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Characterization, Weathering, and Application of Sesquiterpanes to Source Identification of Spilled Lighter Petroleum Products

ZHENDI WANG,*,† CHUN YANG,† MERV FINGAS,† BRUCE HOLLEBONE,† XIANZHI PENG,† ASGER B. HANSEN,‡ AND JAN H. CHRISTENSEN§

Emergencies Science and Technology Division, Environmental Technology Centre, Environment Canada, Ottawa, Ontario, Canada K1A 0H3, Department of Environmental Chemistry and Microbiology, National Environmental Research Institute, Roskilde, Denmark, and Department of Natural Sciences, Royal Veterinary & Agricultural University, Frederiksberg, Denmark

Biomarkers have become increasingly important for identifying the source of spilled oil, due to their specificity and high resistance to biodegradation. The biomarkers most commonly used in forensic investigations are the high molecular weight (MW) tri- and pentacyclic terpanes and steranes. For lighter petroleum products such as jet fuels and diesels, the refining processes remove most high MW biomarkers from the original crude oil feedstock. The smaller bicyclic sesquiterpanes, however, are concentrated in these products. Sesquiterpanes are ubiquitous components of crude oils and ancient sediments. Examination of GC−MS chromatograms of these bicyclic biomarkers using their characteristic fragment ions (*m*/*z* 123, 179, 193, and 207) provides a highly diagnostic means for identifying spilled oil, particularly for lighter refined product samples that are difficult to identify by current techniques. In this work, sesquiterpanes in crude oils and petroleum products are identified and characterized, distributions of sesquiterpanes in oils and refined products are compared, the effects of evaporative weathering on sesquiterpane distributions are examined, and a methodology using diagnostic indices of sesquiterpanes is developed for oil correlation and differentiation. Finally, two case studies are presented to illustrate the unique utility of sesquiterpanes for fingerprinting and identifying unknown diesel spills.

Introduction

Biomarkers are one of the most important hydrocarbon groups in petroleum. They can be detected in low quantities (ppm and sub-ppm levels) in the presence of a wide variety of other types of petroleum hydrocarbons by the use of gas chromatography−mass spectrometry (GC−MS). Relative to other compounds in oil, including the *n*-alkanes and most aromatics, biomarkers are more degradation-resistant in the environment. The relative content of biomarker compounds in source rocks, and hence crude oils and refined products, depends on the source, maturation, and in-reservoir weathering and biodegradation processes (*1*). Thus, biomarkers reveal more information about oil source than do other compounds in oil. Therefore, chemical analysis of biomarkers can be of great importance to environmental forensic investigations for determining the source of spilled oil, differentiating and correlating oils, and monitoring the degradation process and weathering state of oils under a wide variety of conditions (*2*−*9*).

Polymethyl-substituted decalins or decahydronaphthalenes (C_{14}−C_{16} bicyclic alkanes), commonly known as sesquiterpanes, were first reported in 1974 (*10*) and later discovered in crude oils of the Loma Novia and Anastasievsko-Troyitskoe deposits (*11*). Alexander et al. (*12*) identified 8β-(*H*)-drimane and 4β(*H*)-eudesmane in most Australian oils. Noble (*13*) identified a series of C_{14}−C_{16} sesquiterpane isomers using synthesized standards and mass spectral studies. Various sesquiterpanes were also identified by Simoneit et al. (*14*), from fossil resins, sediments, and crude oils with the greatest enrichment in condensate, and by Chen and He (*15*), from an offshore condensate field of Liaodong Bay, Northern China. The bicyclic biomarkers comprise one of the largest of the terpenoid classes (*1*). Sesquiterpanes with the drimane skeleton (Figure 1) are ubiquitous components of crude oils and ancient sediments. Most sesquiterpanes in oil probably originate from higher plants and also from simpler algae or bacteria (*16*−*18*). Philp et al. (*19*) suggested tricyclic diterpanes from higher plants as a source of bicyclic terpanes through the opening of ring C during maturation. Over the course of thermal evolution of an oil reservoir, the relative concentration of C_{14} sesquiterpanes decreases with increasing maturation of organic matter. The concentrations of C_{14} sesquiterpanes are higher in the immature stages, while those of the C_{15} drimanes and C_{16} homodrimanes are lower. As their higher MW precursors dehydroxylate, concentrations of drimanes and homodrimanes gradually increase and the concentrations of C_{14} sesquiterpanes decline (*20*).

Though biomarker sesquiterpanes have found increasing application in petroleum exploration in recent years, there have been few reports of use of these compounds for forensic oil spill identification (*21*). For lighter petroleum products, including jet fuels and most diesels, refining processes remove most high MW biomarkers from the original crude oil feedstock. Thus, the pentacyclic terpanes and steranes are generally absent or in low abundance in lighter petroleum products, while the sesquiterpanes are concentrated in these distillates. The sesquiterpanes are monitored using *m*/*z* 123, a base fragment ion common to all sesquiterpanes. Confirmation ions (Figure 2) include *m*/*z* 179 (the ion after parent $C_{14}H_{26}$ loses CH_3), 193 (the ion after $C_{15}H_{28}$ loses $-CH_3$ and after $C_{16}H_{30}$ loses $-C_2H_5$), and 207 (the ion after $C_{16}H_{30}$ loses $-CH_3$). Examination of GC−MS chromatograms of these characteristic ions of sesquiterpanes provides a highly diagnostic tool for correlation, differentiation, and source identification of light to middle-range petroleum products, in comparison with the use of other hydrocarbon groups.

In this work, sesquiterpanes in crude oils and petroleum products are characterized and their distributions are compared. The effects of evaporative weathering on sesquiterpane distributions are examined. A number of diagnostic indices of sesquiterpanes are developed for oil correlation and differentiation. Finally, two real-world spill case studies are presented to illustrate the unique utility of sesquiterpanes for fingerprinting and identification of unknown diesel fuel spills.

* Corresponding author phone: (613)-990-1597; fax: (613)-991-9485; e-mail: zhendi.wang@ec.gc.ca.
† Environment Canada.
‡ National Environmental Research Institute.
§ Royal Veterinary & Agricultural University.

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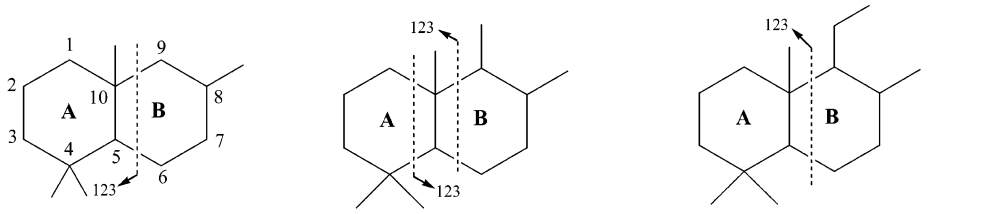

C_{14}: C_4-decalin (peak 1) C_{15}: 8β(H)-drimane (peak 5) C_{16}: 8β(H)-homodrimane (peak 10)

FIGURE 1. Molecular structures of representative sesquiterpanes.

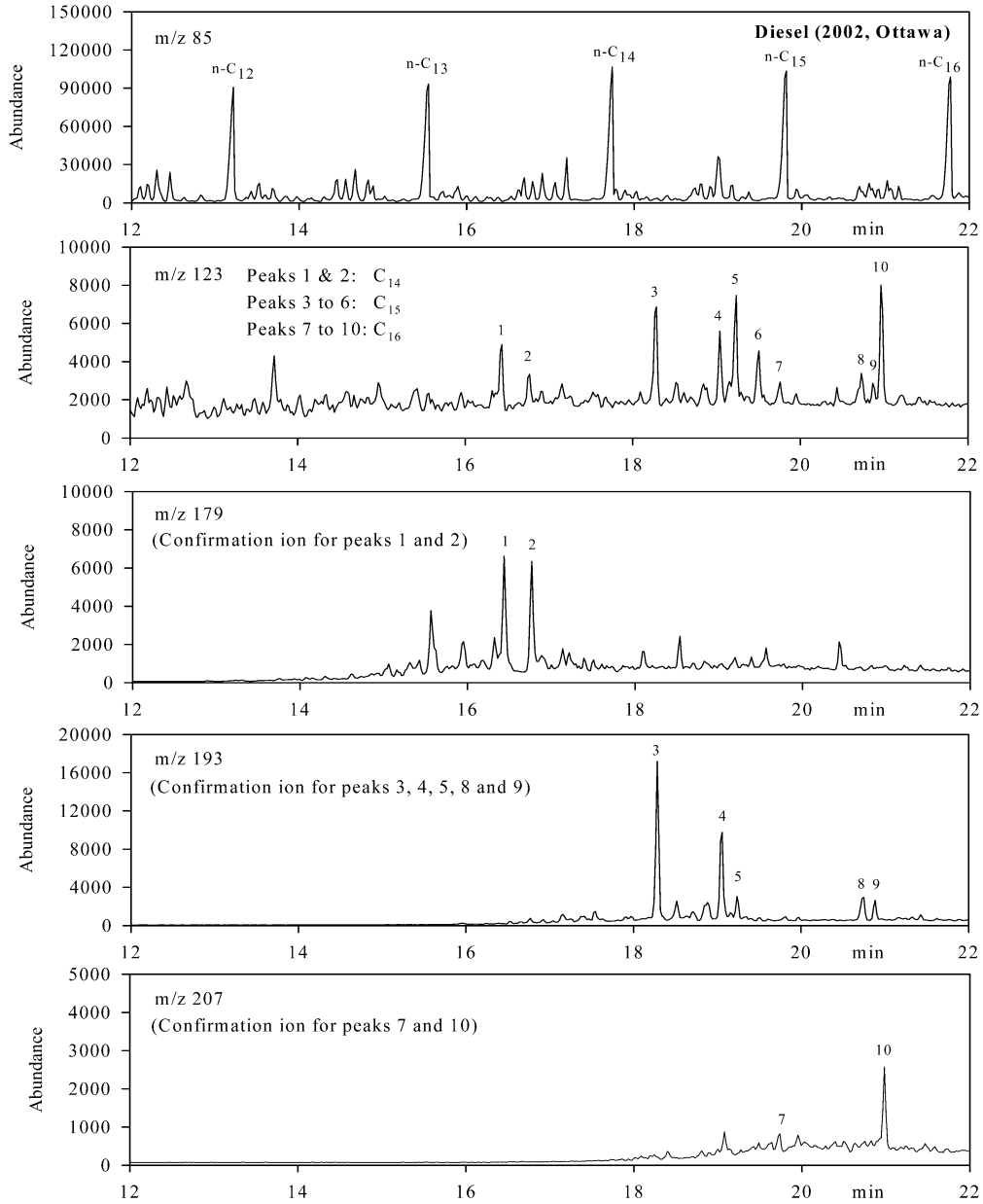

FIGURE 2. GC–MS chromatograms of sesquiterpanes (*m/z* 123, 179, 193, and 207) eluting in the *n*-C_{13} and *n*-C_{16} range.

Experimental Section

Reagents and Materials. Distilled chromatographic solvents were used without further purification. Calibration standards used include *n*-alkane standards from C_8 to C_{36}, polycyclic aromatic hydrocarbon (PAH) standards (SRM 1491) from the National Institute of Standards and Technology (NIST), and biomarker standards (hopanes and steranes) from Chiron Laboratory of Norway. Calibration standards for the sesquiterpanes, *cis*-decahydronaphthalene-d_{18}, *cis*-decahydronaphthalene, and 1-methyldecaline, were purchased from Aldrich.

Oil and Petroleum Product Samples. The oils and petroleum products were obtained from various oil companies and refineries and stored at 5 °C. The API gravities of these crude oils cover a wide range from 11 to 40. A laboratory oil-weathering technique (*22*) by rotary evaporation was used to artificially weather oils with varying degrees of weight loss. Typically, three weathered fractions were prepared for each

oil sample. This weathering technique used allows for precise control of the evaporative weight loss for the target oil and can be directly correlated to compositional changes of the oil.

Analytical Methods. A chromatographic column (10.5 mm i.d. × 200 mm length), dry-packed with 3 g of activated silica gel and topped with anhydrous sodium sulfate, was used for sample cleanup. The column was conditioned with 20 mL of hexane.

Aliquots of approximately 16 mg of oil in hexane, spiked with surrogates (*23*), were transferred into preconditioned silica gel columns. Hexane (12 mL) and 50% dichloromethane (DCM) in hexane (v/v, 15 mL) were used to elute the saturated and aromatic hydrocarbon fractions, respectively. The hexane fraction was used for analysis of aliphatics, *n*-alkanes, and biomarker compounds including sesquiterpanes, while a 50% DCM/hexane fraction was used for analysis of PAHs. The two fractions were spiked with internal standards and adjusted to a preinjection volume of 1.00 mL for GC−MS and GC/FID analyses (*9*, *23*).

Characterization of *n*-alkanes and TPH (total petroleum hydrocarbons) was performed on an HP 5890 gas chromatograph with a flame-ionization detector (GC/FID). Analyses of PAHs and biomarkers, including sesquiterpanes, were performed on an Agilent 6890 GC with an Agilent 5973 mass selective detector (MSD) using Agilent G1701 BA MSD ChemStation. An HP-5MS fused silica column (30 m × 0.25 mm i.d., 0.25 μm film thickness) was used for GC−MS analyses. For detailed chromatographic conditions and temperature programs, analysis quality control, and quantitation methodology, refer to Wang et al. (*9*).

Certified sesquiterpane standards are not commercially available. Thus, we used the bicylic standard compounds *cis*-decahydronaphthalene and 1-methyldecaline, which have similar molecular structures to those of the sesquiterpanes for method development. The average response factors of *cis*-decahydronaphthalene ($C_{10}H_{18}$, m/z 138) and 1-methyldecaline ($C_{11}H_{20}$, m/z 152) relative to the internal standard decahydronaphthalene-d_{18} (m/z 156) were used for quantitation of sesquiterpanes.

Results and Discussion

Distribution and Diagnostic Ratios of Sesquiterpanes. The sesquiterpanes ranging from C_{14} to C_{16} usually elute out between *n*-C_{13} and *n*-C_{16} (bp 235−287 °C) in the GC−MS/SIM chromatogram. Peaks 1 and 2, 3−6, and 7−10 (Figure 2) were identified as C_{14}, C_{15}, and C_{16} sesquiterpanes, respectively. Identification of individual sesquiterpanes was based upon comparison of mass spectral data, GC retention data, and the distribution pattern of sesquiterpanes to published literature data at m/z 123 (*12*, *13*, *16*) and further confirmed with the m/z 179, 193, and 207 mass chromatograms. Of the 10 identified sesquiterpanes, peaks 5 and 10 were identified to be 8β(*H*)-drimane (C_{15}) and 8β(*H*)-homodrimane (C_{16}), respectively.

GC−MS analyses demonstrate different distribution patterns of sesquiterpanes in crude oils and refined products of different origins. The left panel of Figure 3 shows GC−MS/SIM chromatograms of sesquiterpanes at m/z 123 for representative light (API > 35), medium (API = 25−35), and heavy (API < 25) crude oils, while the right panel compares sesquiterpane distributions in representative petroleum products from light kerosene to heavy fuel oil.

Ten sesquiterpanes were found in the oils examined. Oils of different origins exhibit differences in both the absolute concentrations and relative distributions of sesquiterpanes. Lighter oils Alaska North Slope (ANS), Arabian Light, and Scotia Light have high concentrations of sesquiterpanes, with peak 10 being the most abundant for the ANS and Arabian Light and peak 3 for the Scotia Light, respectively. The Arabian Light has the lowest concentration of C_{14} sesquiterpanes (peaks 1 and 2), indicating that this oil is highly mature. In contrast, the Californian API 11 heavy oil has the highest concentration of C_{14} sesquiterpanes, indicating that this oil is relatively immature.

Sesquiterpanes are absent in kerosene and in heavy lubricating oils. However, the refined products IFO-180 and HFO-6303 (a Bunker C fuel) contain high concentrations of sesquiterpanes. Jet A is characterized with peaks 1, 3, and 5 being the most abundant, while peak 10 is the most abundant in the diesel sample followed by peaks 5 and 3. Sesquiterpanes are abundant in middle-range distillates such as diesels due to concentration of lower MW biomarkers from the original crude oil feedstock. The differences in distribution patterns and concentrations of sesquiterpanes are often apparent between diesels (*21*).

We have developed and calculated diagnostic ratios of selected paired sesquiterpanes from a large number of oils and petroleum products. Diagnostic ratios vary greatly between oils from different regions. The cross-plot of peak 4/peak 5 (P4:P5) versus peak 3/peak 5 (P3:P5) is shown in Figure 4 for more than 50 crude oils and refined products. There is a large scatter in this set of oils in the cross-plot data: P4:P5 and P3:P5 fall in ranges of 0.2−1.2 and 0.1−2.1, respectively. Furthermore, related oils, such as the circle for Orimulsion samples from different batches and for the original Orinoco bitumen, produce tight clusters on the plot. This implies that the sesquiterpane ratios, in combination with other fingerprinting data, may be used to discriminate different oils and to identify the source of spill samples.

Effects of Evaporatative Weathering on Sesquiterpane Distributions. When crude oil or petroleum products are released to the environment, they are immediately subject to a wide variety of changes in physical and chemical properties that are aggregated as "weathering" (*3*). The most important weathering processes include evaporation, dissolution, dispersion, and biodegradation. In the short term after a spill, evaporation is the single most important and dominant weathering process. For the lighter petroleum products particularly, evaporation has a great effect on the amount of oil remaining on water or land (*24*).

To better understand the effects of evaporative weathering on oil sesquiterpanes in the environment, we weathered several oils by rotary evaporation to varying degrees. Table 1 summarizes the concentrations of nine sesquiterpane compounds in Cook Inlet and Maya oils at four evaporative weathering levels (peak 9 was below our detection limit). The sums of the nine target sesquiterpanes were 2112 (0%), 2513 (11.4%), 2831 (25.0%), and 3116 μg/g of oil (34.4% weathered) for the Cook Inlet oil series and 964 (0%), 1022 (5.5%), 1098 (11.4%), and 1186 μg/g of oil (16.7% weathered) for the Maya oil series, respectively. The diagnostic indices of a selection of paired sesquiterpanes in the C_{14}−C_{16} groups and between groups for these two oils are also listed in Table 1. Our elevated-temperature rotary evaporation method has been found to be equivalent to shallow-pool evaporation at ambient temperatures (*25*).

Table 1 illustrates that light to moderate evaporative weathering has little effect on the relative abundances of sesquiterpanes due to the moderately high boiling point range of these compounds. The concentrations of sesquiterpanes increase in proportion with increased weathering. In highly weathered oil, however, the lighter C_{14} sesquiterpanes, peaks 1 and 2, are preferentially lost. For example, isomers 1 and 2 were only 262 and 267 μg/g of oil for the 34% weathered Cook Inlet oil, slightly lower than the corresponding values (272 and 289 μg/g of oil) for the 25% weathered Cook Inlet oil. More importantly, most diagnostic rations of paired sesquiterpanes are essentially unaltered over the weathering series of the same oil. A double ratio plot of P4:P5 versus

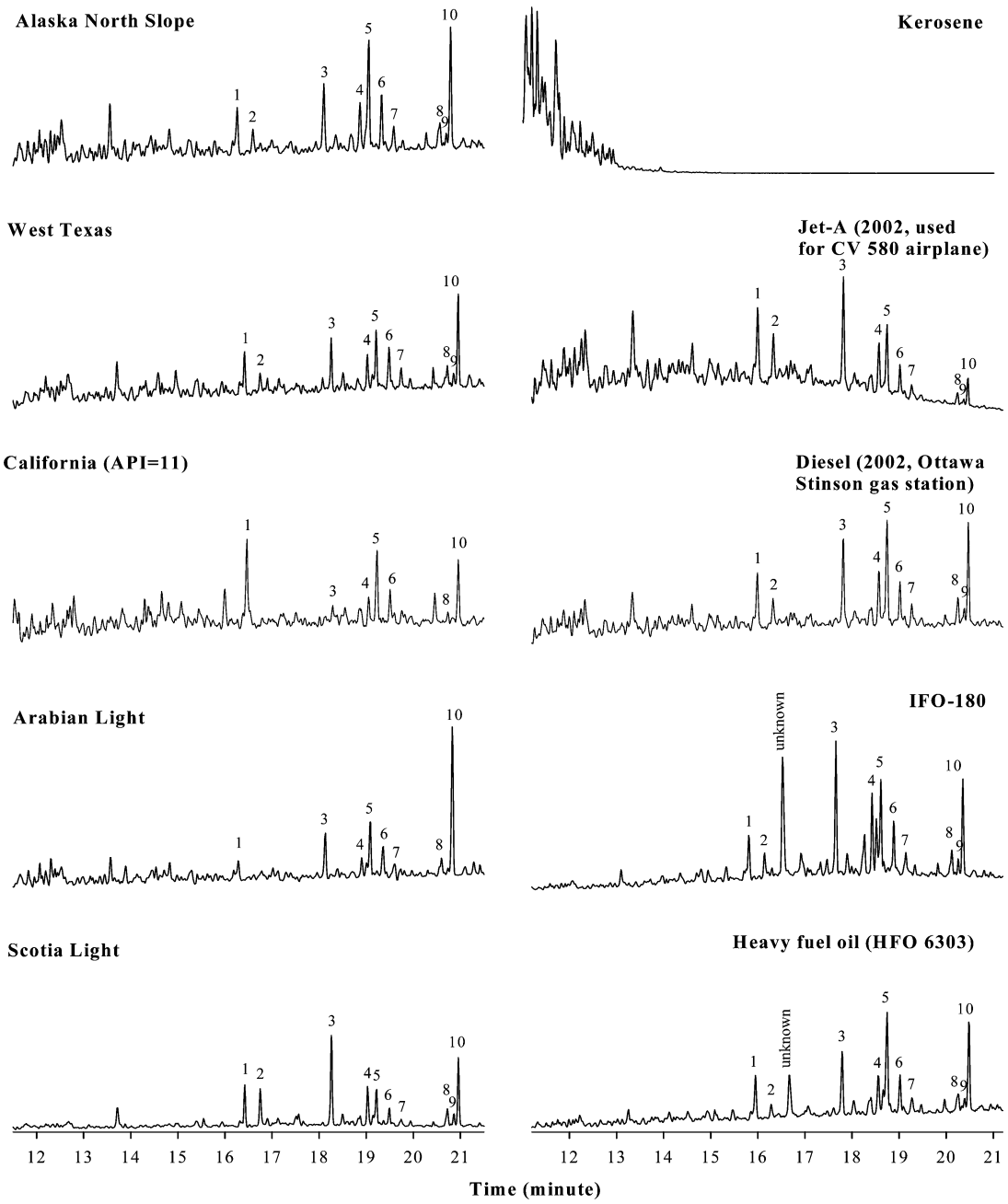

FIGURE 3. (Left panel) GC−MS chromatograms of sesquiterpanes at *m/z* 123 for representative light (API > 35), medium (API = 25−35), and heavy (API < 25) crude oils including ANS, Arabian Light, Scotia Light, West Texas, and California API 11. (Right panel) Comparison of GC−MS chromatograms of sesquiterpanes at *m/z* 123 for representative petroleum products from light kerosene to heavy fuel oil.

P3:P5 for 11 weathered crude oil and 1 weathered diesel series are shown in the right panel of Figure 4. The four weathered samples for each oil series (crude oils and diesel) form tight clusters. For example, no depletion of sesquiterpanes (relative to the most abundant peak 3) was observed for the weathered diesel samples (an Ottawa diesel, 2002) at four weathering percentages of 0, 7.2, 14.2, and 22.0%. This laboratory study indicates that evaporative weathering would not be expected to alter sesquiterpane distributions.

Biodegradation of petroleum hydrocarbons by natural microbes is a primary mechanism by which crude oil and refined products are eliminated from a contaminated environment. The biodegradation is generally a long-term weathering process. Relative to the isoprenoids and PAHs, *n*-alkanes and small aromatic compounds are preferentially biodegraded (*26*−*28*). It is well established, however, that petroleum biomarkers are highly resistant to biodegradation (*2*, *3*, *28*). Noble (*13*) has reported that the distribution patterns of sesquiterpanes were unaltered in biodegraded oil, even after the *n*-alkanes and isoprenoids had been completely depleted. Recently, Stout et al. (*21*) analyzed fresh diesel and its associated, variably weathered, nonaqueous phase liquids, and they found that sesquiterpanes are relatively stable, despite complete biodegradation of *n*-alkanes. These studies suggest that sesquiterpanes can be used to determine weathered percentages for lighter petroleum products, at least when biodegradation is not advanced significantly beyond the stage of *n*-alkane removal.

The estimation of degree of weathering for spilled lighter petroleum products such as diesel is difficult, because diesels do not contain high MW hopanes. Historically, estimation of weathering has mainly relied on the ratios of *n*-C_{17}/pristane and *n*-C_{18}/phytane. These ratios, however, can substantially underestimate the extent of weathering as pristine and

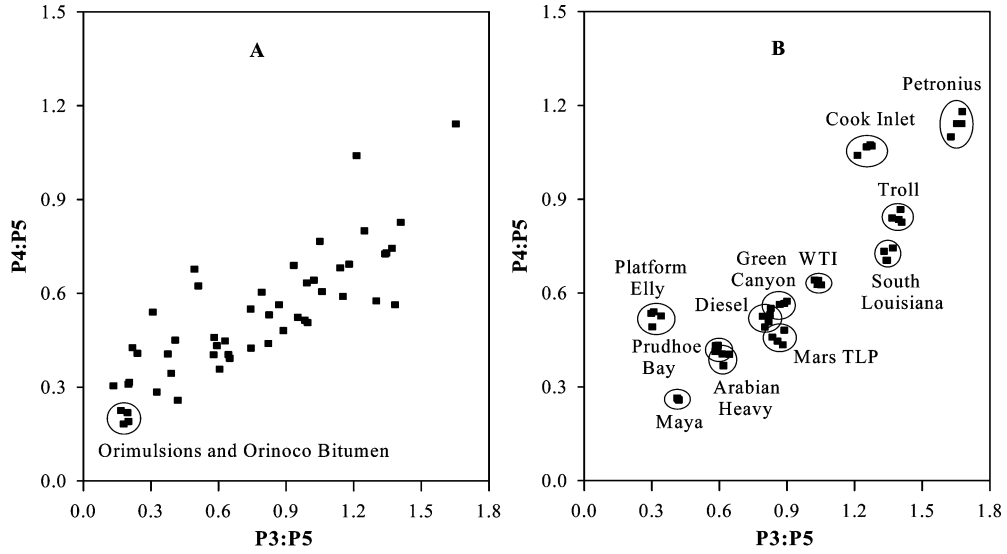

FIGURE 4. (A) Cross-plots of the double ratios of peak 4/peak 5 versus peak 3/peak 5 for over 50 different oils and refined products. The circle indicates related samples from the same origin. (B) Cross-plots of the double ratios of peak 4/peak 5 versus peak 3/peak 5 for 11 weathering oil series and 1 diesel weathering series. Each weathering oil series produces a tight cluster.

TABLE 1. Quantitation Results of Nine Sesquiterpane Compounds in Cook Inlet and Maya Oils at Four Evaporative Weathering Levels

	Cook Inlet				Maya			
	0%	11.4%	25.0%	34.4%	0%	5.5%	11.4%	16.7%
	Sesquiterpanes (μg/g of oil)							
peak 1	198	232	272	262	98.9	104	112	113
peak 2	203	247	289	267	27.1	30.5	32.2	34.0
peak 3	312	384	422	479	97.5	102	108	118
peak 4	267	322	359	406	59.8	64.2	69.4	72.9
peak 5	257	301	337	378	232	246	262	281
peak 6	280	336	358	414	88.1	98.5	104	108
peak 7	93.0	109	130	137	54.0	57.5	64.8	67.2
peak 8	119	134	161	183	48.5	51.4	55.3	62.8
peak 9[a]								
peak 10	384	446	503	590	258	267	289	328
total	2112	2513	2831	3116	964	1022	1098	1186
	Diagnostic Indices							
C14 group								
P1:P2	0.97	0.94	0.94	0.98	3.65	3.42	3.48	3.33
C15 group								
P3:P5	1.21	1.28	1.25	1.27	0.42	0.42	0.41	0.42
P4:P5	1.04	1.07	1.07	1.07	0.26	0.26	0.26	0.26
P6:P5	1.09	1.12	1.06	1.10	0.38	0.40	0.40	0.39
C16 group								
P8:P10	0.31	0.30	0.32	0.31	0.19	0.19	0.19	0.19
intergroup								
P1:P5	0.77	0.77	0.81	0.69	0.43	0.42	0.43	0.40
P3:P10	0.81	0.86	0.84	0.81	0.38	0.38	0.37	0.36
P5:P10	0.67	0.68	0.67	0.64	0.90	0.92	0.91	0.86

[a] Not integrated due to low abundance.

phytane readily biodegrade (*29*). We propose a method using the highly abundant and relatively degradation-resistant C_{15} and C_{16} sesquiterpanes as an internal oil reference to estimate the depletion degree of lighter fuels as the following (eq 1)

$$P\ (\%) = (1 - C_s/C_w) \times 100\% \qquad (1)$$

where P is the weathered percentages of the samples, and C_s and C_w are the concentrations of C_{15} or C_{16} sesquiterpanes with high abundance in the source and weathered samples, respectively.

Case Study 1: Spill Source Identification by Comparison of Distribution and Diagnostic Ratios of Sesquiterpanes. Oil spills were reported and sampled on March 17 and 23, 1998, at a sewer outlet flowing into the Lachine Canal in Quebec, Canada. Following the incident, a diesel fuel, the suspected source of the spill, was collected and analyzed. The product-type screening by GC/FID and PAH characterization by GC−MS indicated that the spilled oil was a diesel fuel (*30*).

Sample fingerprinting detected only trace amounts (<10 μg/g of oil) of lower MW C_{19}−C_{24} tricyclic terpanes, regular C_{20}−C_{22} steranes, and diasteranes. Spill samples were found to be lightly weathered and to have alkylated PAH distributions and diagnostic ratios similar to the suspected source diesel. This evidence argued strongly, but not defensibly, that the suspected diesel collected from the pumping station

TABLE 2. Diagnostic Sesquiterpane Ratios of Two Representative 1998 Spill Diesels and One Suspected-Source Diesel

diagnostic indices	spill sample I	spill sample II	suspected source
P5:P3	1.30	1.32	1.28
P10:P3	1.31	1.33	1.29
P8:P10	0.28	0.28	0.29
P2:P1	0.48	0.47	0.50
P1:P3:P5:P10	0.54:1.00:1.30:1.31	0.57:1.00:1.32:1.33	0.58:1.00:1.28:1.29

close to the spill site was the source of the spilled diesel. Both the spill samples and suspected-source diesel, however, contained significant amounts of sesquiterpanes. The diagnostic ratios of selected sesquiterpanes for two spilled diesels and the suspected-source diesel are compared in Table 2. Chromatograms at m/z 123 and diagnostic ratios of sesquiterpanes of the spill samples were almost identical to that of the suspected-source diesel. Note that, compared to the suspected source, the spill sample had higher abundances of sesquiterpanes, because of concentration by weathering. This case study implies that sesquiterpanes may provide useful information for source identification of lighter petroleum products when other typical hydrocarbon fingerprinting groups, such as high-boiling hopanes and steranes, are absent.

Case Study 2: Forensic Comparison and Identification of Round Robin Oil Samples of Very Similar Composition Using a Multicriterion Approach. Three oil samples were collected from a harbor spill in The Netherlands in 2004. A thick layer of oil (Sample 2) was found between a bunker boat and the quay next to the bunker center, and it was suspected that something had gone wrong during the bunkering of the vessel. Employees of the ship and the bunker center, however, both disclaimed responsibility for the spill. Fuel oils from the bunker boat (sample 1) and the bunker center (sample 3) were collected as suspected sources for comparison with the spill sample.

A multicriteria approach was applied to fingerprint these oil samples and to identify whether the spilled oil came from the bunker boat or bunker center. First, we determined the product type from the hydrocarbon distribution patterns. Then we quantified the sesquiterpanes. Finally, we validated our conclusions by comparison of the GC−MS/SIM profiles and diagnostic ratios of conventional "source-specific" PAHs and biomarkers as available.

Triplicate analyses of all three samples were performed. The abundances and selected diagnostic ratios were evaluated by comparing relative standard deviations (RSDs). The relative variations of diagnostic ratios at 95% confidence limit were calculated using the Student's t-test (*7*, *31*). The RSD calculated for each triplicate set and each diagnostic ratio are given in Table 3. The RSDs are less than 5% for all sesquiterpane isomers, indicating a high precision of measurements for the individual analytes used to calculate the diagnostic ratios (*31*).

Product Type-Screening. The samples were all transparent, pale red, with no water phase, indicating all were pure fuel oil. The samples were type-screened from their GC traces: (1) all have similar GC/FID and GC−MS chromatographic profiles (m/z 83 and 85 for alkyl cyclohexanes and n-alkanes, respectively); (2) hydrocarbons ranged between n-C_8 and n-C_{32} with maximal abundances between n-C_{15} and n-C_{17}, and no hydrocarbons heavier than C_{32} were detected; (3) a nearly symmetrical UCM (unresolved complex mixtures of hydrocarbons) of middle-range distillate was apparent; (4) GC-detectable total petroleum hydrocarbons (GC-TPH) ranged from 870 to 920 mg/g of oil, typical of lighter distillate fuels, significantly higher than most crude oils; (5) total n-alkanes including pristane and phytane were 142, 142, and 145 mg/g of oil for the three samples, typical for diesel fuels;

TABLE 3. Quantitation Results and Diagnostic Ratios of Sesquiterpane in Three Round Robin Oil Samples

	sample 1 ($n = 3$)	sample 2 ($n = 3$)	sample 3 ($n = 3$)
	Sesquiterpanes (μg/g of oil)		
peak 1	481 (1.2)[a]	518 (0.2)	527 (0.6)
peak 2	334 (0.5)	355 (2.3)	283 (2.6)
peak 3	1163 (0.9)	1212 (1.4)	965 (2.4)
peak 4	805 (1.0)	836 (1.4)	666 (2.2)
peak 5	1349 (2.4)	1392 (1.5)	1370 (1.0)
peak 6	722 (1.4)	750 (1.2)	658 (2.3)
peak 7	368 (4.8)	377 (2.4)	384 (4.1)
peak 8	625 (2.1)	640 (1.4)	507 (2.6)
peak 9	251 (4.1)	259 (4.6)	220 (4.2)
peak 10	1889 (0.7)	1916 (0.3)	1803 (1.2)
total	7986 (0.7)	8255 (0.5)	7384 (0.6)
	Diagnostic Indices		
C_{14} group			
P1:P2	1.44 (1.6)	1.46 (2.3)	1.87 (3.0)
C_{15} group			
P3:P5	0.86 (3.3)	0.87 (2.5)	0.70 (1.5)
P4:P5	0.60 (1.4)	0.60 (2.7)	0.49 (1.5)
P6:P5	0.54 (1.0)	0.54 (2.7)	0.48 (3.2)
C_{16} group			
P8:P10	0.33 (2.7)	0.33 (1.4)	0.28 (1.5)
intergroup			
P1:P5	0.36 (3.2)	0.37 (1.3)	0.38 (0.6)
P3:P10	0.62 (0.4)	0.63 (1.3)	0.54 (1.9)
P5:P10	0.71 (3.0)	0.73 (1.3)	0.76 (0.8)

[a] The concentrations and diagnostic ratios were determined from three measurements. The values in parentheses are relative standard deviation (%RSD) of three measurements.

(6) all three samples had similar ratios of n-C_{17}/pristane, n-C_{18}/phytane, and pristane/phytane, with sample 1 (bunker boat) being closer to the spill sample 2 than sample 3 (bunker center); (7) spill sample 2 had been slightly weathered, having considerably lower concentrations of n-C_8, n-C_9, and n-C_{10} than the suspected source samples 1 and 3.

The chromatographic evidence suggested that the spilled oil (sample 2) was a diesel-type fuel and slightly weathered. The diagnostic ratios of n-C_{17}/pristane, n-C_{18}/phytane, and pristane/phytane are useful but cannot be considered "diagnostically conclusive" in many cases because of evaporation, biodegradation, and/or other weathering processes. In this case, two questions remain after the product type-screening: (1) Do these three samples come from the same source? (2) Were the minor differences in chemical composition between samples caused by weathering or contamination? To unambiguously answer these questions, characterization of more than one suite of analytes was necessary.

Bicyclic Sesquiterpanes. Figure 5 shows the GC−MS/SIM chromatograms at m/z 123. Table 3 summarizes the sesquiterpane quantitations and diagnostic ratios of paired sesquiterpane isomers. Investigation of Figure 5 and Table 3 reveals that (1) sesquiterpanes are abundant in all three oil samples. The total sesquiterpane concentrations were 7142, 7623, and 6802 μg/g of oil. (2) Samples 1 and 2 have nearly identical distribution patterns of sesquiterpanes. (3) The

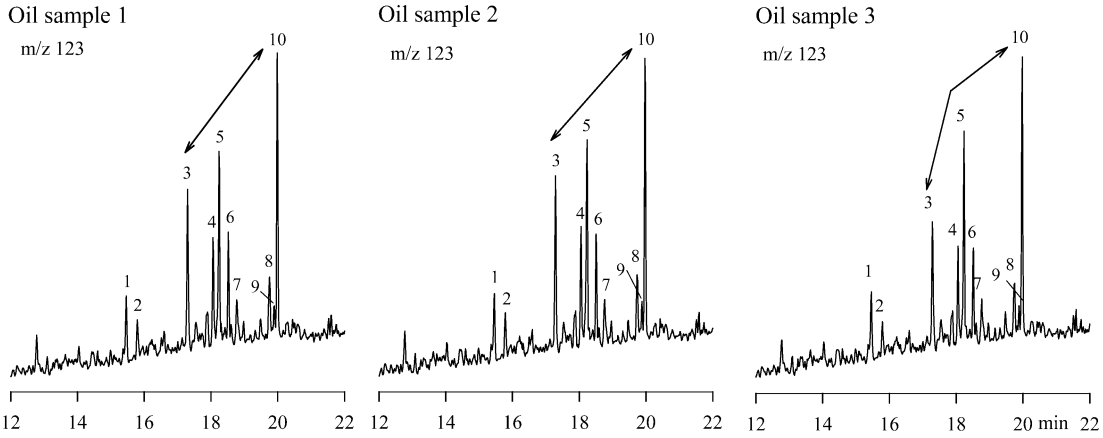

FIGURE 5. Extracted ion chromatograms at *m/z* 123 for the analysis of sesquiterpanes of three round robin oil samples.

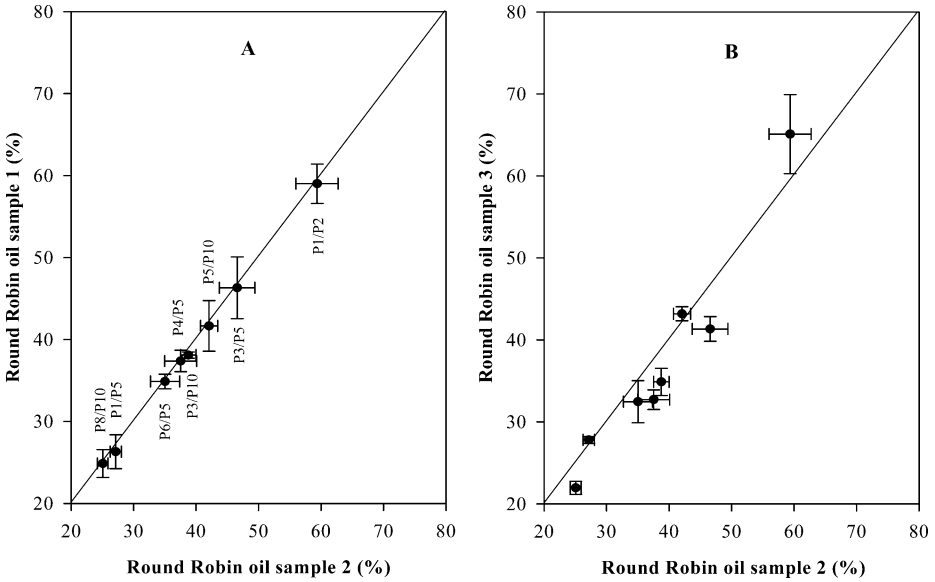

FIGURE 6. Correlation of diagnostic ratios (normalized to %) of sesquiterpanes between spill sample 2 and suspected source samples 1 (left) and 3 (right) at 95% confidence. All the data (A, left panel) overlap the 1:1 line at 95% confidence, representing a "perfect" match between samples 1 and 2. Conversely, most data points (B, right panel) between samples 2 and 3 do not overlap the line at 95% confidence, representing a "nonmatch".

diagnostic ratios of eight sesquiterpane isomeric pairs were nearly identical for samples 1 and 2. (4) Sample 3 is distinctly different from samples 1 and 2 not only in the diagnostic ratios but also in the concentrations of target sesquiterpanes. In particular, the abundances of peaks 2, 3, 4, and 8 of sample 3 are much lower than the corresponding peaks of samples 1 and 2. (5) Furthermore, the diagnostic ratios of P3:P5 and P4:P5 for sample 3 are considerably lower than the corresponding ratio values for sample 1 and 2. Conversely, sample 3 has a much higher ratio of P1:P2 than samples 1 and 2.

Spill sample 2 had slightly higher concentrations of all the observed sesquiterpanes compared to sample 1, which is most likely due to weathering (i.e. evaporation of the spill sample). The evaporative mass-loss of sample 2 relative to sample 1 is estimated by eq 1 to between 4% and 6% based on the sesquiterpane concentrations.

The diagnostic ratios of sesquiterpanes are compared in double-ratio plots at 95% confidence limits (Figure 6). Spill sample 2 is compared to both suspected source oil sample 1 and 3 in parts A and B, respectively, of Figure 6. On the basis of the criteria described in the standardized CEN method (*7*), there is a perfectly "positive match" between the spill sample (sample 2) and the spill source candidate (sample 1), while sample 3 is a "nonmatch" to the spill.

Confirmation of Source Identification from Alkylated PAHs and Pentacyclic Terpanes and Steranes. The source identification by characterization of sesquiterpanes was further validated by quantitative evaluation of five petroleum-characteristic alkylated PAH homologous series (naphthalene, phenanthrene, dibenzothiophene, fluorene, and chrysene) and pentacyclic biomarkers.

PAH fingerprinting results show that (1) the total of alkylated PAHs were 24 902, 25 870, and 21 528 μg/g of oil for samples 1, 2, and 3, respectively; (2) samples 1 and 2 have nearly identical distribution patterns of alkylated PAHs, but the pattern for sample 3, however, is noticeably different; (3) diagnostic ratios of target PAH groups and paired PAH isomers are all very similar for all samples, but the ratios for sample 1 and 2 were more similar to each other than to sample 3.

Biomarker fingerprinting results at *m/z* 191 and 218 reveal that (1) only traces of terpanes and steranes were detected in the samples (157, 181, and 101 μg/g of oil for samples 1, 2, and 3, respectively), mostly lower MW C_{19}−C_{24} terpanes, diasteranes, and C_{27}−C_{29} steranes. No C_{33}−C_{35} pentacyclic hopanes were detected. (2) Samples 1 and 2 have nearly identical terpane and sterane distribution patterns. (3) Sample 3 shows the distribution pattern different from that of samples

1 and 2. The concentrations of tricyclic terpanes ($C_{21}-C_{24}$) in sample 3 are similar to those of samples 1 and 2, but the pentacyclic terpanes ($C_{29}-C_{32}$) and $C_{27}-C_{29}$ steranes have much lower concentrations than for samples 1 and 2. (4) The diagnostic ratios of target hopanes and steranes are similar for samples 1 and 2, while the diagnostic ratios of sample 3, however, are significantly different from either.

The analyses of PAH and terpanes and steranes confirm the conclusion of sesquiterpane analysis, that is, sample 1 (bunker boat) is a positive match to the spill sample 2, while sample 3 (bunker center) is a nonmatch to the spill.

In this study, we demonstrate the great potential of biomarker sesquiterpane analysis for fingerprinting of crude oils and petroleum products, as their chemical distributions (at m/z 123) vary significantly between oils from different sources and between petroleum product types. Furthermore, we show that diagnostic ratios of sesquiterpanes are also largely unaffected by evaporative weathering at percentages up to 25% for light crude oils and diesels. Sesquiterpanes can markedly improve confidence of oil spill identification, especially for lighter petroleum products. They also aid in the identification of lightly weathered crude oils, heavy fuels, and lubricants. It is important to note that, however, there is no single fingerprinting technique that is a definitive and ultimately defensible forensic criterion. Therefore, a "multicriteria" approach, characterization of more than one suite of analytes, for spill source identification is necessary.

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