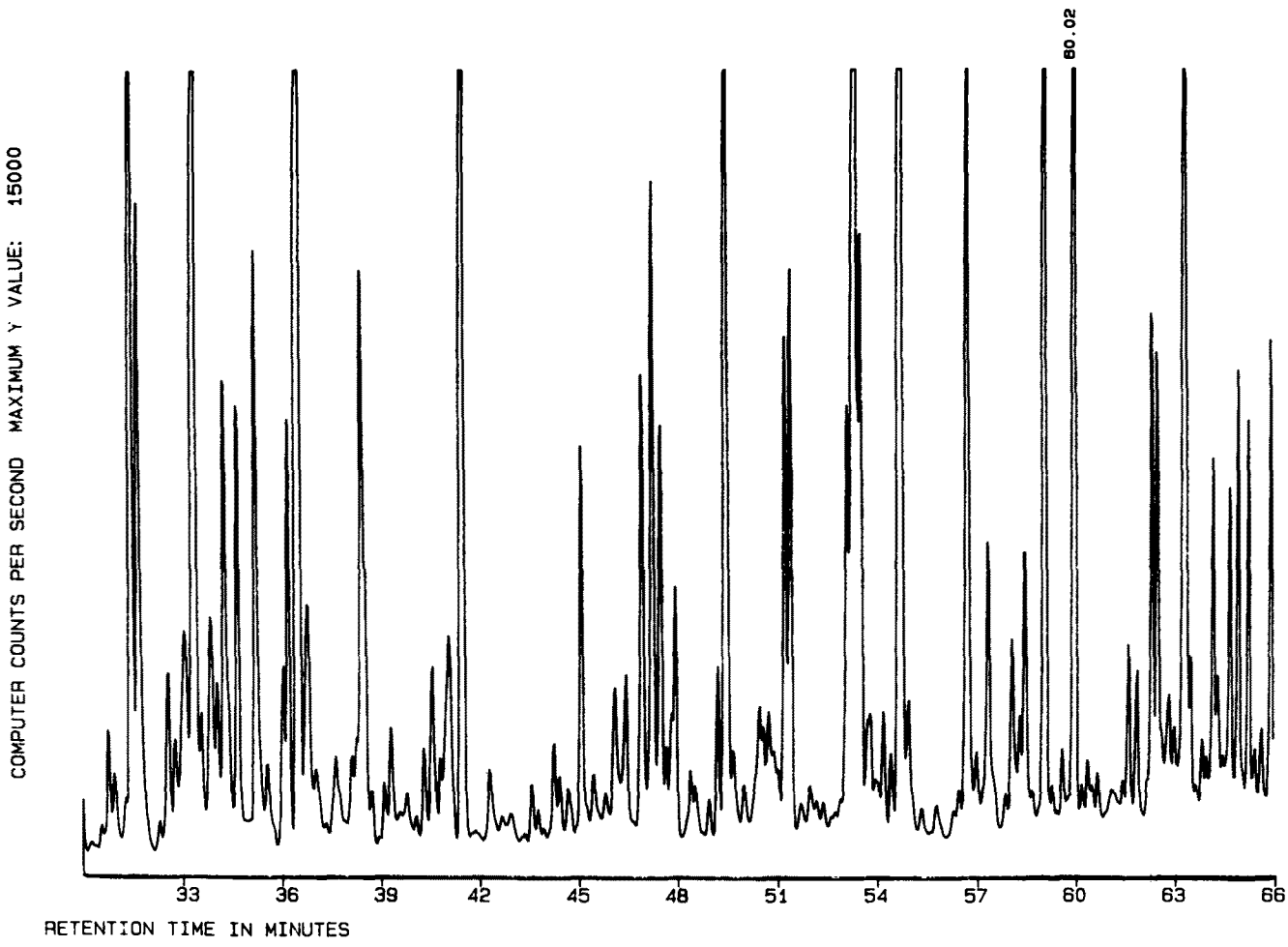

FIG. 1. Profile of oil extract of pond water containing geosmin, with geosmin peak at retention time 60.02 min.

successful in stripping trace amounts of geosmin from aqueous media and fish tissue, but they are tedious and time-consuming.

Steinke (14) developed a method for collection of the volatiles from food aromas, including off-flavor fish, by collecting the volatiles on Tenax-GC and eluting them with diethyl ether. In a separate step, capillary GC was used to resolve the volatiles, including the detection of geosmin in Lake Michigan salmon. Josephson et al. (15) and Olafsdottir et al. (16) have used Steinke's method for determining fish odors and food aromas successfully. In the present work the step involving elution with ether is eliminated. Some aspects of the method presented here for analyzing for geosmin are similar to the method used by Suzuki and Bailey (17) in studying the volatiles from ovine fat.

In the current work it has been found that a small amount of good vegetable oil (about 0.2% based on the volume of water) extracts geosmin at the parts per billion level from aqueous media. Figure 1 illustrates the success of this method. This profile showing the presence of geosmin was obtained using capillary GC. Recoveries of over 95% of geosmin were obtained in experiments with deionized water spiked with varying amounts of geosmin.

Earlier attempts using packed columns containing carbowax 20 M or Tenax-MPE were not satisfactory in resolving the geosmin from other volatile components.

In determination of geosmin in fish tissue, the tissue was heated at 100 C for 1 hr under a reflux condenser to break up the tissue and cause separation of the fish oil. Water was added and the oil phase separated, centrifuged (to remove traces of water), and an aliquot analyzed for geosmin by direct capillary gas chromatography. The geosmin is eluted at 140 C for 20 min to minimize oil decomposition and maximize geosmin elution. Figure 2 shows the profile obtained by application of the method to catfish tissue containing geosmin. Of six fish tested by this method, three were positive for geosmin and had geosmin levels of 3, 9 and 17 ppb, respectively, as determined by peak areas and response factors. The identity of the peak at retention time about 60 min was confirmed by gas chromatography-mass spectrometry (GC-MS) in comparison with an authentic sample of geosmin. Experiments with catfish not positive for geosmin but spiked with 5, 12 and 20 ppb, respectively, of geosmin gave over 90% recovery of the geosmin as determined by this method. A check of the volatiles adsorbed in the Tenax cartridge on the condenser showed that no geosmin was lost by volatilization during the heating. Also, it was determined that the conditions used elute all of the geosmin from the oil by repeated analysis of the residual oil.

The oil extraction procedure for detecting trace

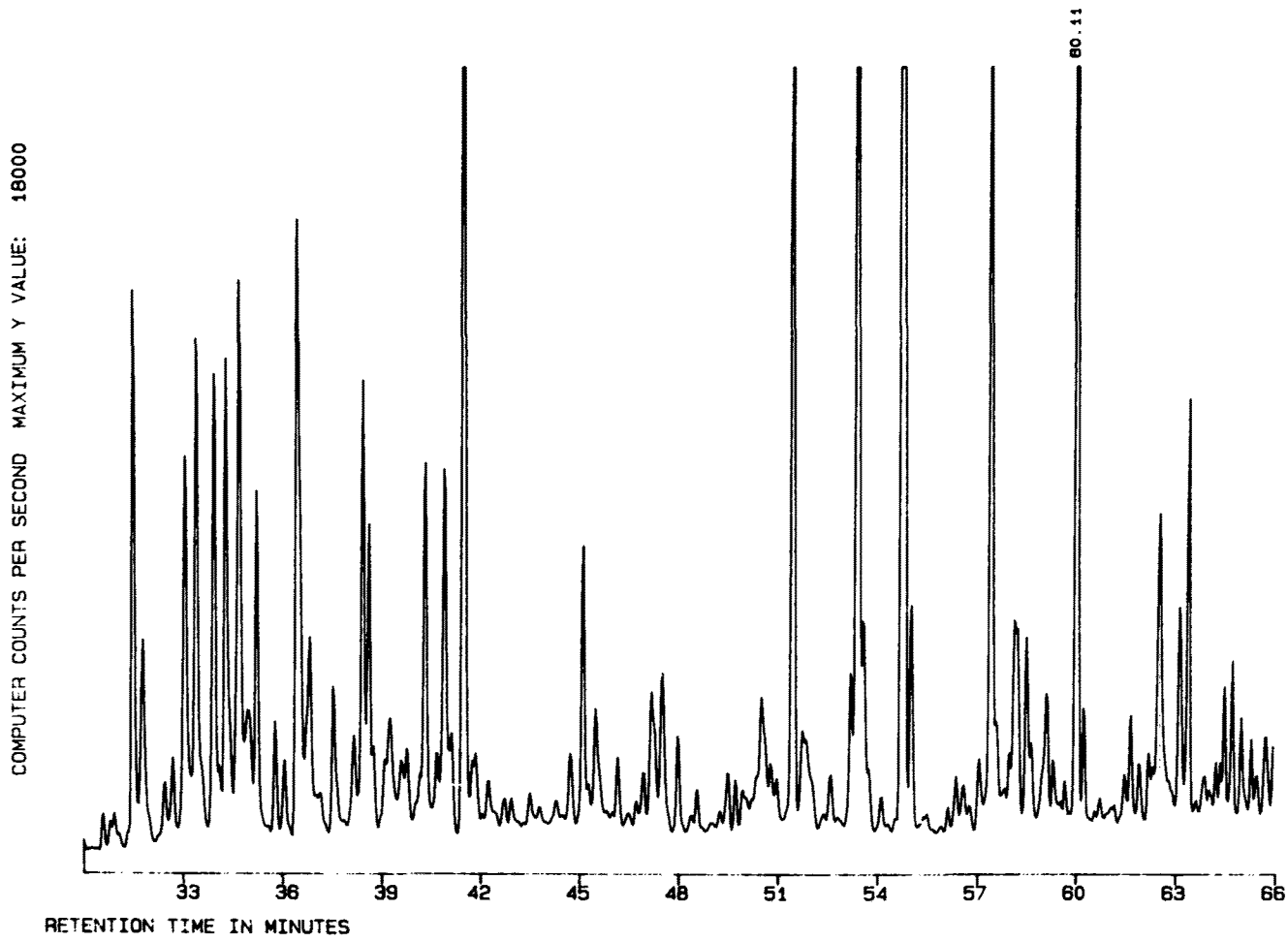

FIG. 2. Profile of volatiles from clear oil left after steaming 200 g of catfish slices found to contain 17 ppb of geosmin.

amounts of geosmin in pond water and fish tissue should be feasible for other products. For example, geosmin was detected at the parts per billion level when samples of sugar beet molasses were examined by oil extraction. Geosmin is a known constituent of beet juice and sometimes causes a flavor problem with table beets (18). Geosmin also is sometimes the cause of a musty off-flavor in dry beans (19,20).

In conclusion, simple rapid methods are presented for concentrating and detecting geosmin in aqueous media and in fish tissue at the parts per billion level.

ACKNOWLEDGMENTS

Michael G. Legendre and Ray H. Liu performed the gas chromatography/mass spectrometry confirmation of the identity of geosmin.

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Synthesis of Fatty 2-Oxazolines from Fatty Methyl 2,3-Epoxy Ester[1]

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Reaction of methyl *trans*-2,3-epoxyhexadecanoate (I) with benzonitrile in presence of boron trifluoride-etherate (BF_3-etherate) as catalyst has yielded *cis*-2-phenyl-4-tridecyl-5-carbomethoxy-2-oxazoline (II), methyl 2-hydroxy-3-benzamidohexadecanoate (IV) and methyl 2,3-dihydroxyhexadecanoate (III). On the other hand, reactions of I with acetonitrile and acrylonitrile have resulted in the formation of their corresponding hydroxyamides, methyl 2-hydroxy-3-acetamidohexadecanoate (VI) and methyl 2-hydroxy-3-acryloamidohexadecanoate (VII), respectively, along with the product (III) only. Pyrolysis of hydroxyamides (IV), (VI) and (VII) afforded their corresponding 2-oxazolines, *cis*-2-phenyl-4-tridecyl-5-carboxy-2-oxazoline (V), *cis*-2-methyl-4-tridecyl-5-carboxy-2-oxazoline (VIII) and *cis*-2-vinyl-4-tridecyl-5-carboxy-2-oxazoline (IX), respectively, in good yields. The products have been characterized with the help of spectral and microanalyses.

The interest in the biological and industrial potential (1) of 2-oxazolines has resulted in various synthetic procedures (1) for the introduction of five-membered nitrogen- and oxygen-containing heterocycles, i.e. 2-oxazoline, into a hydrocarbon chain. Studies of the reactions of various short chain epoxides with nitriles in presence of a catalyst leading to the formation of 2-oxazolines have been described (2-5). Smith et al. (6) have reported the preparation of 2-oxazolines in good yields under mild conditions from the reactions of various short-chain epoxides with acetonitrile and benzonitrile in presence of boron trifluoride-etherate (BF_3-etherate) as catalyst. The 2-oxazolines also have been prepared by the pyrolysis of hydroxyamino compounds (7). Scanning of the literature revealed that the reaction of nitriles with fatty 2,3-epoxy esters and pyrolysis of fatty hydroxyamido esters are not reported. These observations prompted us to carry out the conversion of fatty 2,3- epoxy and hydroxyamido esters into fatty 2-oxazolines using the above methods (6,7).

EXPERIMENTAL PROCEDURES

All melting points were taken on a Koflar hot plate

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