

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

**Jean-François Rontani, Claude Aubert &
Simon T. Belt**

**Journal of The American Society for
Mass Spectrometry**

The official journal of The American
Society for Mass Spectrometry

ISSN 1044-0305

J. Am. Soc. Mass Spectrom.
DOI 10.1007/s13361-015-1157-3

Volume 26 Number 7

**ONLINE
FIRST**

Journal of The American Society for
MASS SPECTROMETRY

In this Issue:

Critical Insight:
Gas-phase Protein
Structure by ECD

Articles on:

- Proteins and Peptides
- Gas-phase HDX
- Non-covalent Binding
- Charge Determination
- Ion-Ion Reactions
- APPI Response

Congratulations, Graham Cooks, for your election to the
National Academy of Sciences, see page 1057.

ISSN 1044-0305 • 26 (7) 1047–1258 (2015) • 13361

**AS
MS**

Your article is protected by copyright and all rights are held exclusively by American Society for Mass Spectrometry. This e-offprint is for personal use only and shall not be self-archived in electronic repositories. If you wish to self-archive your article, please use the accepted manuscript version for posting on your own website. You may further deposit the accepted manuscript version in any repository, provided it is only made publicly available 12 months after official publication or later and provided acknowledgement is given to the original source of publication and a link is inserted to the published article on Springer's website. The link must be accompanied by the following text: "The final publication is available at link.springer.com".

RESEARCH ARTICLE

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

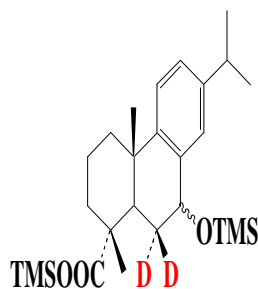
Jean-François Rontani,^{1,2} Claude Aubert,³ Simon T. Belt⁴

¹Mediterranean Institute of Oceanography (MIO), Aix-Marseille Université, 13288, Marseille, Cedex 9, France

²Université du Sud Toulon-Var, 83957, CNRS-INSU/IRD UM 110, La Garde Cedex, France

³Laboratoire de Pharmacocinétique et Toxicocinétique (EA 3286), Faculté de Pharmacie, 13385, Marseille, France

⁴Biogeochemistry Research Centre, School of Geography, Earth and Environmental Sciences, University of Plymouth, Drake Circus, Plymouth, Devon PL4 8AA, UK



Abstract. EI mass fragmentation pathways of TMS derivatives of 7 α / β -hydroxy-dehydroabietic acids resulting from NaBH⁴-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 7 α / 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: 7 α / β -Hydroxy-dehydroabietic acids, TMS derivatives, EI fragmentations, MRM quantification, Natural samples

Received: 12 March 2015/Revised: 30 March 2015/Accepted: 30 March 2015

Introduction

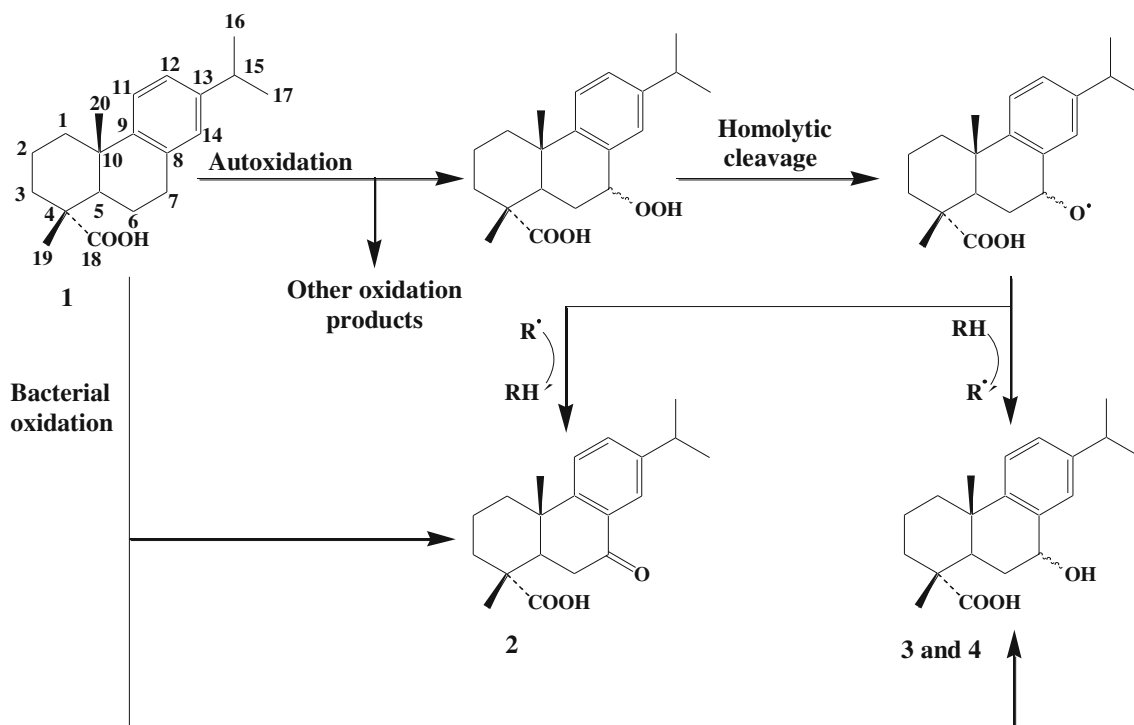
Understanding the fate of terrestrial organic matter (OM) in the ocean has intrigued scientists for decades and is necessary for understanding the global carbon cycle and its anthropogenic perturbations [1]. Since riverine particulate OM consists, in part, of already highly degraded residues from terrestrial 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.

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), 7 α / 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 7 α / 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

Correspondence to: Jean-François Rontani; e-mail: jean-francois.rontani@mio.osupytheas.fr



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%), NaBH₄, NaBD₄ (98.0 atom% D), deuterium oxide (99.9 atom% D), sodium, acetic anhydride, chromium oxide, Pd/C, potassium hydroxide, anhydrous dioxane and pyridine, BF₃/methanol (10%), and solvents were purchased from Sigma-Aldrich (St. Quentin Fallavier, France). Unlabeled and perdeuterated (99.0 atom% D) *N,O*-bis(trimethylsilyl)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 N₂ at 240°C affording methyl dehydroabietate (**6**) (85% yield), (2) oxidation of **6** with CrO₃ 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).

7 α - and 7 β -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 NaBH₄ (90% yield) (Scheme 2).

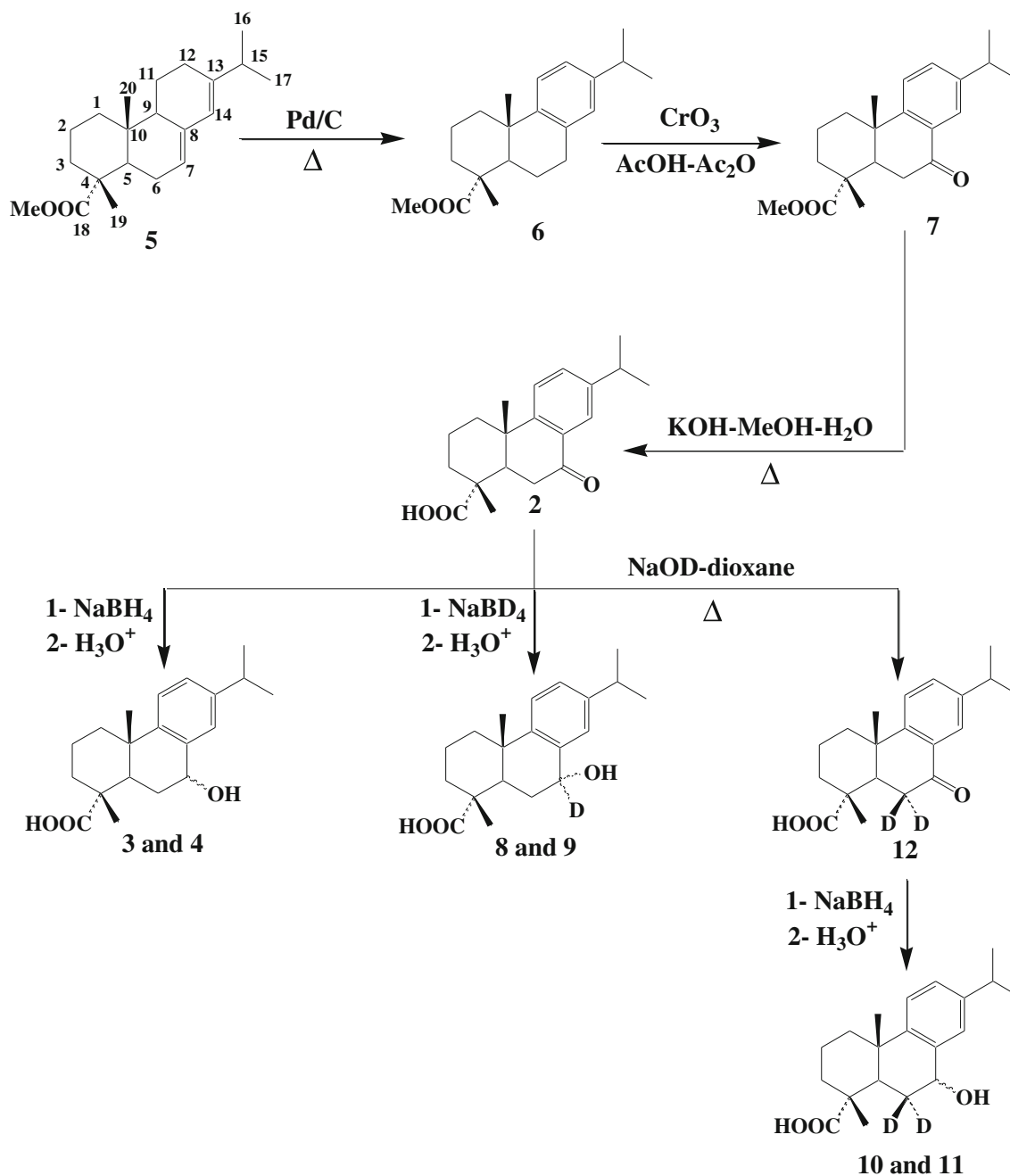
Synthesis of Deuterium-Labeled Dehydroabietic Acid Oxidation Products

[7-²H]-7 α - and [7-²H]-7 β -Hydroxy-dehydroabietic acids (**8** and **9**) were obtained by reduction of the ketoacid **2** with NaBD₄ instead of NaBH₄ as described above (85% yield) (Scheme 2).

[6-²H₂]-7 α - and [6-²H₂]-7 β -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 NaBH₄-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).

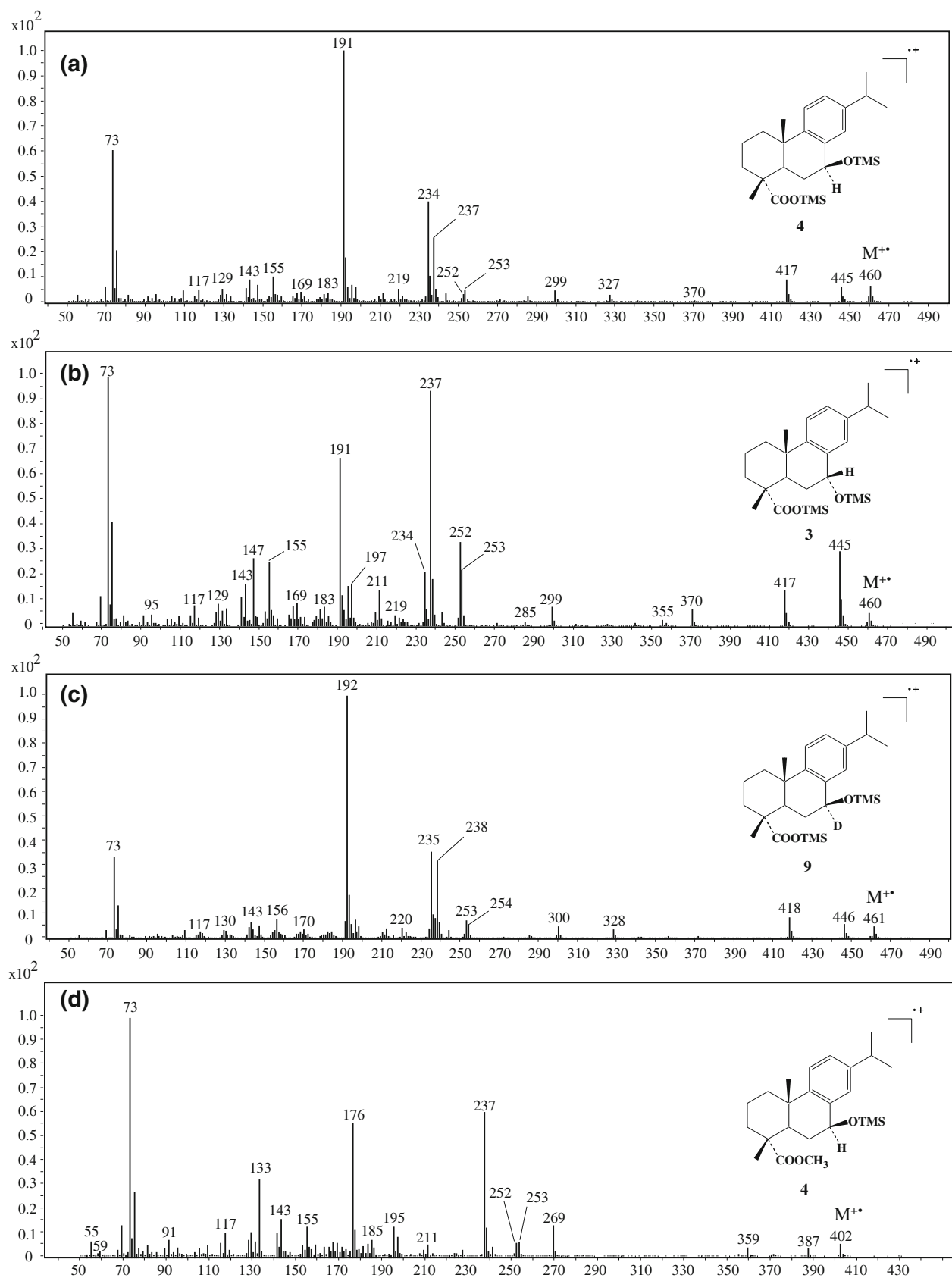


Figure 1. EI mass spectra of 7β-hydroxy-dehydroabietic acid (**4**) TMS derivative (**a**), 7α-hydroxy-dehydroabietic acid (**3**) TMS derivative (**b**), [7-²H]-7β-hydroxy-dehydroabietic acid (**9**) TMS derivative (**c**), and methyl 7β-hydroxy-dehydroabietic acid (**4**) TMS derivative (**d**)

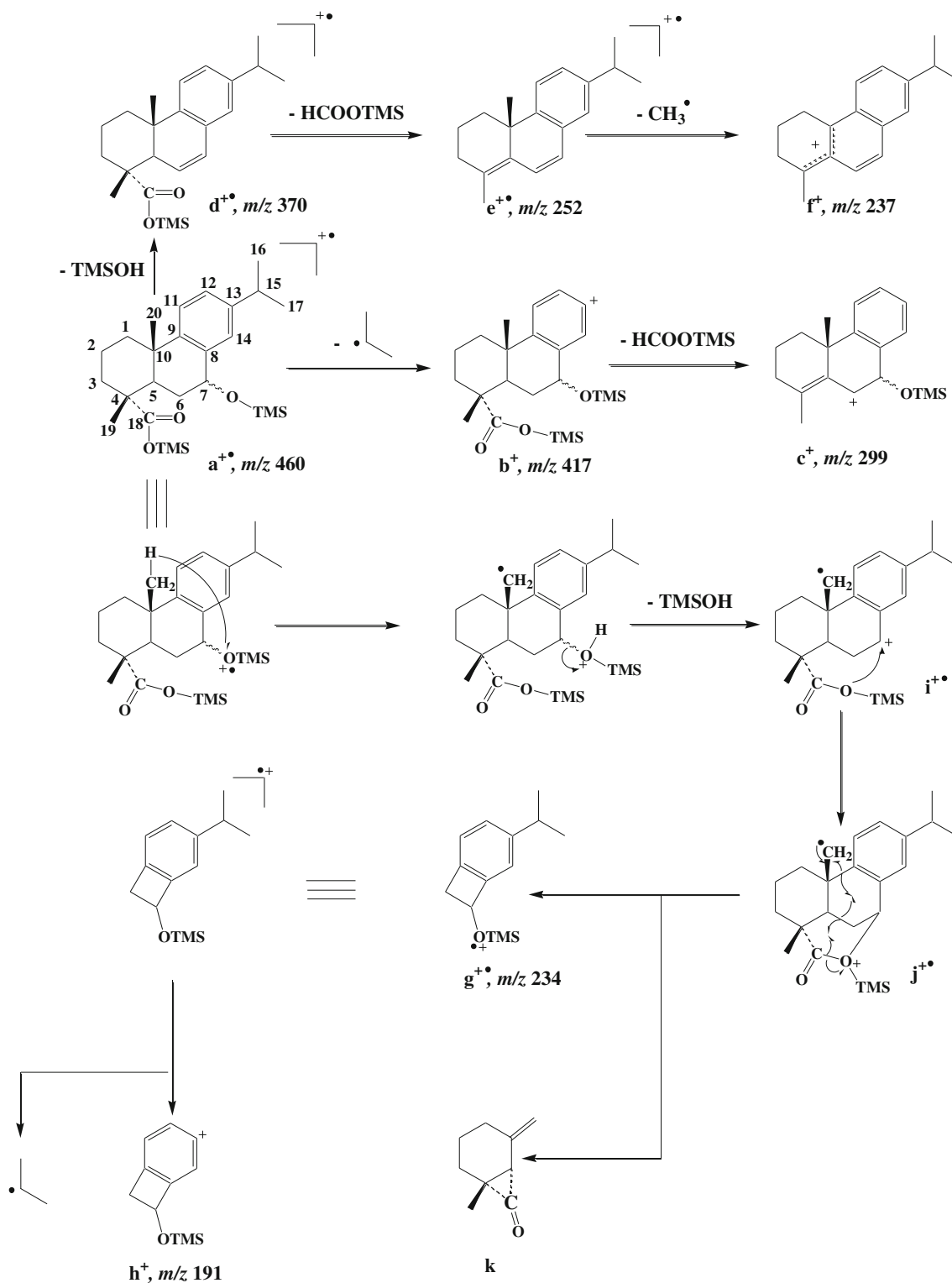
Table 1. CID Analyses of Labeled and Unlabeled Fragment Ions

| Code | Precursor ion | <i>m/z</i> | Collision energy (eV) | Product ions |
|-----------------------|---------------|------------|-----------------------|---|
| a⁺⁺ | | 460 | 10 | 460(5),417(93),299(35),243(17),237(2),234(7),191(100) |
| a⁺⁺ | | 460 | 10 | 460(11),417(100),299(13),243(9),234(5),237(2),191(38) |
| d⁺⁺ | | 370 | 10 | 370(4),252(12),237(38),224(17),210(17),209(17),197(100),195(10),181(26),173(25) |
| a⁺⁺ | | 402 | 10 | 402(14),359(62),241(16),237(21),185(16),176(100),133(24) |
| a⁺⁺ | | 461 | 10 | 461(15),418(100),300(8),244(10),235(14),192(55) |
| a⁺⁺ | | 478 | 10 | 478(21),453(100),308(11),243(18),200(60) |
| b⁺ | | 417 | 10 | 417(100),299(97),243(84),217(4),209(6),73(5) |
| b⁺ | | 419 | 10 | 419(70),300(100),244(61),243(11),73(8),69(3) |
| e⁺⁺ | | 252 | 10 | 252(5),237(100),211(14),210(12),209(14),195(27),169(10),155(12) |
| g⁺⁺ | | 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 0.69×10⁵ Pa until the end of the temperature program and then programmed from 0.69×10⁵ Pa to 1.49×10⁵ Pa at 0.04×10⁵ 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 (N₂) flow, 1.5 ml min⁻¹; quench gas (He)

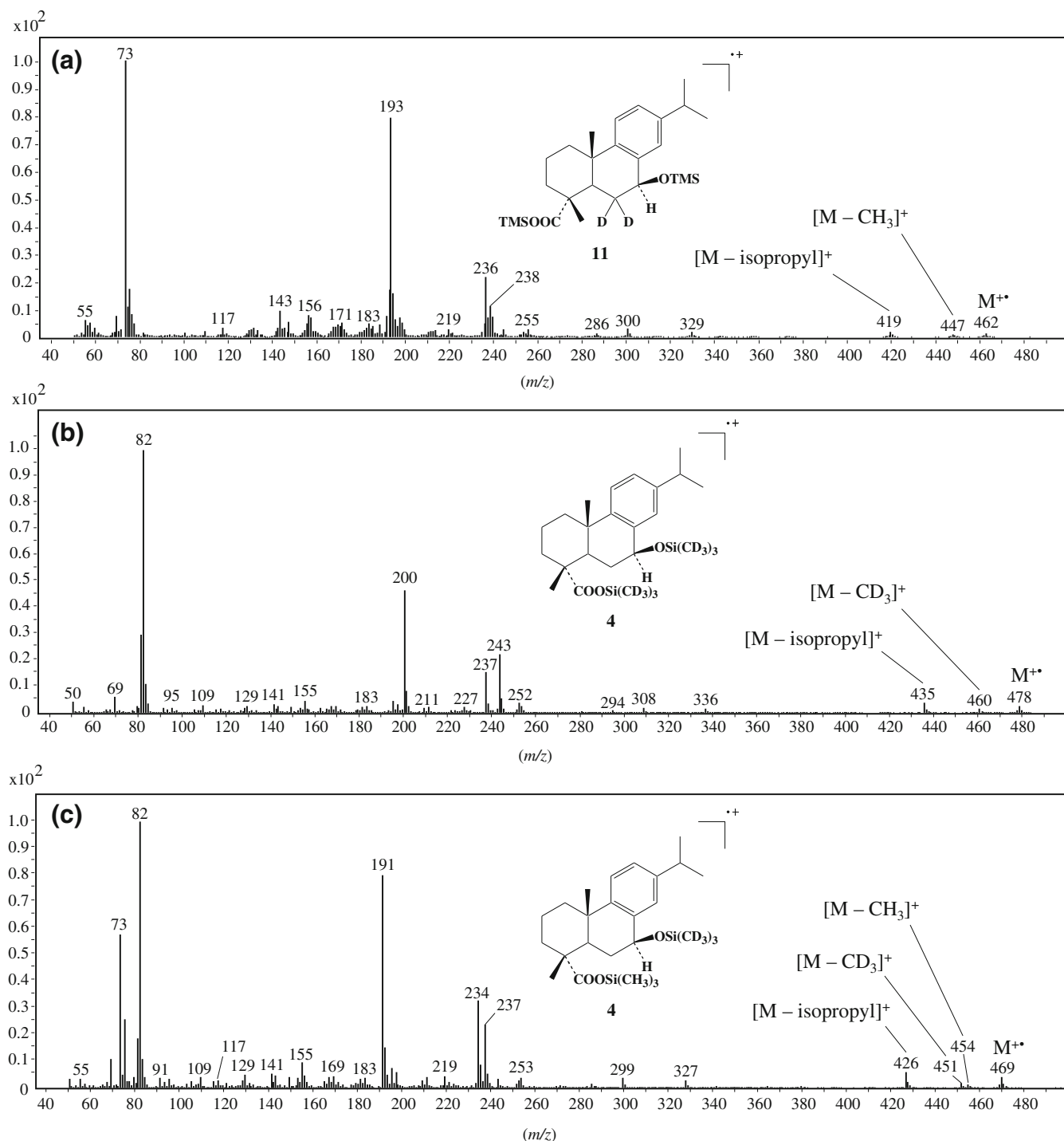


Figure 2. EI mass spectra of [6-²H₂]-7β-hydroxy-dehydroabietic acid (11) TMS derivative (a), 7β-hydroxy-dehydroabietic acid (4) perdeuterated TMS derivative (b), and 7β-hydroxy-dehydroabietic acid (4) TMS derivative perdeuterated on the ether group but not on the ester group (c)

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.

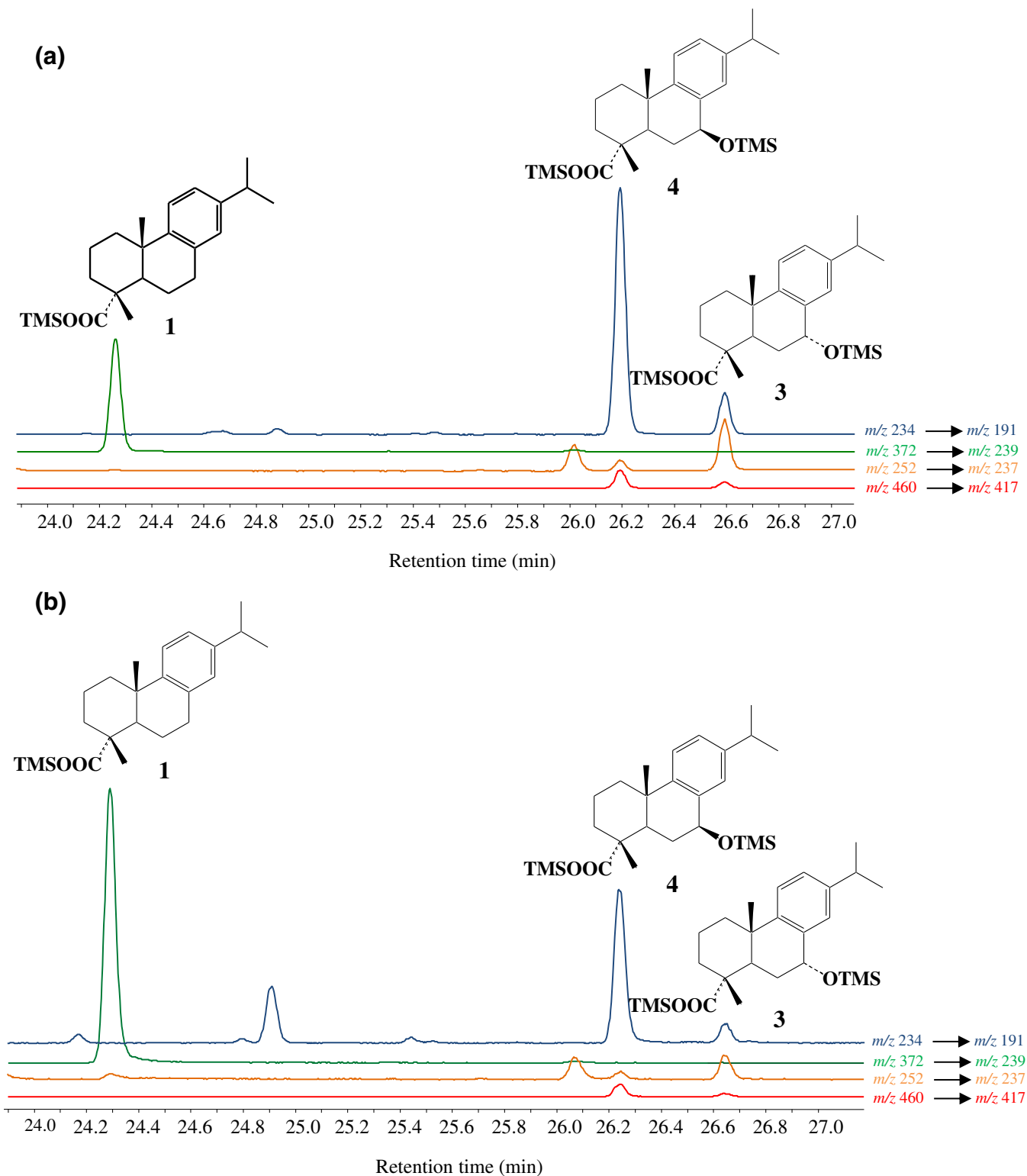


Figure 3. MRM chromatograms (m/z 234→191, m/z 252→237, m/z 372→239, and m/z 460→417) for a sea ice sample (a), and the 0–1 cm sediment layer (b)

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

| Code | Formula | <i>m/z</i> observed | <i>m/z</i> calculated | Δ (ppm) |
|------------------------|--|---------------------|-----------------------|----------------|
| h ⁺ | C ₁₁ H ₁₅ OSi | 191.0889 | 191.0886 | +1.6 |
| g ⁺⁺ | C ₁₄ H ₂₂ OSi | 234.1433 | 234.1433 | 0 |
| f ⁺ | C ₁₈ H ₂₁ | 237.1634 | 237.1637 | -1.3 |
| e ⁺⁺ | C ₁₉ H ₂₄ | 252.1871 | 252.1871 | 0 |
| c ⁺ | C ₁₉ H ₂₇ OSi | 299.1827 | 299.1824 | +1.0 |
| d ⁺⁺ | C ₂₃ H ₃₄ O ₂ Si | 370.2316 | 370.2320 | -1.1 |
| b ⁺ | C ₂₃ H ₃₇ O ₃ Si ₂ | 417.2279 | 417.2274 | +1.2 |
| a ⁺⁺ | C ₂₆ H ₄₄ O ₃ Si 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 μ m 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 0.69×10⁵ 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 7 α / β -Hydroxy-Dehydroabietic Acids (**3** and **4**)

The EI mass spectra of the TMS derivatives of 7 β -hydroxy-dehydroabietic (**4**) and 7 α -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 (**b**⁺) results from the loss of the isopropyl chain from the molecular ion (**a**⁺⁺) (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 **b**⁺ ion represents the source of the fragment ion **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) **b**⁺ confirmed that the hydrogen (deuterium) atom involved in this loss was located at C-6 (Table 1).

The molecular ion **a**⁺⁺ can also lose neutral trimethylsilanol to produce the fragment ion **d**⁺⁺ (*m/z* 370). Subsequent loss of trimethylsilyl formate from **d**⁺⁺ yields the highly conjugated ion **e**⁺⁺ (*m/z* 252), which can then lose the methyl group at C-10 to give the fragment ion **f**⁺ (*m/z* 237), strongly stabilized by conjugation (Scheme 3). This fragmentation assignment was confirmed by CID analyses of **d**⁺⁺ and **e**⁺⁺ (Table 1) and supported further by the shift of **f**⁺ to *m/z* 238 in the EI mass spectra of [7-²H]-7 β -hydroxy-dehydroabietic acid (**9**) (Figure 1c) and [6-²H₂]-7 β -hydroxy-dehydroabietic acid (**11**) (Figure 2a).

The formation pathways of the fragment ions **g**⁺⁺ (*m/z* 234) and **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 7 β -hydroxy-dehydroabietic perdeuterated trimethylsilyl derivative (Figure 2b) did, however, allow us to demonstrate the presence of a TMS-O- group in both. Interestingly, **g**⁺⁺ and **h**⁺ shifted by 58 Da in the EI mass spectrum of methyl 7 β -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-7 β OH ^b (4) (pg mL ⁻¹) | DHAA-7 α OH ^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 7 β -Hydroxy-dehydroabietic acid.

^c 7 α -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-dehydroabiatic acid.^b 7α-Hydroxy-dehydroabiatic acid.

(C-18) and not the OTMS of the ether group (C-7), which is present in ions **g**⁺ and **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 **a**⁺ to the ionized OTMS group at C-7 and subsequent elimination of trimethylsilanol to yield the distonic radical cation **i**⁺, which can cyclize via the ester group at C-18 to produce **j**⁺. Concerted cleavage of **j**⁺ produces ion **g**⁺ (*m/z* 234) and the neutral fragment **k**. Further support for such assignments comes from the observed transformation of **g**⁺ (*m/z* 234) to **h**⁺ (*m/z* 191) attributed to the loss of the isopropyl group and confirmed by CID analysis (Table 1). The shift of **g**⁺ and **h**⁺ to *m/z* 235 and 192, and to *m/z* 236 and 193 in the EI mass spectra of the trimethylsilyl derivatives of [7-²H]-7β-hydroxy-dehydroabiatic acid (**9**) (Figure 1c) and [6-²H₂]-7β-hydroxy-dehydroabiatic 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 **g**⁺ (*m/z* 234) and **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 Dehydroabiatic 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 NaBH₄-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-dehydroabiatic acids were analyzed in the form of 7α/β-hydroxy-dehydroabiatic 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-dehydroabiatic 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-dehydroabiatic acids (**3** and **4**) derived from NaBH₄-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.

Acknowledgments

The authors acknowledge support for this work by the LEFE-CYBER (Les Enveloppes Fluides et l'Environnement) national program, as part of the MORTIMER (réévaluation de la labilité biotique et abiotique de la Matière Organique Terrestre rejetée par les fleuves et les rivières en MER) research program. It is part of the transversal research axis DEBAT of the

Mediterranean Institute of Oceanography, Marseille, France. Thanks are due to the FEDER OCEANOMED for the funding of the apparatus employed during this work. The authors also thank the officers and crew of the CCGS Amundsen for help in obtaining sediment samples.

References

- Hedges, J.I., Keil, R.G.: Sedimentary organic matter preservation: an assessment and speculative synthesis. *Mar. Chem.* **49**, 81–115 (1995)
- de Leeuw, J.W., Largeau, C.: A Review of Macromolecular Organic Compounds That Comprise Living Organisms and Their Role in Kerogen, Coal, and Petroleum Formation. In: Engel, M.H., Macko, S.A. (eds.) *Organic Geochemistry Principles and Applications*, pp. 23–72. New York pp, Plenum Publishing (1993)
- Wakeham, S.G., Canuel, E.A.: Degradation and preservation of organic matter in marine sediments. In: Volkman, J.K., Ed., *Marine Organic Matter: Biomarkers, Isotopes and DNA. The Handbook of Environmental Chemistry*, Vol. 2, pp. 295–321, part N. Springer Verlag, Berlin (2006)
- Hedges, J.I., Keil, R.G., Benner, R.: What happens to terrestrial organic matter in the ocean? *Org. Geochem.* **27**, 195–212 (1997)
- van Dongen, B.E., Zencak, Z., Gustafsson, Ö.: Differential transport and degradation of bulk organic carbon and specific terrestrial biomarkers in the surface waters of a sub-arctic brackish bay mixing zone. *Mar. Chem.* **112**, 203–214 (2008)
- Vonk, J.E., Sanchez-Garcia, L., Semiletov, I., Dudarev, O., Eglinton, T., Andersson, A., Gustafsson, Ö.: Molecular and radiocarbon constraints on sources and degradation of terrestrial organic carbon along the Kolyma paleoriver transect, East Siberian Sea. *Biogeosciences* **7**, 3153–3166 (2010)
- Bourgeois, S., Pruski, A.M., Sun, M.-Y., Buscail, R., Lantoiné, F., Kerhervé, P., Vétion, G., Rivière, B., Charles, F.: Distribution and lability of land-derived organic matter in the surface sediments of the Rhône prodelta and the adjacent shelf (Mediterranean Sea, France): a multi proxy study. *Biogeosciences* **8**, 3107–3125 (2011)
- Rontani, J.-F., Charriere, B., Sempéré, R., Doxaran, D., Vaultier, F., Vonk, J.E., Volkman, J.K.: Degradation of sterols and terrestrial organic matter in waters of the Mackenzie Shelf, Canadian Arctic. *Org. Geochem.* **75**, 61–73 (2014)
- Brassell, S.C., Eglinton, G., Maxwell, J.R.: The geochemistry of organic terpenoids and steroids. *Biochem. Soc. Trans.* **11**, 575–586 (1983)
- Oros, D.R., Simoneit, B.R.T.: Identification and emission factors of molecular tracers in organic aerosols from biomass burning. Part 1. Temperate climate conifers. *Appl. Geochem.* **16**, 1513–1544 (2001)
- Simoneit, B.R.T.: Biomass burning—a review of organic tracers for smoke from incomplete combustion. *Appl. Geochem.* **17**, 129–162 (2002)
- Shao, L.P., Gäfvert, E., Nilsson, U., Karlberg, A.-T., Nilsson, J.L.G.: 15-Hydroperoxydehydroabietic acid—a contact allergen in colophony from *Pinus* species. *Phytochemistry* **38**, 853–857 (1995)
- Martin, V.J.J., Mohn, W.W.: Genetic investigation of the catabolic pathway for degradation of abietane diterpenoids by *Pseudomonas abietaniphila* BKME-9. *J. Bacteriol.* **182**, 3784–3793 (2000)
- Cartoni, G.P., Russo, M.V., Spinelli, F., Talarico, F.: GC-MS identification and characterization of natural terpenic resins employed in works of art. *Anal. Chim. Acta.* **94**, 767–782 (2004)
- Doménech-Carbó, M.T., Osete-Cortina, L., De la Cruz Cañizares, J., Bolívar-Galiano, F., Romero-Noguera, J., Fernández-Vivas, M.A., Martín-Sánchez, I.: Study of the microbiodegradation of terpenoid resin-based varnishes from easel painting using pyrolysis-gas chromatography–mass spectrometry and gas chromatography–mass spectrometry. *Anal. Bioanal. Chem.* **385**, 1265–1280 (2006)
- González, M.A., Pérez-Guaita, D., Correa-Royero, J., Zapata, B., Agudelo, L.: Mesa-Arango, A., Betancur-Galvis, L.: Synthesis and biological evaluation of dehydroabietic acid derivatives. *Eur. J. Med. Chem.* **45**, 811–816 (2010)
- Lund, E., Budzikiewicz, H., Wilson, J.M., Djerassi, C.: Mass spectrometry in structural and stereochemical problems. XXI. Fragmentation and hydrogen transfer reactions after electron impact on β -decalones. *J. Am. Chem. Soc.* **85**, 1528 (1963)
- Belt, S.T., Brown, T.A., Ringrose, A.E., Cabedo-Sanz, P., Mundy, C.J., Gosselin, M., Poulin, M.: Quantitative measurements of the sea ice diatom biomarker IP₂₅ and sterols in Arctic sea ice and underlying sediments: further considerations for palaeo sea ice reconstructions. *Org. Geochem.* **62**, 33–45 (2013)
- Rontani, J.-F., Belt, S.T., Brown, T., Vaultier, F., Mundy, C.J.: Identification of sequential photo- and autoxidation of diatom lipids in Arctic sea ice. *Org. Geochem.* **77**, 59–71 (2014)
- Pierce, A.E.: *Silylation of Organic Compounds*, p. 487. Pierce Chemical Co, Rockford (1982)
- Budzikiewicz, H., Djerassi, C., Williams, D.H.: Interpretation of Mass Spectra of Organic Compounds, p. 212. Holden-Day, Inc, San Francisco (1965)
- Petersson, G.: Mass spectrometry of hydroxydicarboxylic acids as trimethylsilyl derivatives. *Rearrangement Reac. Org. Mass Spectrom.* **6**, 565–576 (1972)
- Dittmar, T., Kattner, G.: The biogeochemistry of the river and shelf ecosystem of the Arctic Ocean: a review. *Mar. Chem.* **83**, 103–120 (2003)
- Russo, M.V., Avino, P.: Characterization and identification of natural terpenic resins employed in 'Madonna con Bambino e angeli' by Antonello da Messina using gas chromatography–mass spectrometry. *Chem. Central J.* **6**, 59–80 (2012)