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RESEARCH ARTICLE

EIMS Fragmentation Pathways and MRM Quantification of 7α/β-Hydroxy-Dehydroabietic Acid TMS Derivatives

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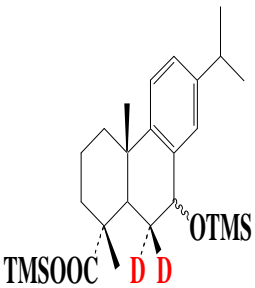

Abstract. EI mass fragmentation pathways of TMS derivatives of 7α/β-hydroxy-dehydroabietic acids resulting from $NaBH^4$-reduction of oxidation products of dehydroabietic acid (a component of conifers) were investigated and deduced by a combination of (1) low energy CID-GC-MS/MS, (2) deuterium labeling, (3) different derivatization methods, and (4) GC-QTOF accurate mass measurements. Having identified the main fragmentation pathways, the TMS-derivatized 7α/β-hydroxy-dehydroabietic acids could be quantified in multiple reaction monitoring (MRM) mode in sea ice and sediment samples collected from the Arctic. These newly characterized transformation products of dehydroabietic acid constitute potential tracers of biotic and abiotic degradation of terrestrial higher plants in the environment.

Keywords: 7α/β-Hydroxy-dehydroabietic acids, TMS derivatives, EI fragmentations, MRM quantification, Natural samples

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Introduction

Understanding the fate of terrestrial organic matter (OM) in the ocean has intrigued scientists for decades and is necessary for understanding the global carbon cycle and its anthropogenic perturbations [1]. Since riverine particulate OM consists, in part, of already highly degraded residues from terrestrial higher plants, it is generally considered to be refractory with respect to further decomposition in the ocean [2, 3]. It is thus surprising that only a small fraction of the OM preserved in marine sediments appears to be derived from terrestrial sources [4]. This suggests that global budgets and distribution estimates are either in error, or that OM of terrestrial origin undergoes rapid and extensive remineralization within the marine environment [1]. Indeed, several recent studies have demonstrated that under some oceanographic conditions, particulate organic matter (POM) delivered by rivers may be sensitive to microbial degradation [5–8] and autoxidation [8] in shelf areas. To investigate the significance of such degradation processes, there is a clear need to identify specific tracers of the degradation of organic matter from terrestrial higher plants.

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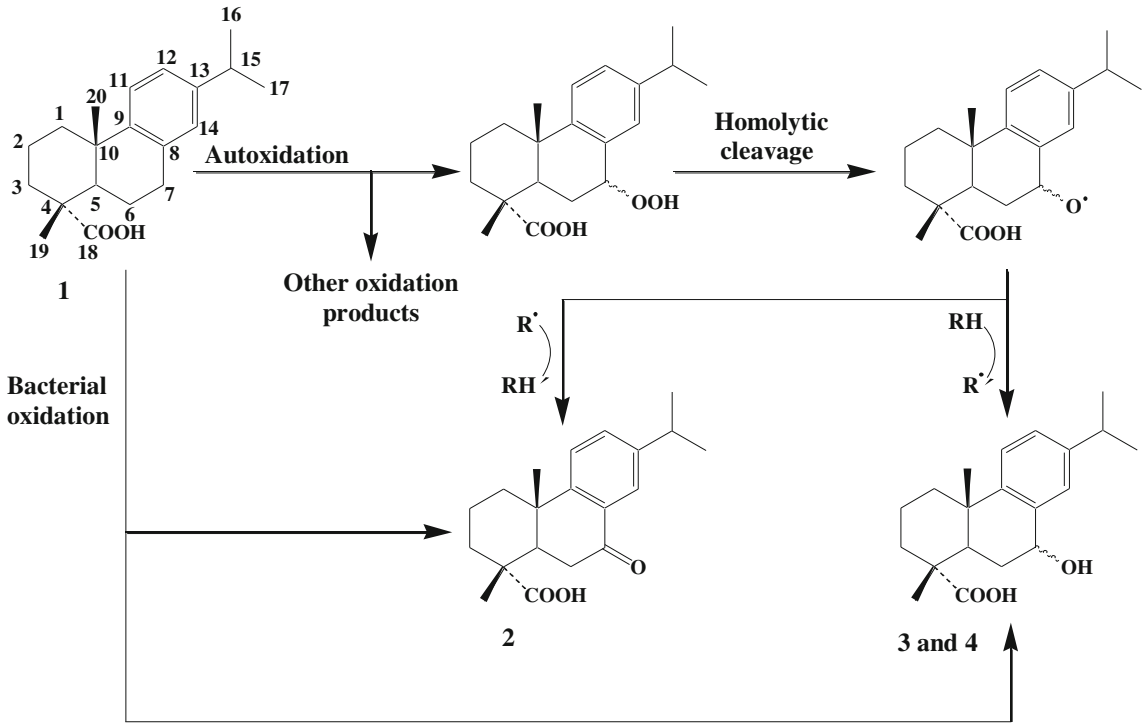
Dehydroabietic acid (8,11,13-abietatrien-18-oic acid) (DHAA) (**1**) is a biomarker of coniferous residues in sediments [9] and biomass burning [10, 11]. Although only a minor component in the fresh resin of conifers, its abundance increases on aging at the expense of the corresponding abietadienic acids. Further, if oxygen is available, DHAA can be oxidized abiotically (Scheme 1) to 7-oxo-dehydroabietic acid (**2**), 7α/β-hydroxy-dehydroabietic acids (**3** and **4**), and numerous other degradation products [12–14]. The oxidation mechanisms are believed to involve the formation of peroxide groups at the thermodynamically favored allylic (carbon 7) position (Scheme 1). In addition, several bacteria are also able to oxidize DHAA to 7-oxo-dehydroabietic (**2**) and 7-hydroxy-dehydroabietic acids (**3** and **4**) [15] (Scheme 1).

Derivatives of DHAA, with various functionalities at C-7 (hydroperoxy, keto, and hydroxy), could thus provide useful and selective tracers of the degradation of organic matter from conifers in the environment. However, owing to the thermal instability of some of the initially formed hydroperoxides, it is first necessary to reduce these primary oxidation products to the corresponding 7α/β-hydroxy-dehydroabietic acids in order to quantify them in natural samples using gas chromatography–mass spectrometry.

In the present paper, we describe (1) the elucidation of EI mass fragmentation pathways of trimethylsilyl derivatives of



Scheme 1.

these two isomeric hydroxyacids following different derivatization methods, low energy CID-GC/MS/MS, isotopic labeling and accurate mass measurements; (2) quantification of the same hydroxyacids in Arctic sea ice and sediment samples in multiple reaction monitoring (MRM) mode using transitions based on the main fragmentation pathways.

Experimental

Chemicals

Dehydroabietic acid (**1**) (95%), methyl abietate (**5**) (99%), $NaBH_4$, $NaBD_4$ (98.0 atom% D), deuterium oxide (99.9 atom% D), sodium, acetic anhydride, chromium oxide, Pd/C, potassium hydroxide, anhydrous dioxane and pyridine, BF_3/methanol (10%), and solvents were purchased from Sigma-Aldrich (St. Quentin Fallavier, France). Unlabeled and perdeuterated (99.0 atom% D) *N,O*-bis(trimethysilyl)trifluoroacetamide (BSTFA) were obtained from Supelco (St. Quentin Fallavier, France) and Campro Scientific (Veenendaal, The Netherlands), respectively.

Synthesis of Dehydroabietic Acid Oxidation Products

The synthesis of 7-oxo-dehydroabietic acid (**2**) from methyl abietate (**5**) required three steps: (1) heating of **5** with Pd/C under N_2 at 240°C affording methyl dehydroabietate (**6**) (85% yield), (2) oxidation of **6** with CrO_3 in acetic acid/acetic anhydride producing methyl 7-oxo-dehydroabietate (**7**) (66% yield), and (3) alkaline hydrolysis of **7** to the corresponding ketoacid **2** (75% yield) [16] (Scheme 2).

7α- and 7β-Hydroxy-dehydroabietic acids (**3** and **4**) were obtained by reduction of the ketoacid **2** (room temperature, 1 h) in diethyl ether:methanol (4:1, v/v; 5 mL) with excess $NaBH_4$ (90% yield) (Scheme 2).

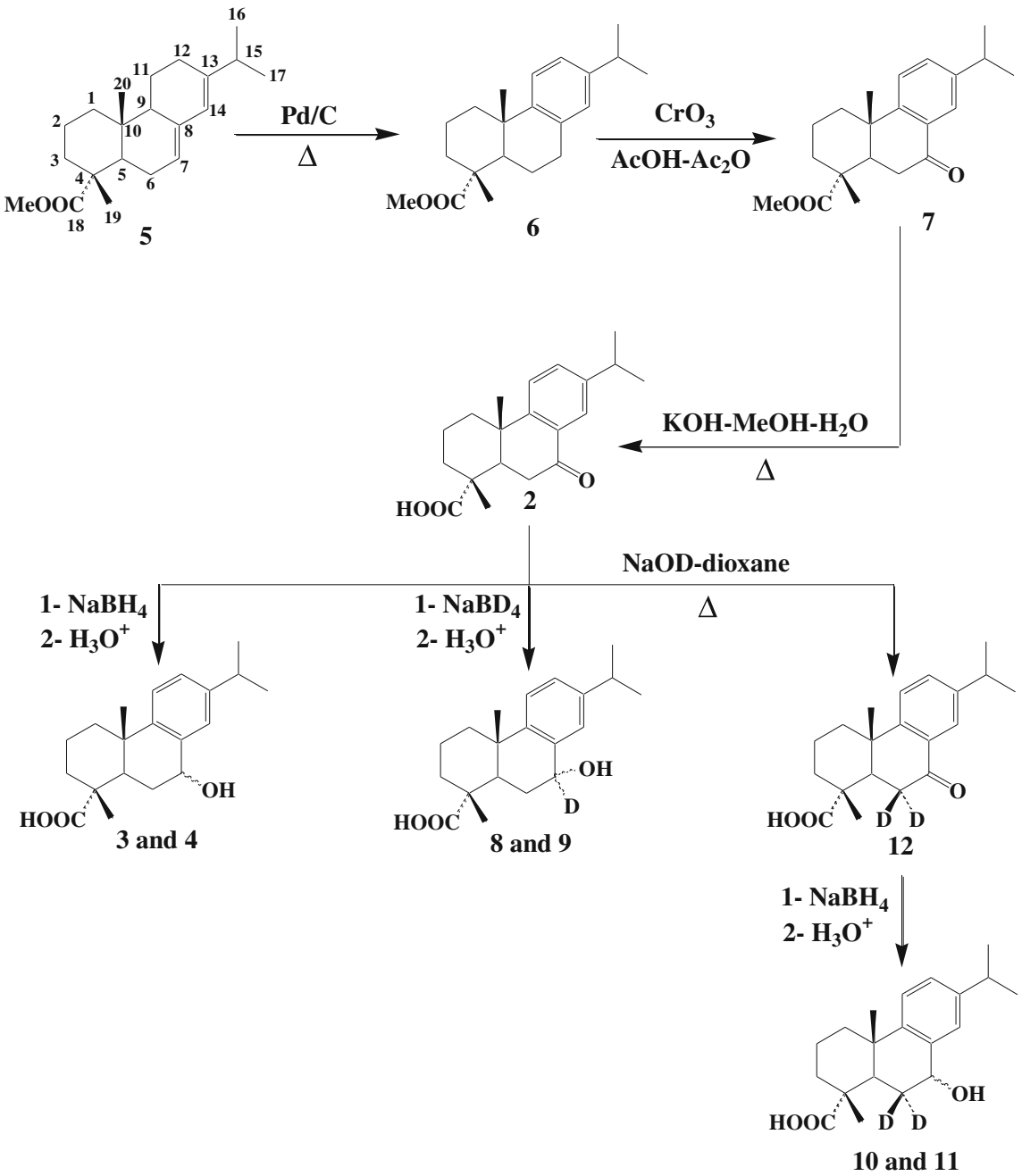
Synthesis of Deuterium-Labeled Dehydroabietic Acid Oxidation Products

[7-^{2}H]-7α- and [7-^{2}H]-7β-Hydroxy-dehydroabietic acids (**8** and **9**) were obtained by reduction of the ketoacid **2** with $NaBD_4$ instead of $NaBH_4$ as described above (85% yield) (Scheme 2).

[6-$^2H_2$]-7α- and [6-$^2H_2$]-7β-Hydroxy-dehydroabietic acids (**10** and **11**) were synthesized by reacting ketoacid **2** with a mixture of sodium (50 mg), deuterium oxide (2 mL), and anhydrous dioxane (2 mL) at 70°C under nitrogen for 20 min [17]. Under these conditions, isotopic exchange of enolic hydrogens (C-6) was >90%, giving deuterium-labeled ketone **12**. Subsequent $NaBH_4$-reduction of **12** afforded compounds **10** and **11** (Scheme 2).

Sea Ice and Sediment Samples

Arctic sea ice samples were collected from Resolute Passage (74.73 °N; 95.56 °W) in May/June 2012 as described by Belt et al. (2013) [18]. Sediment samples were obtained from a box core retrieved from a location slightly to the west of Resolute Passage, within the central channels of the Canadian Arctic islands (74.12 °N; 103.07 °W) as part of the IPY-CFL program in 2007 on board the CCGS Amundsen [18]. Sectioned subsamples were stored (<0°C) prior to analysis.



Scheme 2.

Sample Treatment

Chemical treatment of the samples ($NaBH_4$-reduction, alkaline hydrolysis) and subsequent purification (column chromatography) were carried out as described previously [19].

Derivatizations

Small quantities (5–20 µg) of compounds to be silylated were dissolved in 300 µL of a mixture of pyridine and BSTFA (2:1, v:v) and reacted at 50°C for 1 h. After evaporation to dryness under a stream of N_2, the TMS derivatives were redissolved in a mixture of ethyl acetate and BSTFA (to avoid desilylation of the samples) and analyzed by gas chromatography/electron impact mass spectrometry (GC-EIMS). Perdeuterated silyl derivatives were obtained using perdeuterated BSTFA (Figure 2b). Further, hydrolysis of unlabeled silyl derivatives of 7-hydroxydehydroabietic acids (by exposure to atmospheric moisture for a few hours) and subsequent silylation with perdeuterated BSTFA yielded deuterated silyl derivatives at the alcohol group only (Figure 2c).

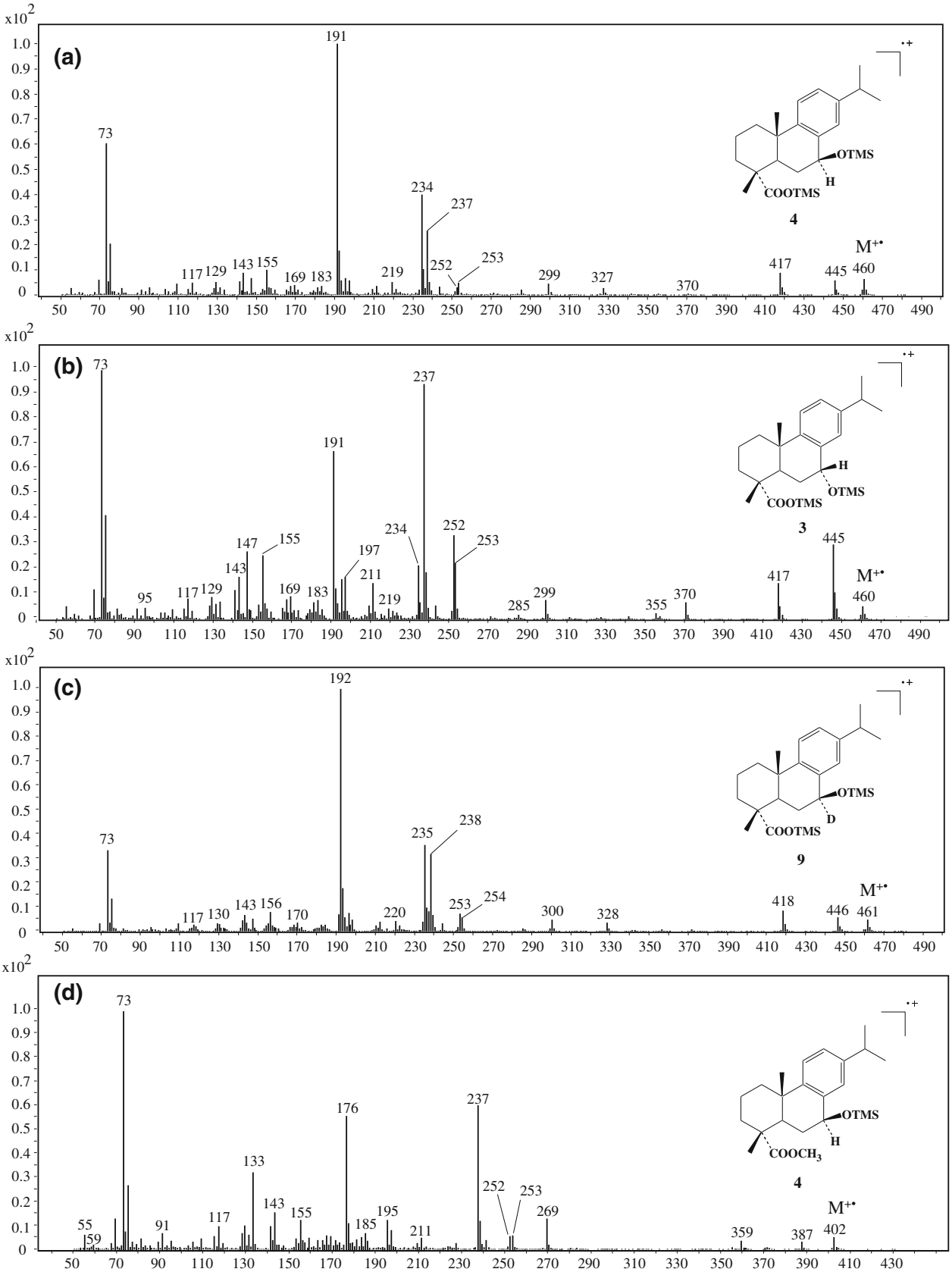
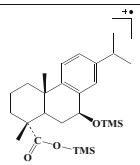
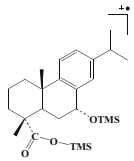
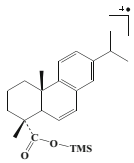
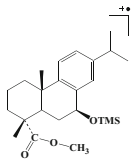
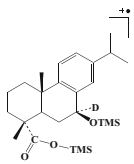
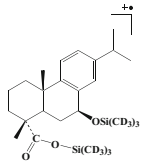
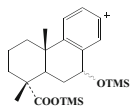
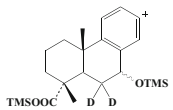
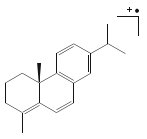
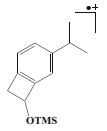


Figure 1. EI mass spectra of 7β-hydroxy-dehydroabietic acid (**4**) TMS derivative **(a)**, 7α-hydroxy-dehydroabietic acid (**3**) TMS derivative **(b)**, [7-^{2}H]-7β-hydroxy-dehydroabietic acid (**9**) TMS derivative **(c)**, and methyl 7β-hydroxy-dehydroabietate (**4**) TMS derivative **(d)**

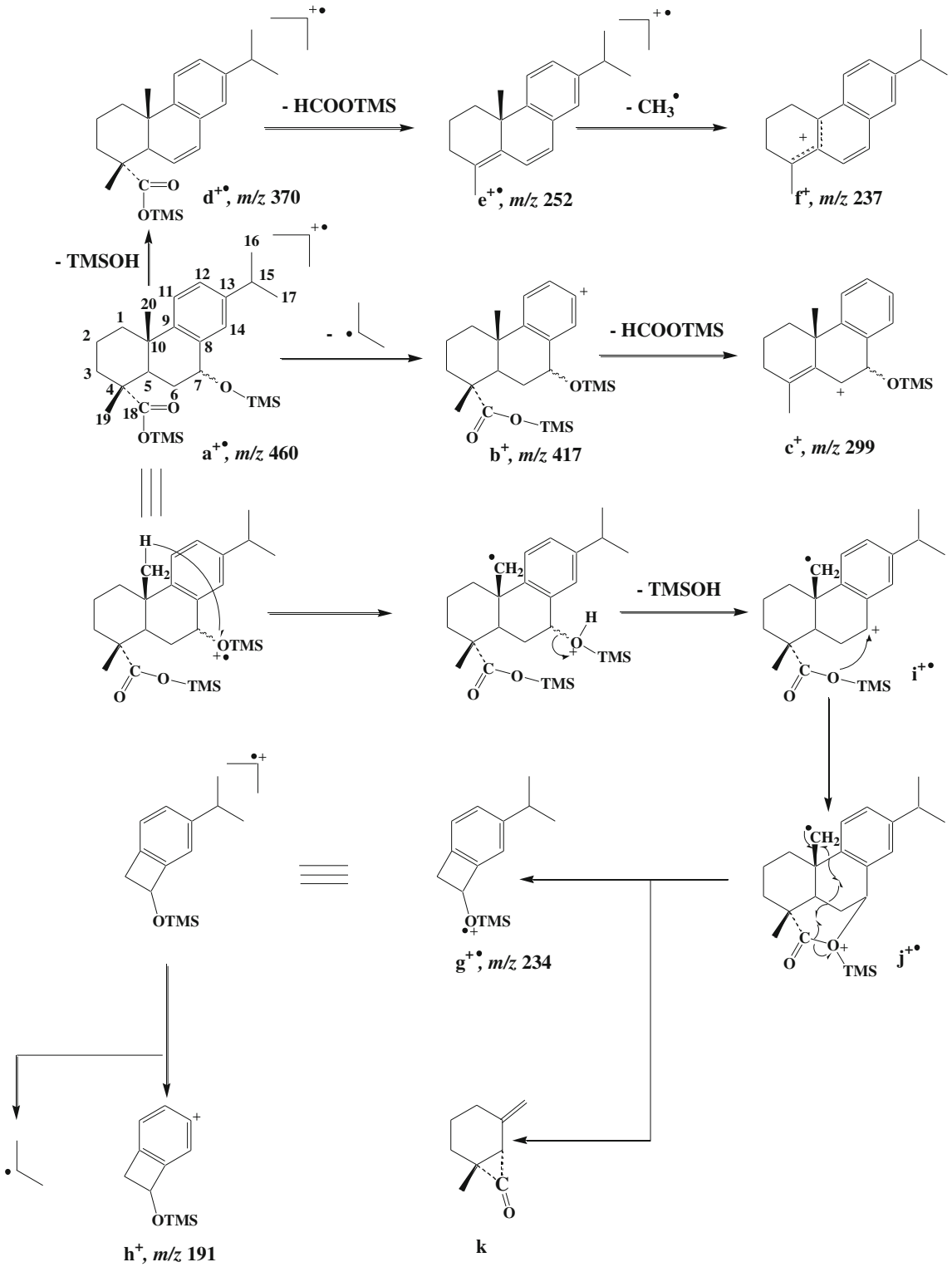
Table 1. CID Analyses of Labeled and Unlabeled Fragment Ions

Code	Precursor ion	*m/z*	Collision energy (eV)	Product ions
$\mathbf{a}^{+\bullet}$		460	10	460(5),417(93),299(35),243(17),237(2),234 (7),191(100)
$\mathbf{a}^{+\bullet}$		460	10	460(11),417(100),299(13),243(9),234(5), 237(2),191(38)
$\mathbf{d}^{+\bullet}$		370	10	370(4),252(12),237(38),224(17),210(17), 209(17),197(100),195(10),181(26),173(25)
$\mathbf{a}^{+\bullet}$		402	10	402(14),359(62),241(16),237(21),185(16), 176(100),133(24)
$\mathbf{a}^{+\bullet}$		461	10	461(15),418(100),300(8),244(10),235(14), 192(55)
$\mathbf{a}^{+\bullet}$		478	10	478(21),453(100),308(11),243(18),200(60)
$\mathbf{b}^{+}$		417	10	417(100),299(97),243(84),217(4),209(6),73 (5)
$\mathbf{b}^{+}$		419	10	419(70),300(100),244(61),243(11),73(8),69 (3)
$\mathrm{e}^{+\bullet}$		252	10	252(5),237(100),211(14),210(12),209(14), 195(27),169(10),155(12)
$\mathrm{g}^{+\bullet}$		234	5	234(6),219(2),191(100)

Compounds to be methylated were dissolved in 2 mL of BF_3/methanol (10%) and heated at 80°C for 1 h in a screw cap flask. After cooling, an excess of water was added and methyl esters were extracted three times with hexane, dried over anhydrous Na_2SO_4, filtered, and concentrated using rotary evaporation.

Gas Chromatography/Electron Ionization Tandem Mass Spectrometry

GC/EIMS and GC/EIMS/MS experiments were performed using an Agilent 7890A/7000A tandem quadrupole gas chromatograph system (Agilent Technologies, Parc Technopolis - ZA



Scheme 3.

Courtaboeuf, Les Ulis, France). A cross-linked 5% phenylmethylpolysiloxane (Agilent; HP-5MS) (30 m×0.25 mm, 0.25 µm film thickness) capillary column was employed. Analyses were performed with a multi-mode injector operating in splitless mode (with 0.5 min splitless period) set at 270°C and the oven temperature programmed from 70°C to 130°C at 20°C min^{-1}, then to 250°C at 5°C min^{-1} and then to 300°C at 3°C min^{-1}. The pressure of the carrier gas (He) was maintained at 0.69×10^5 Pa until the end of the temperature program and then programmed from 0.69×10^5 Pa to 1.49×10^5 Pa at 0.04×10^5 Pa min^{-1}. The following mass spectrometric conditions were employed: electron energy, 70 eV; source temperature, 230°C; quadrupole 1 temperature, 150°C; quadrupole 2 temperature, 150°C; collision gas (N_2) flow, 1.5 ml min^{-1}; quench gas (He)

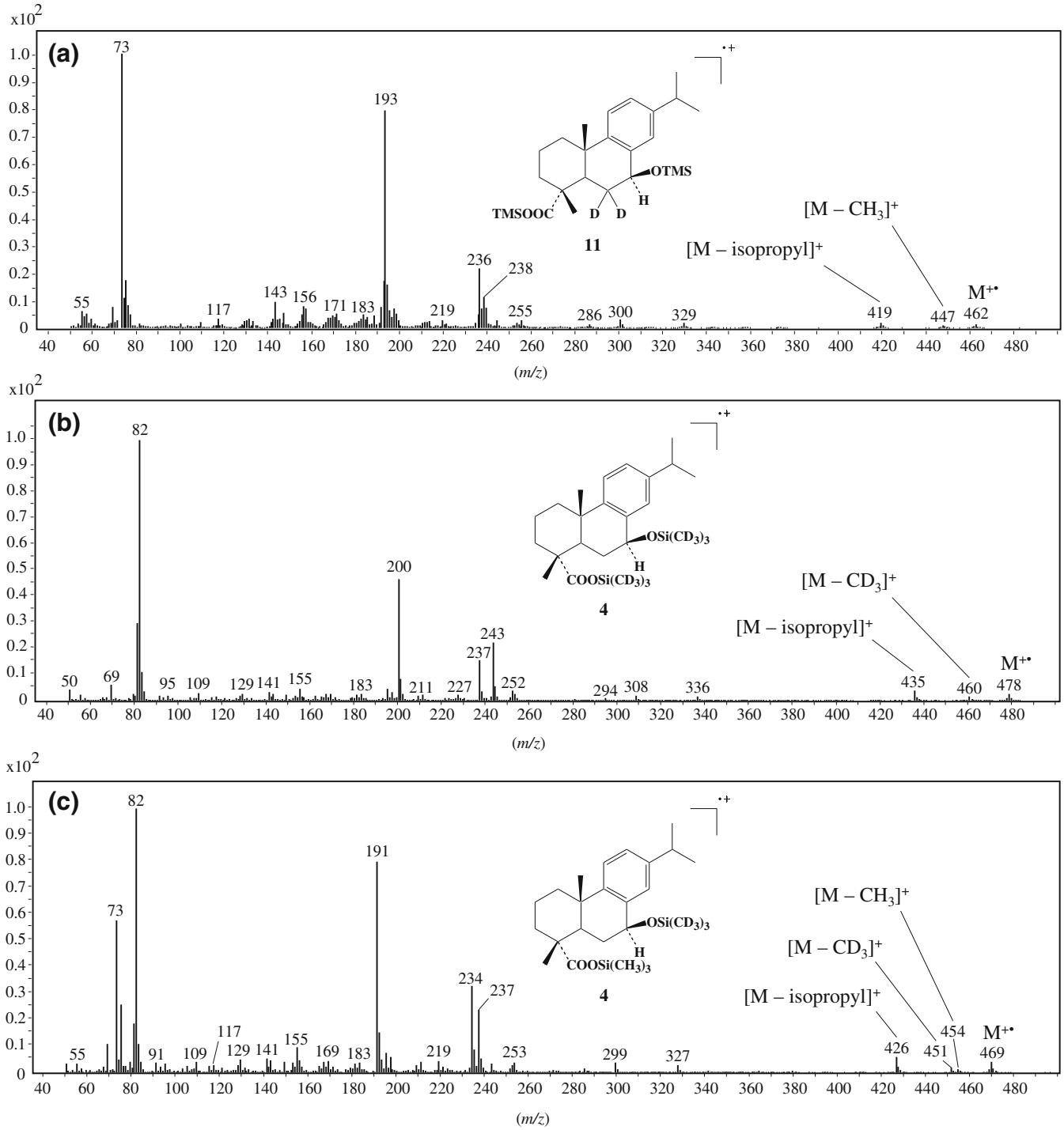


Figure 2. EI mass spectra of [6-$^2H_2$]-7β-hydroxy-dehydroabietic acid (**11**) TMS derivative **(a)**, 7β-hydroxy-dehydroabietic acid (**4**) perdeuterated TMS derivative **(b)**, and 7β-hydroxy-dehydroabietic acid (**4**) TMS derivative perdeuterated on the ether group but not on the ester group **(c)**

flow, 2.25 ml min^{-1}; mass range, 50–700 Da; cycle time, 313 ms. The use of collision induced dissociation (CID) was optimized by collision energies adjusted at 5, 10, 15, and 20 eV. Quantification of dehydroabietic acid (**1**) and its oxidation products (**3** and **4**) was carried out with external standards in MRM mode using the *m/z* 272→239, *m/z* 234→191, and *m/z* 252→237 transitions.

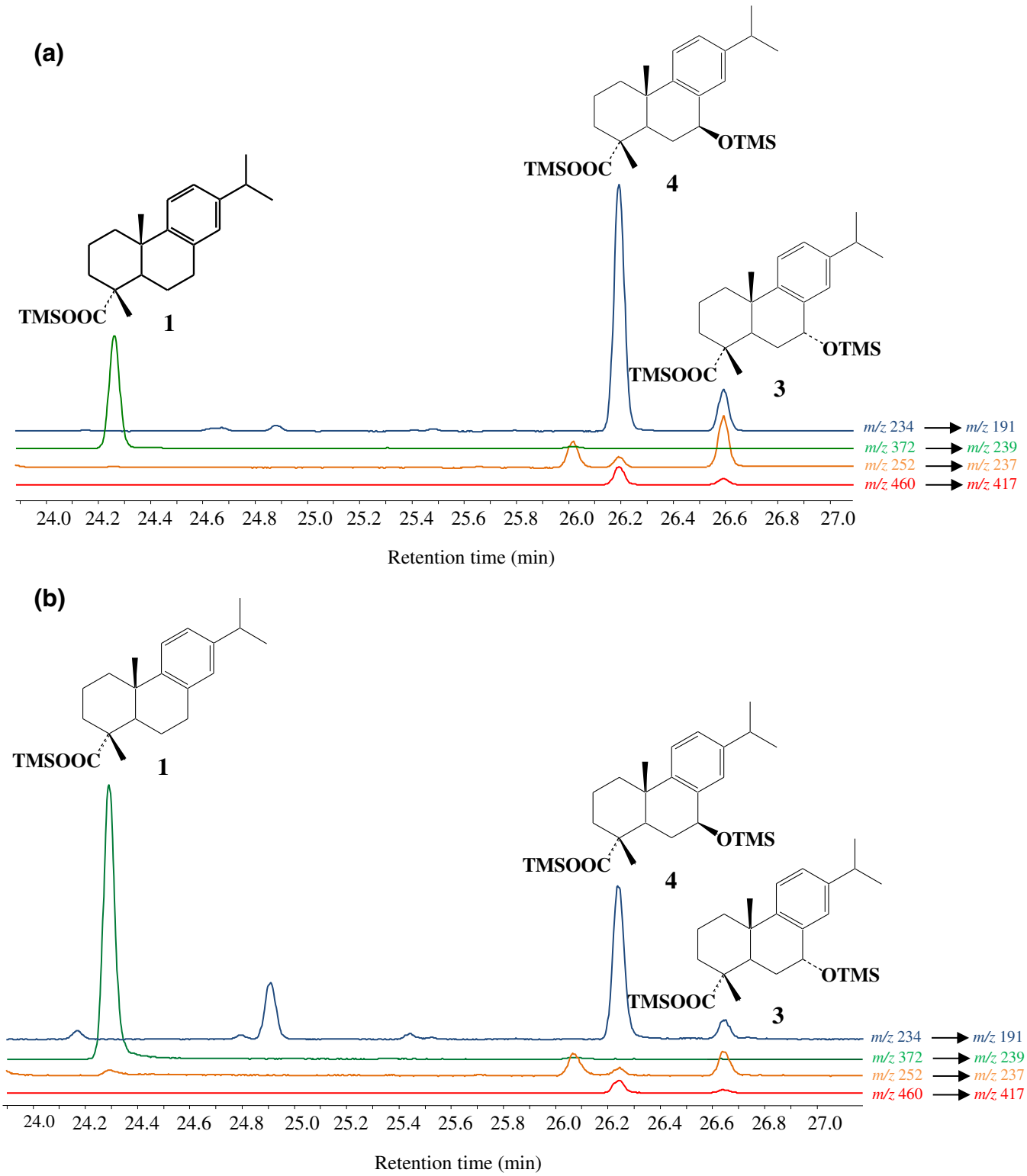


Figure 3. MRM chromatograms (*m/z* 234→191, *m/z* 252→237, *m/z* 372→239, and *m/z* 460→417) for a sea ice sample **(a),** and the 0–1 cm sediment layer **(b)**

Table 2. High-Accuracy Mass Spectral Data for Ions **a-h**

Code	Formula	*m/z* observed	*m/z* calculated	Δ (ppm)
$\mathbf{h}^+$	$C_{11}H_{15}OSi$	191.0889	191.0886	+1.6
$\mathbf{g}^{+\bullet}$	$C_{14}H_{22}OSi$	234.1433	234.1433	0
$\mathbf{f}^+$	$C_{18}H_{21}$	237.1634	237.1637	−1.3
$\mathbf{e}^{+\bullet}$	$C_{19}H_{24}$	252.1871	252.1871	0
$\mathbf{c}^+$	$C_{19}H_{27}OSi$	299.1827	299.1824	+1.0
$\mathbf{d}^{+\bullet}$	$C_{23}H_{34}O_2Si$	370.2316	370.2320	−1.1
$\mathbf{b}^+$	$C_{23}H_{37}O_3Si_2$	417.2279	417.2274	+1.2
$\mathbf{a}^{+\bullet}$	$C_{26}H_{44}O_3Si_2$	460.2819	460.2821	−0.4

Precursor ions were selected from the more intense ions (and specific fragmentations) observed in EI mass spectra.

Gas Chromatography/Electron Ionization Quadrupole Time of Flight Mass Spectrometry

Accurate mass measurements were carried out in full scan mode with an Agilent 7890B/7200 GC/QTOF System (Agilent Technologies, Parc Technopolis - ZA Courtaboeuf, Les Ulis, France). A cross-linked 5% phenyl-methylpolysiloxane (Agilent; HP-5MS ultra inert) (30 m×0.25 mm, 0.25 μm film thickness) capillary column was employed. Analyses were performed with an injector operating in split mode (1:10) set at 280°C and the oven temperature programmed from 70 to 130°C at 20°C min^{-1}, then to 250°C at 5°C min^{-1}, and then to 300°C at 3°C min^{-1}. The pressure of the carrier gas (He) was maintained at 0.69×10^5 Pa until the end of the temperature program. Instrument temperatures were 300°C for transfer line and 230°C for the ion source. Accurate mass spectra were recorded across the range *m/z* 50–700 at 4 GHz. The QTOF-MS instrument provided a typical resolution ranging from 8009 to 12252 from *m/z* 68.9955 to 501.9706. Perfluorotributylamine (PFTBA) was utilized for daily MS calibration.

Results and discussion

EIMS Fragmentations of TMS Derivatives of 7α/β-Hydroxy-Dehydroabietic Acids (3 and 4)

The EI mass spectra of the TMS derivatives of 7β-hydroxy-dehydroabietic (**4**) and 7α-hydroxy-dehydroabietic (**3**) acids (Figure 1a and b) both exhibit a small molecular ion at *m/z* 460 and a fragment ion at *m/z* 445 resulting from the classic loss of a methyl radical from the ionized TMS groups [20]. Further ions at *m/z* 191, 234, 237, 252, 299, 370, and 417 are also observed, although the relative abundances differ strongly between the two isomers.

As confirmed by CID analysis (Table 1), the fragment ion at *m/z* 417 ($\mathbf{b}^+$) results from the loss of the isopropyl chain from the molecular ion ($\mathbf{a}^{+\bullet}$) (Scheme 3). Although generally considered as energetically unfavorable, this type of bond cleavage between a substituent and the aromatic ring can occur especially in the case of α-branched aromatic rings [21] such as for **3** and **4**. The CID analyses also showed that the $\mathbf{b}^+$ ion represents the source of the fragment ion $\mathbf{c}^+$ at *m/z* 299 (Table 1). This conversion involves the loss of a neutral molecule of trimethylsilyl formate, a common fragmentation in TMS derivatives [22]. In this case, we suggest that trimethylsilyl formate is produced from the trimethylsilyl carboxyl group at C-4 and a hydrogen atom at C-6 (Scheme 3). Indeed, CID analyses of dideuterated (C-6) $\mathbf{b}^+$ confirmed that the hydrogen (deuterium) atom involved in this loss was located at C-6 (Table 1).

The molecular ion $\mathbf{a}^{+\bullet}$ can also lose neutral trimethylsilanol to produce the fragment ion $\mathbf{d}^{+\bullet}$ (*m/z* 370). Subsequent loss of trimethylsilyl formate from $\mathbf{d}^{+\bullet}$ yields the highly conjugated ion $\mathbf{e}^{+\bullet}$ (*m/z* 252), which can then lose the methyl group at C-10 to give the fragment ion $\mathbf{f}^+$ (*m/z* 237), strongly stabilized by conjugation (Scheme 3). This fragmentation assignment was confirmed by CID analyses of $\mathbf{d}^{+\bullet}$ and $\mathbf{e}^{+\bullet}$ (Table 1) and supported further by the shift of $\mathbf{f}^+$ to *m/z* 238 in the EI mass spectra of [7-^{2}H]-7β-hydroxy-dehydroabietic acid (**9**) (Figure 1c) and [6-$^2H_2$]-7β-hydroxy-dehydroabietic acid (**11**) (Figure 2a).

The formation pathways of the fragment ions $\mathbf{g}^{+\bullet}$ (*m/z* 234) and $\mathbf{h}^+$ (*m/z* 191) are more difficult to explain with certainty. The shift of these ions to *m/z* 243 and 200, respectively, in the EI mass spectrum of 7β-hydroxy-dehydroabietic perdeuterated trimethylsilyl derivative (Figure 2b) did, however, allow us to demonstrate the presence of a TMS-O- group in both. Interestingly, $\mathbf{g}^{+\bullet}$ and $\mathbf{h}^+$ shifted by 58 Da in the EI mass spectrum of methyl 7β-hydroxy-dehydroabietate trimethylsilyl derivative (Figure 1d), but did not shift when only the silyl derivative of the alcohol group was perdeuterated (Figure 2c). These observations clearly show that this is the OTMS of the ester group

Table 3. Concentrations (pg mL^{-1}) of DHAA and Its Degradation Products in Sea Ice Samples Corresponding to 3–10 cm Sections

Sample	Depth (cm)[a]	Sampling date	DHAA (**1**) (pg ml^{-1})	DHAA-7βOH[b] (**4**) (pg ml^{-1})	DHAA-7αOH[c] (**3**) (pg ml^{-1})	DHAA degradation percentage
B1	3–10	19/05/12	10.8	23.5	7.8	74.3
B2	3–10	27/05/12	19.0	13.5	3.2	46.8
B3	3–10	31/05/12	18.3	127.8	21.5	89.1
B4	3–10	04/06/12	16.7	13.3	2.4	48.6
B5	3–10	08/06/12	10.8	3.8	1.4	32.5
B6	3–10	12/06/12	9.3	20.5	4.0	72.5

[a] Depth corresponds to distance from the bottom ice-seawater interface.
[b] 7β-Hydroxy-dehydroabietic acid.
[c] 7α-Hydroxy-dehydroabietic acid.

Table 4. Concentrations (ng g^{-1} d.w.) of DHAA and Its Degradation Products in Sediment Samples

Depth (cm)	DHAA (**1**) (ng g^{-1} d.w.)	DHAA-7βOH[a] (**4**) (ng g^{-1} d.w.)	DHAA-7αOH[b] (**3**) (ng g^{-1} d.w.)	DHAA degradation percentage
0–1	0.52	0.29	0.04	38.8
2–3	0.44	0.10	0.01	20.0
4–5	0.26	0.08	0.02	27.7
6–7	1.16	0.24	0.03	18.9
8–9	0.88	0.10	0.02	12.0
10–11	1.12	0.10	0.02	9.7

[a] 7β-Hydroxy-dehydroabietic acid.
[b] 7α-Hydroxy-dehydroabietic acid.

(C-18) and not the OTMS of the ether group (C-7), which is present in ions $\mathbf{g}^{+\bullet}$ and $\mathbf{h}^{+}$. On the basis of these observations and of the higher abundance of these ions in the EI mass spectrum of the β-isomer (Figure 1a and b), we propose the fragmentation pathways shown in Scheme 3. These pathways involve initial transfer of a hydrogen atom from the β-methyl group (C-20) of $\mathbf{a}^{+\bullet}$ to the ionized OTMS group at C-7 and subsequent elimination of trimethylsilanol to yield the distonic radical cation $\mathbf{i}^{+\bullet}$, which can cyclize via the ester group at C-18 to produce $\mathbf{j}^{+\bullet}$. Concerted cleavage of $\mathbf{j}^{+\bullet}$ produces ion $\mathbf{g}^{+\bullet}$ (*m/z* 234) and the neutral fragment **k**. Further support for such assignments comes from the observed transformation of $\mathbf{g}^{+\bullet}$ (*m/z* 234) to $\mathbf{h}^{+}$ (*m/z* 191) attributed to the loss of the isopropyl group and confirmed by CID analysis (Table 1). The shift of $\mathbf{g}^{+\bullet}$ and $\mathbf{h}^{+}$ to *m/z* 235 and 192, and to *m/z* 236 and 193 in the EI mass spectra of the trimethylsilyl derivatives of [7-^{2}H]-7β-hydroxy-dehydroabietic acid (**9**) (Figure 1c) and [6-$^{2}H_2$]-7β-hydroxy-dehydroabietic acid (**11**) (Figure 2a), respectively, enabled us to confirm that carbon atoms 6 and 7 are present in these ions and, thus, further support the proposed fragmentation pathways. The higher abundance of the ions $\mathbf{g}^{+\bullet}$ (*m/z* 234) and $\mathbf{h}^{+}$ (*m/z* 191) in the EI mass spectrum of the β-isomer **4** (Figure 1a) can be rationalized in terms of a more efficient transfer of the hydrogen atom from C-20 to the ether group carried at C-7 when both groups are on the same side of the cyclic structure (i.e., in the β-face).

Accurate mass measurements (GC-QTOF) were also performed to confirm the proposed structures of the main fragment ions resulting from EI fragmentation of the trimethylsilyl derivatives of compounds **3** and **4**. The measured masses of ions **a-h** showed only weak deviations (ranging from 0 to 1.6 ppm) from the calculated molecular masses (Table 2), thus confirming the elemental composition of the fragment ions in each case.

*Application: MRM Analysis of Dehydroabietic Acid (**1**) and Its Oxidation Products (**3** and **4**) in Arctic Sea Ice and Sediment Samples*

The fragmentation pathways described here were employed to identify and quantify traces of DHAA and its oxidation products in natural samples. For this purpose, we analyzed some lipid fractions obtained following $NaBH_4$-reduction and alkaline hydrolysis of some Arctic sea ice samples, shown previously to provide a host for terrestrially derived OM in the marine environment [19]. We also analyzed lipid extracts of underlying sediments from a nearby location since, within this region, significant amounts of terrigenous particulate organic matter are known to be transported from the shelves further offshore and within the network of island channels by different processes such as freezing into, and subsequent release from, drifting sea-ice, ocean currents, and turbidity currents [23]. Due to the use of a reduction step, the combined abundances of 7-hydroperoxy-, 7-oxo-, and 7-hydroxy-dehydroabietic acids were analyzed in the form of 7α/β-hydroxy-dehydroabietic acids. These fractions were analyzed in MRM mode using the *m/z* 234→191 and *m/z* 252→237 transitions for the TMS derivatives of 7α/β-hydroxy-dehydroabietic acids (**3** and **4**) and the *m/z* 472→239 transition for the TMS ester of the parent DHAA (**1**) [24] (Figure 3). Comparison of these mass spectral data with those obtained from standards allowed unambiguous characterization of compounds **3** and **4** in the different extracts. Quantification of the oxidation products relative to the parent compounds (Tables 3 and 4) confirmed the very high in situ reactivity of DHAA (**1**) towards degradation processes.

Conclusions

EIMS fragmentations of TMS derivatives of 7α/β-hydroxy-dehydroabietic acids (**3** and **4**) derived from $NaBH_4$-reduction of oxidation products of DHAA (**1**) (a component of coniferous resin) were investigated. A combination of CID-MS/MS, deuterium labeling, accurate mass measurements, and chemical derivatizations provided a complementary approach for elucidating these fragmentation pathways. On the basis of these fragmentations, some MRM transitions were selected and applied to extracts from Arctic sea ice and sediment samples. The results obtained demonstrate the high reactivity of DHAA (**1**) towards biotic and abiotic degradation processes.

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