



Title	Identification of hydroxy- and keto-dicarboxylic acids in remote marine aerosols using gas chromatography/quadruple and time-of-flight mass spectrometry
Author(s)	Gowda, Divyavani; Kawamura, Kimitaka; Tachibana, Eri
Citation	Rapid communications in mass spectrometry, 30(7), 992-1000 https://doi.org/10.1002/rcm.7527
Issue Date	2016-04-15
Doc URL	http://hdl.handle.net/2115/65114
Rights	"This is the peer reviewed version of the following article: Gowda, Divyavani; Kawamura, Kimitaka; Tachibana, Eri; Identification of hydroxy- and keto-dicarboxylic acids in remote marine aerosols using gas chromatography/quadruple and time-of-flight mass spectrometry Rapid communications in mass spectrometry v.30 pp.992-1000 2016, which has been published in final form at [10.1002/rcm.7527]. This article may be used for non-commercial purposes in accordance with Wiley Terms and Conditions for Self-Archiving."
Type	article (author version)
File Information	Gauda et al., 2016 RCMS main_kk -.pdf



[Instructions for use](#)

1 Identification of hydroxy- and keto-dicarboxylic acids in remote marine aerosols using
2 gas chromatography/quadruple and time-of-flight mass spectrometry

3

4

5 Divyavani Gowda^{1,2}, Kimitaka Kawamura^{2*} and Eri Tachibana²

6

7

8

9

10 ¹Graduate School of Environmental Science, Hokkaido University, Japan

11 ²Institute of Low Temperature Science, Hokkaido University, Japan

12

13

14

15 **Submitted to**

16 ***“Rapid Communication in Mass Spectrometry”***

17

18

19

20

21

22

23 *Corresponding author: Prof. K. Kawamura

24 Institute of Low Temperature Science

25 Hokkaido University, Japan

26 E-mail: kawamura@lowtem.hokudai.ac.jp

27 Tel.: +81 11 706 5457; Fax: +81 11 706 7142

28

29 **RATIONALE:** Identification of hydroxy- and keto-dicarboxylic acids (diacids) in remote
30 marine aerosol samples are important for a better understanding the composition of
31 organic particulate matter, whose chemical composition is essential for predicting the
32 effects on climate, air quality, and human health. Molecular characterization of these
33 compounds provides insights into sources and formation pathways of organic aerosols.

34 **METHODS:** The method of chemical derivatization followed by gas chromatography-
35 flame ionization detection (GC-FID), gas chromatography/quadruple mass spectrometry
36 (GC/QMS) and gas chromatography/time-of-flight mass spectrometry (GC/TOFMS)
37 analysis was used to identify hydroxy- and keto-diacids in remote marine aerosols.
38 Atmospheric samples were collected at Chichijima Island in the western North Pacific and
39 extracted with organic free ultrapure water for diacids and related compounds. A two-step
40 derivatization technique were used in this study using 14% BF_3 /n-butanol for the
41 butylation of carboxyl groups and acidic ketones followed by N,O-
42 bis(trimethylsilyl)trifluoroacetamide (BSTFA) for the trimethylsilylation of hydroxyl
43 groups.

44 **RESULTS:** Several new peaks were detected in the gas chromatogram after
45 trimethylsilylation of the dibutyl ester fraction. Based on mass spectral interpretation with
46 authentic standards, we successfully identified and quantified a homologous series of
47 hydroxydiacids, including tartaric and oxaloacetic acids. In addition, transformation of
48 oxaloacetic acid into its enol form was elucidated.

49 **CONCLUSIONS:** Utilizing GC-FID, GC/QMS and GC/TOFMS, hydroxy- and keto-
50 diacids were identified in the remote marine aerosols. A complete structural
51 characterization was achieved with extensive mass spectral analysis. Molecular
52 distributions of hydroxydiacids generally showed the predominance of malic acid
53 followed by tartronic acid. We consider that these hydroxydiacids are important
54 intermediates in the atmospheric oxidation of organic aerosols to result in smaller diacids.
55 **Keywords:** remote marine aerosol, ketodicarboxylic acids, hydroxydicarboxylic acids,
56 oxaloacetic acid, keto-enol tautomerization, GC/TOFMS.

57

58 Organic aerosols are present not only in the continental atmosphere, but also in the marine
59 atmosphere.^[1] They can significantly contribute to the global budget of atmospheric
60 particles and thus have an impact on the Earth's albedo, climate, atmospheric chemistry,
61 and biogeochemical cycles of carbon and nitrogen.^[2] The fate of hydrophilic organics in
62 the marine atmosphere is highly uncertain. Organic compounds in marine aerosols may act
63 as important precursors and a dominant sink for OH radicals, leading to the formation of
64 various low-molecular-weight diacids by their degradation.^[3] However, water-soluble
65 organic carbon in remote marine aerosols has not been well characterized at the molecular
66 level.^[4]

67 Dicarboxylic acids (diacids) are dominant components of organic aerosols in the
68 atmosphere and have been extensively studied at many locations around the world.^[5-7]
69 Because of their high water-soluble and hygroscopic properties, particles enriched with
70 diacids play an important role as cloud condensation nuclei (CCN) and ice nuclei (IN)
71 affecting the radiative forcing of the earth.^[8-11] Recent studies on marine aerosols have
72 revealed high abundances of diacids with a predominance of oxalic acid followed by
73 malonic and succinic acids.^[2, 7, 12] Their concentrations and relative abundances are
74 controlled by primary emissions and secondary formation in the atmosphere.^[13] Diacids
75 are scavenged from the atmosphere by dry and wet deposition.^[14]

76 Previous studies from the western North Pacific show that diacids contribute 1.6% of
77 the total aerosol mass^[13], suggesting they are important constituents of marine organic
78 aerosols. Further, studies from different sites demonstrate that average ratios of total
79 diacid-C to OC are 1.4% in 14 Chinese cities^[15], 0.95% in Tokyo, Japan^[16], and 3.2% in
80 the western Pacific.^[7] In spite of their ubiquity in the environment and their high
81 reactivity, there are some diacid-related compounds containing hydroxyl- and keto- groups
82 that have been suggested to exist in the atmosphere^[13, 17] but have never been identified in
83 marine aerosols. Due to the enhanced polarity compared to diacids, they should play a
84 crucial role as CCN and IN, and act as key intermediates in the formation of smaller
85 diacids in the atmosphere.^[17] It is also possible that they react further in the aerosol phase
86 through esterification reactions and serve as precursors for oligomeric humic-like
87 substances.^[18] Diacids have a low volatility, are relatively strong acids, and can readily
88 form complexes with transition metals, which are sensitive to photo degradation.^[19]

89 Trifunctional hydroxy- and keto-diacids can give rise to stable homo- and hetero-
90 molecular clusters through hydrogen bonding, being similar to the clusters proposed for

91 bifunctional compounds^[20], and may as such participate in new particle formation and
92 growth.^[21] Since they are highly oxygenated, it is expected that studies of keto- and
93 hydroxy-diacids provide useful information to better understand the oxidative reaction
94 mechanisms of organic matter in the marine atmosphere during long-range transport.
95 Identification and quantification of these compounds will thus help identify the principle
96 mechanisms for the chemical aging of organic aerosols in the marine atmosphere.

97 In this study, many unknown peaks were detected in the gas chromatogram after
98 trimethylsilyl (TMS) derivatization of the dibutyl ester fraction of remote marine aerosols.
99 Mass spectral examination of the unknown peaks suggests that they belong to a
100 homologous series of hydroxydiacids (hC₃di- hC₆di), including tartronic acid, malic acid,
101 3-hydroxyglutaric acid, 2-hydroxyglutaric acid, 2-hydroxyadipic acid, 3-hydroxyadipic
102 acid, and tartaric acid. Some of these compounds are individually detected in laboratory
103 photo-oxidations^[22] and ambient aerosol samples^[23], but there is no report on the
104 identification of the homologous series of hydroxyl diacids formed in marine aerosols.
105 Many isomeric ketodicarboxylic acids have been identified in atmospheric aerosol
106 particles.^[24] Oxaloacetic acid was suggested to exist in aerosols by the photo-oxidation of
107 succinic acid^[25], but has not been detected in ambient aerosols previously. Here,
108 oxaloacetic acid was successfully identified as isomeric forms in marine aerosols.
109 Tentative fragmentation pathways are proposed for the derivatized unknown
110 hydroxydiacids and oxaloacetic acid in marine aerosols.

111 **EXPERIMENTAL**

112 **Reagents and chemicals**

113 Special grade organic solvents and authentic malic, tartaric, and oxaloacetic acids were
114 purchased from Wako Pure Chemical Industries (Tokyo, Japan). Tartronic and 3-
115 hydroxyglutaric acids were purchased from Alfa Aesar (Heysham, England) and Toronto
116 Research Chemicals (Toronto, Canada) respectively. The purities of authentic standards
117 are more than 97%. All the organic solvents were redistilled in the laboratory prior to use.
118 All the glassware was cleaned with soap solution, rinsed with tap water followed by
119 distilled water, dried and baked at 450 °C overnight. Just before usage, the glassware was
120 rinsed three times with organic-free pure water, methanol, and dichloromethane.

121 **Aerosol sampling**

122 Total suspended particles (TSP) were collected on weekly basis from 1 December
123 2010 to 30 November 2011 (n = 53) using pre-combusted (450 °C) quartz fiber filters and

124 a high volume air sampler at a flow rate of $1.0 \text{ m}^3 \text{ min}^{-1}$ at the Ogasawara Downrange
125 Station of the Japan Aerospace Exploration Agency (JAXA) at Chichijima Island
126 ($27^{\circ}04'N$; $142^{\circ}13'E$) in the western North Pacific, an outflow region of the Asian dust and
127 polluted air masses from China and other neighboring countries. Sampling periods were
128 generally five days. After sampling, the filters were placed in precombusted glass jars with
129 a Teflon-lined screw cap, sent to Hokkaido University in Sapporo, and stored in a freezer
130 room at $-20 \text{ }^{\circ}\text{C}$ prior to analysis.

131 **Preparation of dibutyl esters**

132 Filter aliquots were extracted three times with 10 mL organic-free pure water under
133 ultrasonication for 10 min. To remove insoluble particles and filter debris, the extracts
134 were passed through a glass column (Pasteur pipette) packed with quartz wool into a pear-
135 shape flask. The water extracts were pH-adjusted to 8.5-9.0 with 0.05 M aqueous KOH
136 solution and concentrated almost to dryness using a rotary evaporator under a vacuum. A
137 14% BF_3/n -butanol was added to the concentrates in the flask to allow esterification at
138 $100 \text{ }^{\circ}\text{C}$ for 1 h. During the reaction, carboxyl groups are derivatized to their butyl esters,
139 and keto groups to dibutoxy acetals. The derivatives were extracted with 10 mL of n-
140 hexane after adding 10 mL of organic free-water and 0.5 mL of acetonitrile. Acetonitrile
141 transfers the excess n-butanol into the aqueous phase efficiently. The hexane layer was
142 further washed with organic free-water (2 x 10 mL). Using a rotary evaporator under a
143 vacuum and a nitrogen blow-down system, extracts were concentrated and then dissolved
144 in 100 μL of n-hexane.

145 **BSTFA derivatization**

146 An aliquot of the dibutyl ester fraction containing hydroxyl diacids was dried under a
147 gentle stream of nitrogen, and hydroxyl diacid dibutyl esters were derivatized to
148 corresponding TMS ethers with 50 μL of N,O-bis(trimethylsilyl)trifluoroacetamide
149 (BSTFA) at $80 \text{ }^{\circ}\text{C}$ for 30 min. The derivatives were dried under a nitrogen stream,
150 dissolved in n-hexane (50 μL) and analyzed using gas chromatography/quadruple mass
151 spectrometry (GC/QMS) and gas chromatography/time of flight-mass spectrometry
152 (GC/TOFMS). Their concentrations were quantified using gas chromatography-flame
153 ionization detection (GC-FID). Known amounts of authentic standards (tartronic, malic, 3-
154 hydroxyglutaric, tartaric, and oxaloacetic acids) were spiked on a pre-combusted quartz
155 fiber filter and derivatized to butyl esters-TMS ethers by the procedure described above.
156 We found that the recoveries of hydroxyl diacids are more than 80%. The analytical errors

157 in duplicate analysis of the aerosol sample (QFF3769) for hydroxy- and keto-diacids are
158 about 10%.

159 **Instrumentation**

160 GC/TOFMS analyses were performed using an Agilent Technologies model 7890A
161 gas chromatograph system coupled to JEOL Accu TOF-GCv 4G mass spectrometer
162 (JEOL, Tokyo, Japan). The mass spectrometer was operated in the electron ionization (EI)
163 mode at 70 eV and the mass analyzer was scanned in the range m/z 40 - 650. The mass
164 spectrometer resolution is > 8000 at a mass range of m/z 4 - 5000. The gas
165 chromatographic separation was carried out on an Agilent DB-5MS fused silica capillary
166 column (30 m long, 0.25 mm i.d., 0.25 μm film thickness), (Agilent Technologies, Tokyo,
167 Japan). For gas chromatograph the temperature was programmed from 50 $^{\circ}\text{C}$ for 2 min,
168 increased from 50 to 120 $^{\circ}\text{C}$ at 15 $^{\circ}\text{C min}^{-1}$, then to 305 $^{\circ}\text{C}$ at 5 $^{\circ}\text{C min}^{-1}$ with a final
169 isothermal hold. The splitless mode was used at an injection temperature of 280 $^{\circ}\text{C}$ and
170 16.3 psi pressure. This mass spectrometer provides high sensitivity, fast detection, and a
171 high dynamic range.

172 GC-FID analyses were performed using a Hewlett Packard HP 6890 gas
173 chromatograph system. GC/MS analyses were carried out on an Agilent 5975C mass
174 spectrometer linked to an Agilent 7890A gas chromatograph system. The mass analyzer
175 was scanned in the range m/z 50-650 and the ion source was in EI mode. In both systems,
176 similar gas chromatographic conditions were maintained as described above. The GC/MS
177 data were acquired and processed with the Agilent GC/MSD Chemstation software.

178 **RESULTS AND DISCUSSION**

179 **Hydroxy- and keto-dicarboxylic acid butyl ester TMS-derivatives**

180 In order to distinguish dicarboxylic acids with and without hydroxyl groups, the water
181 extracts of marine aerosols were subjected to a two-step derivatization procedure, i.e.,
182 butylation followed by trimethylsilylation, by which carboxyl groups are converted to
183 butyl esters whereas hydroxyl groups are converted to TMS ethers. Methylation TMS-
184 derivatization has been often used for hydroxydicarboxylic acids ^[24] but in this study the
185 butylation TMS method was used because butyl ester derivatization is highly selective for
186 bifunctional compounds ^[26] and the recoveries are high even for small diacids. ^[16] On the
187 other hand, methyl esters of diacids are sometimes highly volatile and thus are lost by
188 evaporation during the analytical procedure. ^[27]

189 Figure 1(a) and 1(b) shows reconstructed total ion chromatogram of butylated-TMS
190 derivatives from Chichijima Island in the western North Pacific (QFF3769) and a blank
191 filter chromatogram. In the gas chromatogram (Fig. 1(a)), nine new peaks were observed
192 after TMS derivatization of the diacid butyl ester fraction. They are unknown structures
193 and thus named as compound U1 to U9, which are considered to contain additional
194 hydroxyl- and keto- groups in their diacid structures. The detailed interpretation and
195 characterization for unknown compounds U1 to U9 are presented below.

196 **Tartronic acid**

197 The EI mass spectra for the butyl ester TMS-derivative of unknown compound U1
198 allow us to infer the molecular weight (MW) of the underivatized compound as 120
199 (retention time (RT): 16.5 min; MW of 304 for the butyl ester TMS-derivative; $[M - CH_3]^+$
200 ion at m/z 289; $C_{13}H_{25}O_5Si$; measured mass: 289.14569, error: -5 ppm). These results
201 suggest that U1 is tartronic acid (2-hydroxymalonic acid). Tartronic acid has been
202 postulated to exist in marine aerosols via the photooxidation of malonic acid^[13], but no
203 evidence has been provided for its presence in ambient aerosols samples. In the present
204 study, we successfully identified tartronic acid (Fig. 5(a)) in remote marine aerosols.

205 The mass spectrum of tartronic acid derivative (Fig. 2(a)) shows fragment ions at m/z
206 289, 233, 203, 177, 147, 131, 73, 57, and 41. Structurally informative ions include m/z 289
207 $[M-15]^+$, due to loss of a methyl radical from the TMS moiety,^[28] while the peak at m/z
208 203 $[M-101]^+$, could be explained by loss of a butoxycarbonyl radical ((CO)OC₄H₉) via an
209 α -cleavage, which is not detectable in the EI mode,^[29] and the peak at m/z 233 $[M-15-56]^+$
210 may be due to elimination of a neutral molecule of butene (C₄H₈, 56 u) by a McLafferty
211 rearrangement. Proposed fragmentation pathways that are structurally informative for the
212 butyl ester TMS-derivative of unknown U1 (tartronic acid) are summarized in Scheme S1
213 (see Supporting Information). The TOFMS spectrum of a tartronic acid standard was
214 compared with that of the unknown compound U1 (Fig. 2a) showed good agreement and a
215 similar retention time, allowing to confirm U1 as tartronic acid.

216 To our knowledge, this is the first identification of tartronic acid in ambient aerosols.
217 We found that the measured concentration of tartronic acid ranged from 0.01 to 5.27 ng m⁻³
218 in total suspended particles collected at Chichijima Island during the period of 1
219 December 2010 to 30 November 2011 (n = 53). This wide range is due to seasonal
220 changes in the air masses. The sampling site is strongly influenced by westerly winds in
221 winter to spring, oceanic clean air masses in summer, and both oceanic and continental air

222 masses in autumn ^[7], affecting the concentration of tartronic acid in remote marine
223 aerosols.

224 **Malic acid**

225 The EI mass spectral data for the butyl ester TMS-derivative of unknown compound
226 U2 allow us to infer that the MW of the underivatized compound is 134 (RT: 18.2 min;
227 MW of 318 for butyl ester TMS-derivative; $[M - CH_3]^+$ ion at m/z 303; $C_{14}H_{27}O_5Si$;
228 measured mass: 303.16563, error: 9 ppm). These results suggest that compound U2 is
229 malic acid (2-hydroxysuccinic acid) (Fig. 5 (b)). Malic acid has been proposed to result
230 from the hydroxylation of succinic acid in marine aerosols using a 14% BF_3/n -butanol as
231 derivatizing agent. ^[13] We found that it is the most abundant hydroxyl diacid in marine
232 aerosols. Because the hydroxyl group was underivatized in the previous technique for
233 diacid dibutyl ester derivatization, malic acid dibutyl ester often showed peak tailing in the
234 chromatogram, which may lead to underestimate the concentrations of this compound. ^[30]
235 Here, we addressed this problem by derivatizing hydroxyl groups to TMS ethers with
236 BSTFA. The malic acid concentration in all the measured samples ($n = 53$) ranged from
237 0.03 to 14.7 $ng\ m^{-3}$, which is ca. 3 times higher than those reported in previous studies
238 without TMS derivatization.

239 The mass spectrum of the malic acid derivative shows fragment ions at m/z 303, 247,
240 217, 173, 161, 145, 117, 101, 73, 57, and 41 (Fig. 2(c)). Structurally informative ions
241 include m/z 303 $[M-15]^+$, m/z 217 $[M-101]^+$, and the peak at m/z 247 $[M-15-56]^+$ could be
242 explained by a similar pathway as described above in the case of dibutyl ester-TMS
243 derivative of tartronic acid. Proposed fragmentation pathways that are structurally
244 informative for the butyl ester TMS-derivative of unknown U2 (malic acid) are
245 summarized in Scheme S2 (see Supporting Information). A Malic acid standard TOFMS
246 spectrum was obtained (Fig. 2(d)) and compared with that of the unknown compound U2
247 (Fig. 2(c)), which showed a good agreement and similar retention time, allowing the
248 confirmation of the U2 as malic acid.

249 **3- and 2-hydroxyglutaric acids**

250 The EI mass spectra for the butyl ester TMS-derivatives of both unknown compounds
251 U4 and U5 suggest that the MW of the underivatized compounds is 148 with a MW of 332
252 for butyl ester TMS-derivatives and m/z 317 for $[M - CH_3]^+$ ion. The RT of U4 and U5 are
253 19.1 min and 20.03 min, and the measured m/z values of $C_{15}H_{29}O_5Si$ are at 317.17713 and
254 317.17765 with errors of -4 ppm and -2 ppm, respectively. These results suggest U4 and

255 U5 are positional isomers, which are assigned to 3-hydroxyglutaric and 2-hydroxyglutaric
256 acids (Figs. 5(c) and 5(d)). Based on the mass spectral fragmentation patterns we could
257 distinguish both isomers. 3-hydroxy and 2-hydroxyglutaric acids have been reported in
258 fine PM_{2.5} aerosols from rural K-pusztá, Hungary as photo-oxidation products of α -pinene.
259 [23]

260 Compound U4 butyl ester TMS-derivative shows fragment ions at m/z 317, 261, 259,
261 243, 217, 205, 187, 169, 145, 113, 101, 73, 57, and 41 (Fig. 2 (e)). Structurally
262 informative ions include m/z 317 [M-15]⁺, and m/z 261 [M-15-56]⁺, while the peak at m/z
263 259 [M-73]⁺, may be due to the loss of a butoxy radical (OC₄H₉) from the molecular ion
264 by α -cleavage. The m/z 217 [M-115]⁺ could be explained by loss of a butyl ethanoate
265 radical [CH₂COOC₄H₉] from the molecular ion by β -cleavage, suggesting that the position
266 of hydroxyl group is at the third carbon. [31] The 3-hydroxyglutaric acid standard TOFMS
267 spectrum was obtained (Fig. 2 (f)) and compared with that of U4 (Fig. 2 (e)). The results
268 showed a good agreement and similar retention time allowing the confirmation of U4 as 3-
269 hydroxyglutaric acid. Proposed fragmentation pathways that are structurally informative
270 for the butyl ester TMS-derivative of 3-hydroxyglutaric acid are summarized in Scheme
271 S3 (see Supporting Information). The atmospheric concentration of 3-hydroxyglutaric acid
272 in all measured samples ($n = 53$) ranged from 0.01 to 4.98 ng m⁻³.

273 Compound U5 butyl ester TMS-derivative shows fragment ions at m/z 317, 259, 231,
274 203, 187, 175, 159, 129, 85, 73, 57, and 41 (Figure 3(a)). Structurally informative ions
275 include m/z 317 [M-15]⁺, m/z 259 [M-73]⁺. The peak at m/z 231 [M-101]⁺ or via [M-73-
276 28]⁺ could be due to a loss of neutral molecule of carbon monoxide (CO, 28 u) from the
277 [M-73]⁺ ion, and m/z 203 could be explained by the elimination of neutral molecule of
278 ethene (C₂H₄, 28 u) from the ion of m/z 231. Tentative fragmentation pathways that are
279 structurally informative for the butyl ester TMS-derivative of unknown U5 (2-
280 hydroxyglutaric acid) are summarized in Scheme 1. The fragment ion of m/z 231 [M-101]⁺
281 suggests that the position of hydroxyl group is at the second carbon and hence, helping us
282 to distinguish 2-hydroxyglutaric acid from 3-hydroxyglutaric acid. Based on these
283 experimental observations, U5 is assigned to 2-hydroxyglutaric acid. The atmospheric
284 concentration of 2-hydroxyglutaric acid in all the measured samples ($n = 53$) ranged from
285 0.03 to 4.79 ng m⁻³.

286 **2-hydroxyadipic and 3-hydroxyadipic acids**

287 The EI mass spectra for the butyl ester TMS-derivatives of both U7 and U8 inferred

288 that the MW of the underivatized compounds is 162 with a MW of 346 for butyl ester
289 TMS-derivatives and with a $[M - CH_3]^+$ ion at m/z 331. The RT of U7 and U8 are 21.7 and
290 21.8 min and the measured m/z values of $C_{16}H_{31}O_5Si$ are at 331.19289 and 331.19226 with
291 errors of -4 ppm and -5 ppm, respectively. Thus, the mass spectral information indicates
292 that compounds U7 and U8 are positional isomers, which are attributed to 2-
293 hydroxyadipic and 3-hydroxyadipic acids, respectively (Fig. 5(e) and 5(f)). Based on the
294 mass spectral fragmentation patterns we could distinguish the two isomers. 2-
295 Hydroxyadipic and 3-hydroxyadipic acids have not been identified in marine aerosols. On
296 the other hand, 3-hydroxyadipic acid has been identified as a reaction product during
297 laboratory photo-oxidation of cyclohexane.^[32]

298 The butyl ester TMS-derivative of compound U7 shows fragment ions at m/z 331, 273,
299 245, 201, 173, 129, 99, 73, 57, and 41 (Fig. 3(b)). Structurally informative ions include
300 m/z 331 $[M-15]^+$, m/z 273 $[M-73]^+$, and m/z 245 $[M-73-28]^+$ or $[M-101]^+$ Tentative
301 fragmentation pathways that are structurally informative for the butyl ester TMS-
302 derivative of U7 (2-hydroxyadipic acid) are summarized in Scheme 2.

303 The butyl ester TMS-derivative of compound U8 shows fragment ions at m/z 331, 273,
304 231, 217, 201, 157, 127, 85, 73, 57, and 41 (Fig. 3(c)). Structurally informative ions
305 include m/z 331 $[M-15]^+$ and 273 $[M-73]^+$. The peak at m/z 217 may be due to a neutral
306 loss of carbon monoxide (CO, 28 u) and ethene (C_2H_4 , 28 u) from the $[M-73]^+$ ion whereas
307 m/z 231 $[M-115]^+$ could be explained by loss of a butyl ethanoate radical $[CH_2COOC_4H_9]$
308 from the molecular ion by β -cleavage, suggesting that the position of the hydroxyl group
309 is at the third carbon. Tentative fragmentation pathways that are structurally informative
310 for the butyl ester TMS-derivative of unknown U8 (3-hydroxyadipic acid) are summarized
311 in Scheme 3. The fragment ions at m/z 245 $[M-101]^+$ and m/z 231 $[M-115]^+$ in U7 and U8
312 indicates the location of hydroxyl group.^[31] Based on these experimental observations U7
313 and U8 are assigned to 2-hydroxyadipic and 3-hydroxyadipic acids, respectively. The
314 concentrations of 2-hydroxyadipic and 3-hydroxyadipic acids in all the measured samples
315 ($n=53$) ranged from 0.04 to 0.73 $ng\ m^{-3}$ and 0.19 to 0.82 $ng\ m^{-3}$, respectively.

316 Tartaric acid

317 The EI mass spectra for the butyl ester TMS-derivative of unknown compound U6
318 suggested that the MW of the underivatized compound is 150 (RT; 20.7 min) and the MW
319 is 406 for the butyl ester TMS-derivative with $[M - CH_3]^+$ ion of m/z 391. The measured

320 m/z value of $C_{17}H_{35}O_6Si_2$ is 391.19572 with an error of -4 ppm. This information suggests
321 that compound U6 is tartaric acid (2,3-dihydroxysuccinic acid) (Fig. 5(g)). Tartaric acid
322 has been reported in continental aerosols,^[30] however, there is no report in marine
323 aerosols. This is the first report on the presence of tartaric acid in continentally influenced
324 remote marine aerosols.

325 The mass spectrum of the butyl ester TMS-derivative of tartaric acid presents
326 characteristic fragment ions at m/z 391, 335, 305, 276, 233, 189, 147, and 73 (Fig. 2(g)).
327 Structurally informative ions include m/z 391 $[M-15]^+$, m/z 335 $[M-15-56]^+$ and m/z 305
328 $[M-101]^+$. The peak at m/z 233 could be caused by the combined loss of a butoxycarbonyl
329 $((C=O)OC_4H_9)$ and a hydrogen radical by α -cleavage from the ion of m/z 335. The
330 abundant fragment ions at m/z 147 and 73 are commonly seen in ditrimethylsilylethers.
331^[33,34] Tartaric acid standard TOFMS spectrum was obtained (Fig. 2(h)) and compared
332 with that of U6 (Fig. 2(g)), showing a good agreement and similar retention time allowing
333 the confirmation of U6 as tartaric acid. Proposed fragmentation pathways that are
334 structurally informative for the butyl ester TMS-derivatives of tartaric acid are
335 summarized in Scheme S4 (see Supporting Information). The concentration of tartaric
336 acid in all the studied samples ($n = 53$) ranged from 0.01 to 2.65 $ng\ m^{-3}$.

337 **Oxaloacetic acid**

338 Many isomeric keto-dicarboxylic acids were reported in a previous study of
339 atmospheric particles with their sources and formation mechanisms.^[24] However,
340 oxaloacetic acid (3-oxodicarboxylic acid) has not been detected in atmospheric aerosols,
341 possibly due to its rapid decarboxylation. In the present study, we successfully identified
342 unknown compounds U3 and U9 as isomers of oxaloacetic acid in the remote marine
343 aerosol samples. The EI mass spectra for the butyl ester TMS-derivatives of both
344 unknown compounds U3 and U9 suggest that the MW of underivatized compounds is 132.
345 The RT of U3 and U9 are 19.5 and 24.0 min, respectively. The derivative of U3 provides a
346 fragment ion at m/z 301 $[M - CH_3]^+$ with measured m/z value of $C_{14}H_{25}O_5Si$ at 301.14764
347 with an error of 2 ppm, whereas U9 provides a fragment ion at m/z 273 $[M-101]^+$ with a
348 measured m/z value of $C_{15}H_{29}O_4$ at 273.20609 with an error of -2 ppm. This mass spectral
349 information indicates that compounds U3 and U9 are isomers of oxaloacetic acid; U3 is
350 the enolic form of oxaloacetic acid whereas U9 is the keto form of oxaloacetic acid (Figs.
351 5(h) and 5(i)).

352 Authentic oxaloacetic acid was derivatized by the procedures described above,

353 followed by GC/MS measurements. The gas chromatogram shows three peaks (Fig. S1,
354 see Supporting Information). The mass spectral analysis of these peaks demonstrated that
355 two peaks are keto and enolic forms of oxaloacetic acid. The third peak was confirmed as
356 the butyl ester of pyruvic acid. Based on the comparison of QMS and TOFMS spectra for
357 authentic oxaloacetic acid with those of unknown U3 and U9, we confirmed that
358 oxaloacetic acid may exist in the atmospheric aerosols as three different forms; the keto
359 form (U9), the enolic form (U3) and its decarboxylated form (pyruvic acid). The
360 transformation of oxaloacetic acid in aqueous solution may be possible because of the
361 presence of a highly acidic α -hydrogen, which exhibits keto-enol isomerism with the enol-
362 form being more stable than the keto-form due to the intermolecular forces of attraction.
363 Dicarboxylic acids with the keto-group at the β -position undergo decarboxylation
364 spontaneously in the aqueous phase to result in pyruvic acid that can be detected in
365 ambient aerosols.^[35]

366 Detailed interpretation of the high-resolution mass spectra led to the structural
367 proposal of oxaloacetic acid. The derivative of enolic oxaloacetic acid (U3) shows
368 fragment ions at m/z 301, 245, 215, 189, 171, 145, 117, 73, 57, and 41 (Fig. 4(a)).
369 Structurally informative ions include m/z 301 $[M-15]^+$, 245 $[M-15-56]^+$, and 215 $[M-101]^+$.
370 Proposed fragmentation pathways for the butyl ester TMS-derivative of unknown U3
371 (enol-oxaloacetic acid) are summarized in Scheme S5 (see Supporting Information). The
372 proposed structure was subsequently confirmed by comparison of its gas chromatographic
373 and mass spectral data (Fig. 4(a)) with those of an authentic standard (Fig. 4(b)).

374 The derivative of the keto form of oxaloacetic acid (U9) shows fragment ions at m/z
375 273, 245, 217, 189, 161, 105, 57, and 41 (Fig. 4(c)). Structurally informative ions include
376 m/z 273 $[M-101]^+$. The peak at m/z 245 $[M-73-56]^+$ could be explained by the loss of a
377 butoxy radical (C_4H_9O) and butene (C_4H_8 , 56 u) from the molecular ion by α -cleavage
378 followed by a McLafferty rearrangement. The fragment ion of m/z 217 was derived by the
379 loss of carbon monoxide from the ion of m/z 245. Proposed fragmentation pathways for
380 the butyl esters derivative of unknown U5 (keto-oxaloacetic acid) are summarized in
381 Scheme S6 (see Supporting Information). The proposed structure was subsequently
382 confirmed by comparison of its gas chromatographic and mass spectral data (Fig. 4 (c))
383 with those of an authentic standard (Fig. 4(d)). This is the first report on the existence of
384 two forms of oxaloacetic acid in marine aerosols. Oxaloacetic acid may be the
385 intermediate in the oxidation of dicarboxylic acids such as succinic acid to result in

386 pyruvic acid, which is further oxidized to oxalic acid. The concentrations of the enol and
387 keto form of oxaloacetic acid in all the samples (n = 53) ranged from 0.01 to 1.52 ng m⁻³
388 and 0.01 to 0.51 ng m⁻³, respectively.

389 SUMMARY AND CONCLUSIONS

390 Unknown intermediates of diacid formation have been successfully identified in
391 remote marine aerosols as tartronic acid, malic acid, 3-hydroxyglutaric acid, 2-
392 hydroxyglutaric acid, 2-hydroxyadipic acid, 3-hydroxyadipic acid, tartaric acid and the
393 enol and keto form of oxaloacetic acid by detailed analysis of chromatographic and mass
394 spectral data. All the identified compounds were further quantified, indicating that malic
395 acid is the most abundant hydroxydiacid followed by tartronic acid in the remote marine
396 atmosphere. This is the first report to identify and quantify homologous series of
397 hydroxydiacids (hC₃di-hC₆di) including tartaric acid. We also demonstrate that
398 oxaloacetic acid is present as two forms and is in part converted to pyruvic acid in remote
399 marine aerosol samples. A further study is required to better understand the seasonal
400 variations of hydroxydiacids in remote marine aerosols and their sources and
401 transformation pathways.

402 ACKNOWLEDGEMENTS

403 This study was in part supported by the Japan Society for the Promotion of Science
404 (JSPS) through Grant-in-Aid No. 24221001. Divyavani thanks to the FY2013 GSES
405 Takuetsu-Research Assistantship 2013 of Hokkaido University for the financial support.

406 REFERENCES

- 407 [1].P. Fu, K. Kawamura, K. Usukura, K. Miura. Dicarboxylic acids, ketocarboxylic acids
408 and glyoxal in the marine aerosols collected during a round-the-world cruise. *Mar.*
409 *Chem.* **2013**, *148*, 22.
- 410 [2].C. D. O'Dowd, M. C. Facchini, F. Cavalli, D. Ceburnis, M. Mircea, S. Decesari, S.
411 Fuzzi, Y. J. Yoon, J. P. Putaud. Biogenically driven organic contribution to marine
412 aerosol. *Nature.* **2004**, *431*, 676.
- 413 [3].X. L. Zhou, A. J. Davis, D. J. Kieber, W. C. Keene, J. R. Maben, H. Maring, E. E Dahl,
414 M. A. Izaguirre, R. Sander, L. Smoydzyn. Photochemical production of hydroxyl
415 radical and hydroperoxides in water extracts of nascent marine aerosols produced by
416 bursting bubbles from Sargasso seawater. *Geophys. Res. Lett.* **2008**, *35*, L20803, DOI:

- 417 10.1029/2008GL035418.
- 418 [4].M. Rinaldi, S. Decesari, E. Finessi, L. Giulianelli, C. Carbone, S. Fuzzi, C. D. O'Dowd,
419 D. Ceburnis, M. C. Facchini. Primary and secondary organic marine aerosol and
420 oceanic biological activity: Recent results and new perspectives for future studies. *Adv.*
421 *Meteorol.* **2010**, 310682, DOI: 10.1155/2010/310682.
- 422 [5].G. H. Wang, S. L. Niu, C. Liu, L. S. Wang. Identification of dicarboxylic acids and
423 aldehyde of PM10 and PM2.5 aerosols in Nanjing, China. *Atmos. Environ.***2002**, *36*,
424 1941.
- 425 [6].K. F. Ho, S. C. Lee, S. S. H. Ho, K. Kawamura, E. Tachibana, Y. Cheng, T. Zhu.
426 Dicarboxylic acids, ketocarboxylic acids, α -dicarbonyls, fatty acids, and benzoic acid
427 in urban aerosols collected during the 2006 Campaign of Air Quality Research in
428 Beijing (CAREBeijing-2006). *J. Geophys. Res. -Atmos.* **2010**, *115*, D19312, DOI:
429 10.1029/2009 JD013304.
- 430 [7].M. Mochida, A. Kawabata, K. Kawamura, H. Hatsushika, K. Yamazaki. Seasonal
431 variation and origins of dicarboxylic acids in the marine atmosphere over the western
432 North Pacific. *J. Geophys. Res. -Atmos.* **2003**, *108*, 4193, DOI:
433 10.1029/2002JD002355.
- 434 [8].C. L. Cheng, G. H. Wang, B. H. Zhou, J. J. Meng, J. J. Li, J. J. Cao, S. Xiao.
435 Comparison of dicarboxylic acids and related compounds in aerosol samples collected
436 in Xi'an, China during haze and clean periods. *Atmos. Environ.* **2013**, *81*, 443.
- 437 [9].A. Limbeck, H. Puxbaum, L. Otter, M.C. Scholes. Semivolatile behavior of
438 dicarboxylic acids and other polar organic species at a rural background site (Nylsvley,
439 RSA). *Atmos. Environ.* **2001**, *35*, 1853.
- 440 [10]. T. Novakov, J. E. Penner. Large contribution of organic aerosols to cloud-
441 condensation-nuclei concentrations. *Nature* **1993**, *365*, 823.
- 442 [11]. P. Saxena, L. M. Hildemann, P. H. McMurry, J. H. Seinfeld. Organics alter
443 hygroscopic behavior of atmospheric particles. *J. Geophys. Res.* **1995**, *100*, 18755.
- 444 [12]. R. Sempéré, K. Kawamura. Low molecular weight dicarboxylic acids and related
445 polar compounds in the remote marine rain samples collected from western Pacific.
446 *Atmos. Environ.* **1996**, *30*, 1609.
- 447 [13]. K. Kawamura, F. Sakaguchi. Molecular distributions of water soluble dicarboxylic
448 acids in marine aerosols over the Pacific Ocean including tropics. *J. Geophys. Res.* -

- 449 *Atmos.* **1999**, *104*, 3501.
- 450 [14]. D. Grosjean, J. H. Seinfeld. Parameterization of the formation potential of
451 secondary organic aerosols. *Atmos. Environ.* **1989**, *23*, 1733.
- 452 [15]. G. H. Wang, K. Kawamura, T. Watanabe, S. Lee, K. Ho, J. J. Cao. High loadings
453 and source strengths of organic aerosols in China. *Geophys. Res. Lett.* **2006**, *33*,
454 L22801, DOI: 10.1029/2006GL027624.
- 455 [16]. K. Kawamura, O. Yasui. Diurnal changes in the distribution of dicarboxylic acids,
456 ketocarboxylic acids and dicarbonyls in the urban Tokyo atmosphere. *Atmos. Environ.*
457 **2005**, *39*, 1945.
- 458 [17]. K. Kawamura, K. Ikushima. Seasonal changes in the distribution of dicarboxylic
459 acids in the urban atmosphere. *Environ. Sci. Technol.* **1993**, *27*, 2227.
- 460 [18]. M. P. Tolocka, M. Jang, J. M. Ginter, F. J. Cox, R. M. Kamens, M. V. Johnston.
461 Formation of oligomers in secondary organic aerosol. *Environ. Sci. Technol.* **2004**, *38*,
462 1428.
- 463 [19]. H. Okochi, P. Brimblecombe. Potential trace metal-organic complexation in the
464 atmosphere. *Scientific World Journal* **2002**, *2*, 767.
- 465 [20]. T. Hoffmann, R. Bandur, U. Marggraf, M. Linscheid. Molecular composition of
466 organic aerosols formed in the α -pinene/O₃ reaction: Implications for new particle
467 formation processes. *J. Geophys. Res.* **1998**, *103*, 25569.
- 468 [21]. M. Kulmala. How particles nucleate and grow. *Science* **2003**, *302*, 1000.
- 469 [22]. J. F. Hamilton, A. C. Lewis, J. C. Reynolds, L. J. Carpenter, and A. Lubben.
470 Investigating the composition of organic aerosol resulting from cyclohexene
471 ozonolysis: low molecular weight and heterogeneous reaction products *Atmos. Chem.*
472 *Phys.* **2006**, *6*, 4973.
- 473 [23]. M. Claeys, R. Szmigielski, I. Kourtchev, P. van der Veken, R. Vermeylen, W.
474 Maenhaut, M. Jaoui, T. E. Kleindienst, M. Lewandowski, J. Offenberg, E. O. Edney.
475 Hydroxydicarboxylic acids: Markers for secondary organic aerosol from the
476 photooxidation of α -pinene. *Environ. Sci. Technol.* **2007**, *41*, 1628.
- 477 [24]. A. Rompp, R. Winterhalter, G. K. Moortgat. Oxodicarboxylic acids in atmospheric
478 aerosol particles. *Atmos. Environ.* **2006**, *40*, 6846.

- 479 [25]. T. Charbouillot, S. Gorini, G. Voyard, M. Parazols, M. Brigante, L. Deguillaume,
480 A. Delort, G. Mailhot. Mechanism of carboxylic acid photooxidation in atmospheric
481 aqueous phase: Formation, fate and reactivity. *Atmos. Environ.* **2012**, *56*, 1.
- 482 [26]. L. Bao, M. Matsumoto, T. Kubota, S. Kazuhiko, Q. Wang, K. Sakamoto.
483 Gas/particle partitioning of low-molecular-weight dicarboxylic acids at a suburban site
484 in Saitama, Japan. *Atmos. Environ.* **2012**, *47*, 546.
- 485 [27]. I. El. Haddada, N. Marchanda, J. Drona, B. Temime-Roussela, E. Quiveta, H.
486 Worthama, J. L. Jaffrezob, C. Baduelb, D. Voisinb, J. L. Besombesc, G. Gilled.
487 Comprehensive primary particulate organic characterization of vehicular exhaust
488 emissions in France. *Atmos. Environ.* **2009**, *43*, 6190.
- 489 [28]. G. Petersson. Mass spectrometry of hydroxy dicarboxylic acids as trimethylsilyl
490 derivatives rearrangement fragmentations. *Org. Mass spectrom.* **1972**, *6*, 565.
- 491 [29]. K. Kawamura, R. B. Gagosian. Midchain ketocarboxylic acids in the remote
492 marine atmosphere - Distribution patterns and possible formation mechanisms. *J.*
493 *Atmos. Chemistry.* **1990**, *11*, 107.
- 494 [30]. A. Rohrl, G. Lammel. Determination of malic acid and other C-4 dicarboxylic
495 acids in atmospheric aerosol samples. *Chemosphere.* **2002**, *46*, 1195.
- 496 [31]. V. Zaikin, J. Halket. *A Handbook of Derivatives for Mass Spectrometry*. IM
497 Publications, **2009**, pp. 14-15.
- 498 [32]. M. Kalberer, J. Yu, D. R. Cocker, R. C. Flagan, J. H. Seinfeld. Aerosol formation
499 in the cyclohexene-ozone system. *Environ. Sci. Technol.*, **2000**, *34*, 4894.
- 500 [33]. J. F. Rontani, C. Aubert. Trimethylsilyl transfer during electron ionization mass
501 spectral fragmentation of some ω -hydroxycarboxylic and ω -dicarboxylic acid
502 trimethylsilyl derivatives and the effect of chain length. *Rapid. Commun. Mass*
503 *Spectrom.* **2004**, *17*, 1889.
- 504 [34]. E. D. Hoffmann, V. Stroobant. *Mass spectrometry: Principles and Applications*.
505 John Wiley & Sons, **2007**, 3rd edition, pp. 296.
- 506 [35]. M. Frosch, A.A. Zardini, S.M. Platt, L. Muller, M.C Reinnig, T. Hoffmann, M.
507 Bilde. Thermodynamic properties and cloud droplet activation of a series of oxo-acids.
508 *Atmos. Chem. Phys.* **2010**, *10*, 5873.

Figures

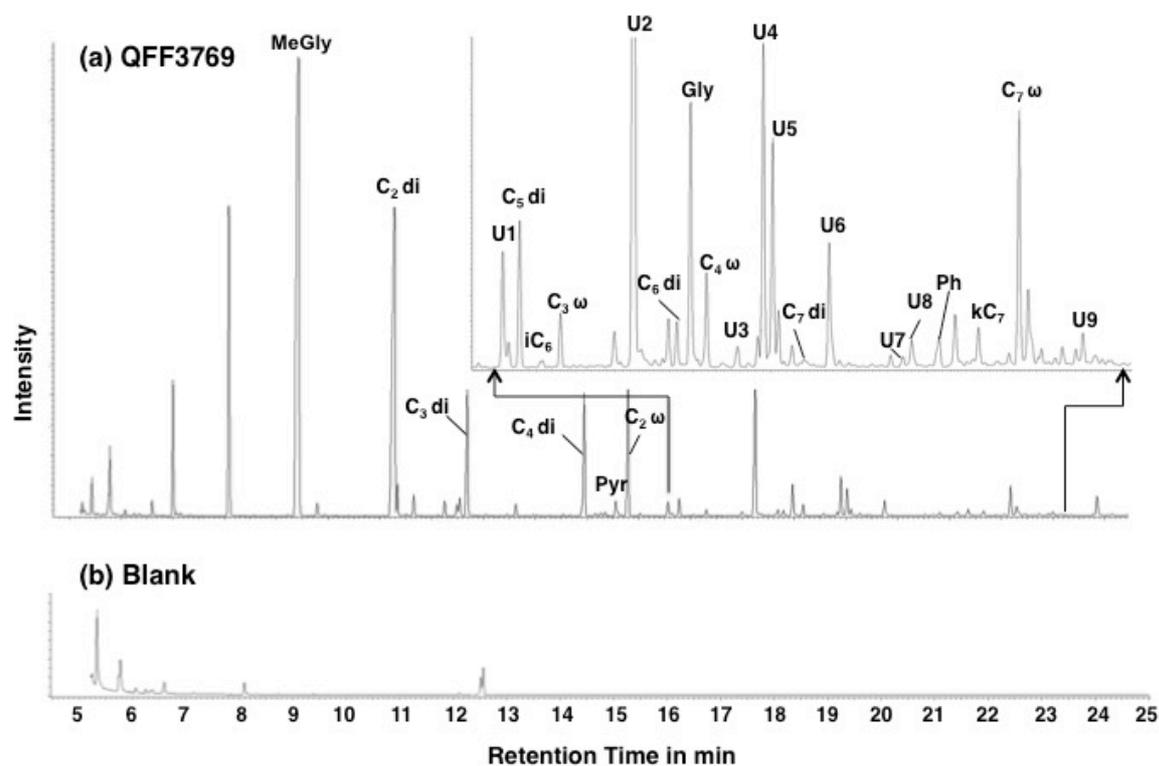


Figure 1. Reconstructed (GC/TOFMS) total ion chromatogram obtained for (a) butyl ester TMS-derivatized of the marine aerosol sample (QFF3769) and (b) blank filter obtained from Chichijima Island in the western North Pacific. Identification: methylglyoxal (MeGly), oxalic acid (C₂ di), malonic acid (C₃ di), succinic acid (C₄ di), pyruvic acid (Pyr), glyoxylic acid (C₂ ω), glutaric acid (C₅ di), methylglutaric acid (iC₆), 3-oxopropanoic acid (C₃ ω), adipic acid (C₆ di), glyoxal (Gly), 4-oxobutanoic acid (C₄ ω), pimelic acid (C₇ di), phthalic acid (Ph), 4-oxopimelic acid (kC₇), and 7-oxoheptanoic acid (C₇ ω). Chemical structures of the novel compounds identified in the present study: U1, tartronic acid; U2, malic acid; U3, enol oxaloacetic acid; U4, 3-hydroxyglutaric acid; U5, 2-hydroxyglutaric acid; U6, tartaric acid; U7, 2-hydroxyadipic acid; U8, 3-hydroxyadipic acid; U9, and keto oxaloacetic acid.

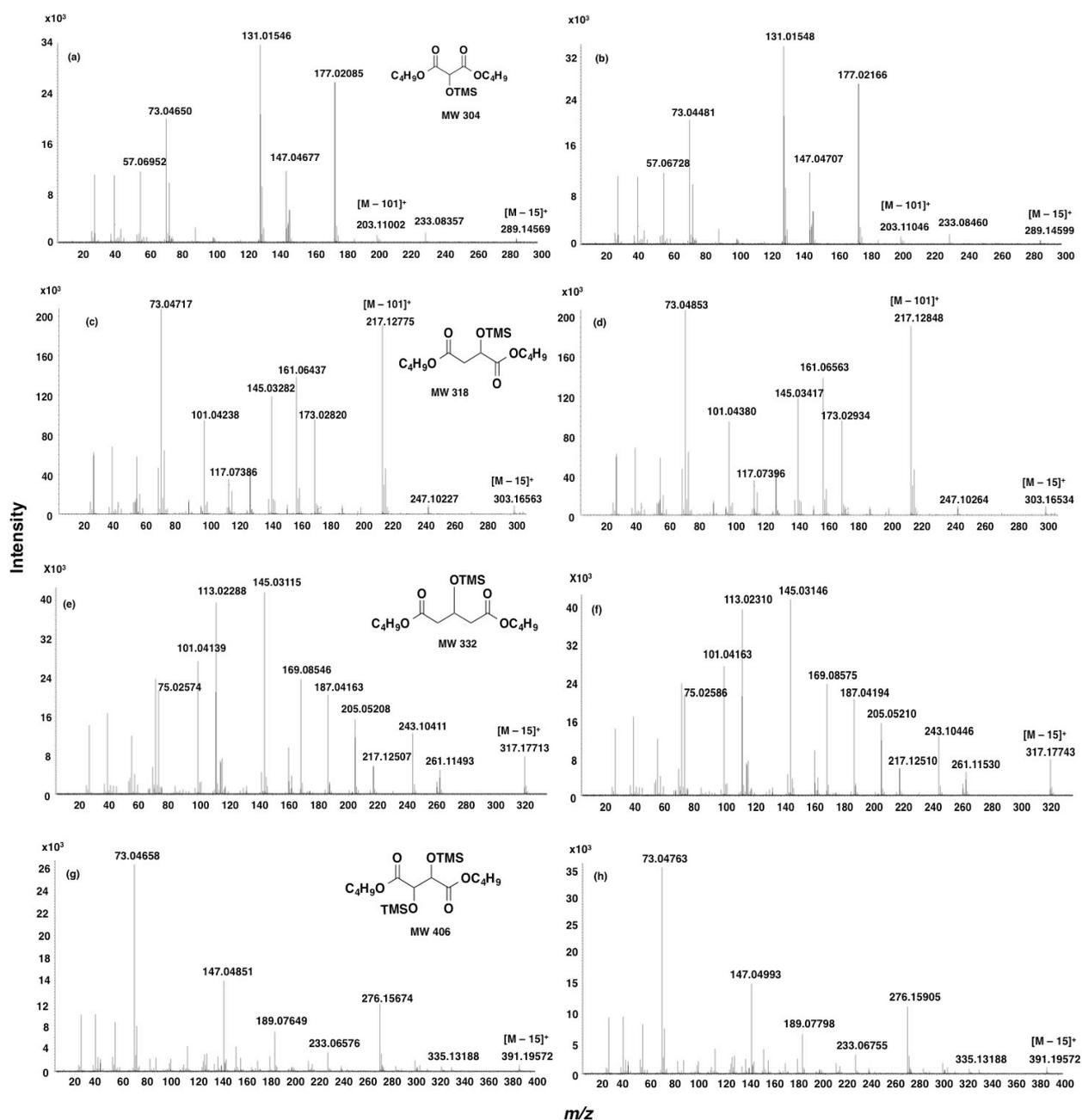


Figure 2. Mass spectra of dibutyl ester TMS-derivatives of (a) tartronic acid (U1), (b) tartronic acid (standard), (c) malic acid (U2), (d) malic acid (standard), (e) 3-hydroxyglutaric acid (U4), (f) 3-hydroxyglutaric acid (standard), (g) tartaric acid (U6), (h) tartaric acid (standard).

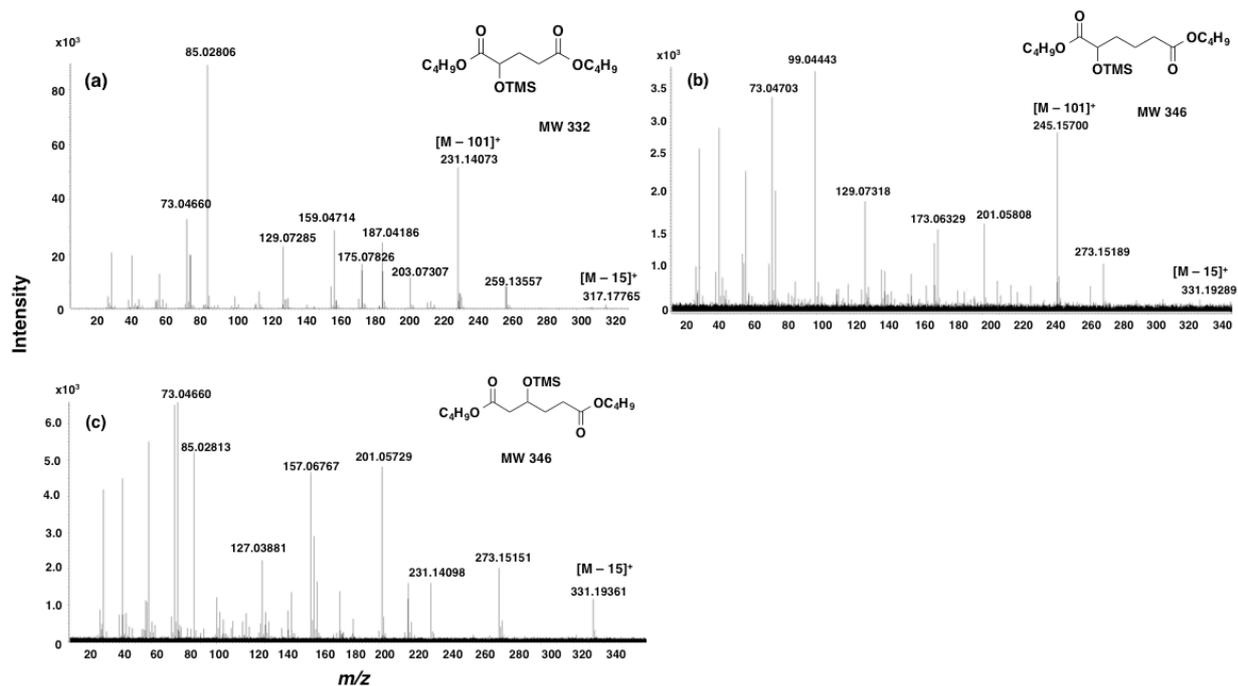


Figure 3. Mass spectra of dibutyl ester TMS-derivatives of (a) 2-hydroxyglutaric acid, (b) 2-hydroxyadipic acid, (c) 3-hydroxyadipic acid, isolated from the remote marine aerosol samples collected in the western North Pacific.

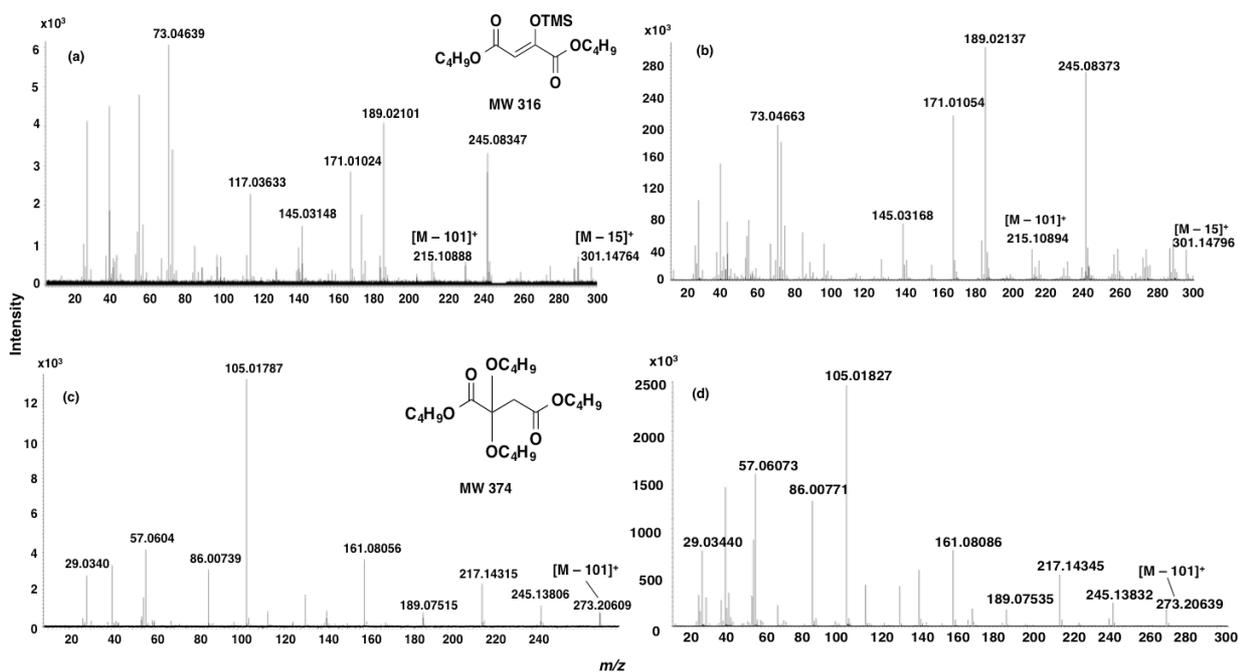
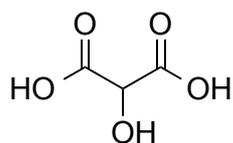
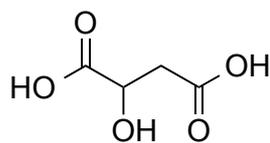


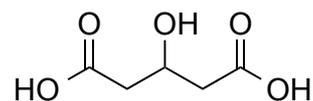
Figure 4. Mass spectra of dibutyl ester TMS-derivatives of (a) enol-oxaloacetic acid (U3), (b) enol-oxaloacetic acid (standard), (c) keto-oxaloacetic acid (U9), and (d) keto-oxaloacetic acid (standard).



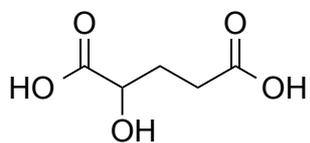
(a) tartronic acid



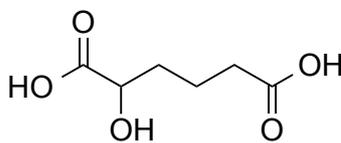
(b) malic acid



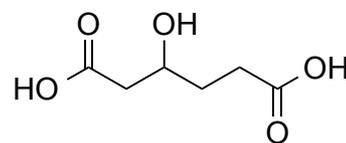
(c) 3-hydroxyglutaric acid



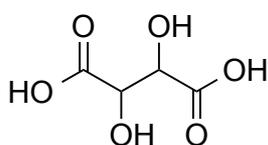
(d) 2-hydroxyglutaric acid



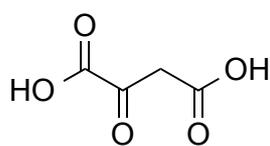
(e) 2-hydroxyadipic acid



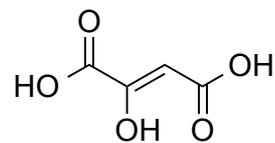
(f) 3-hydroxyadipic acid



(g) tartaric acid

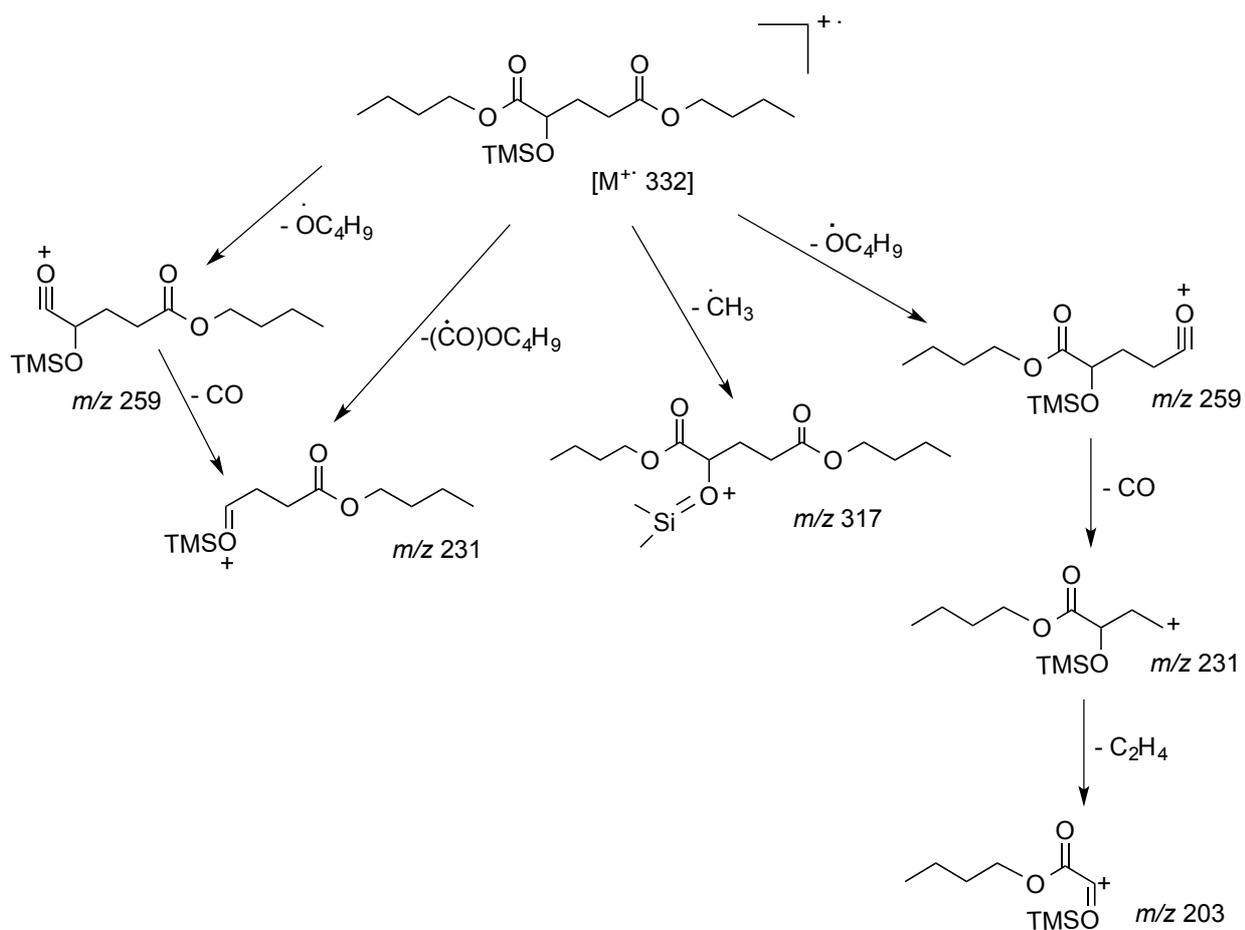


(h) keto-oxaloacetic acid

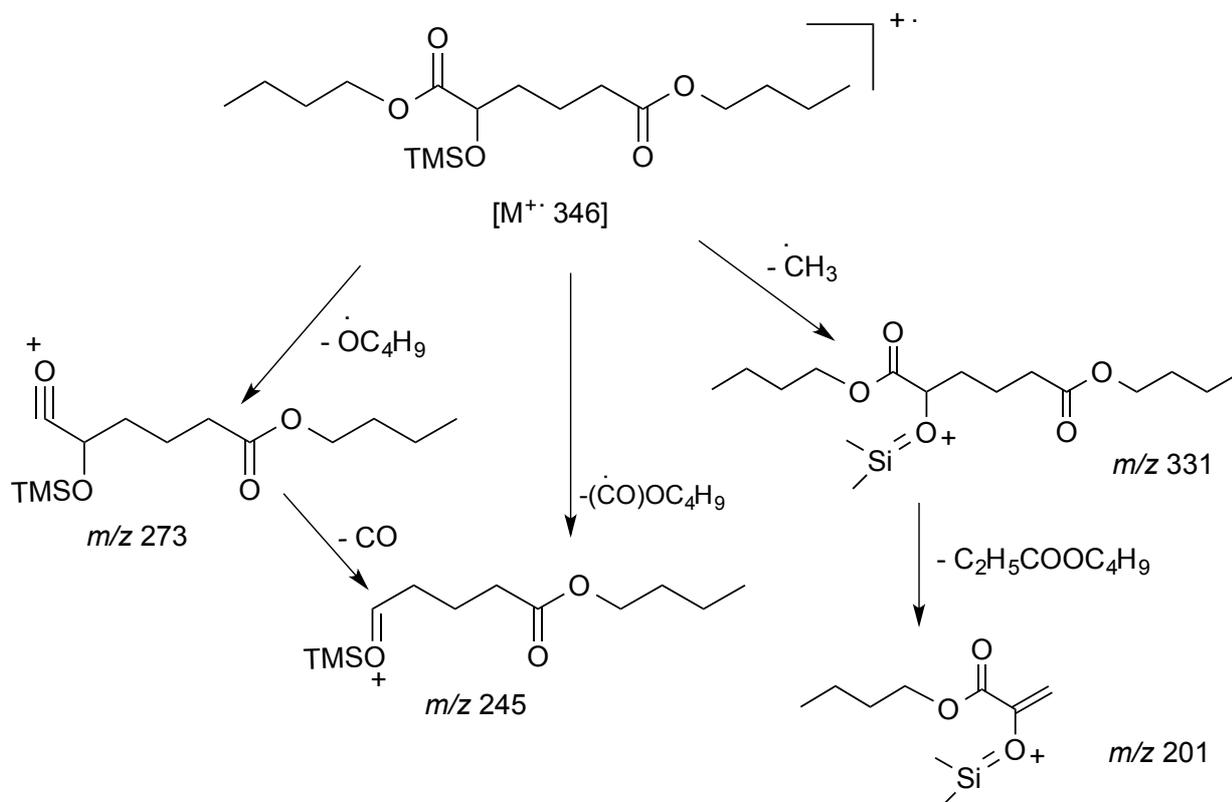


(i) enol-oxaloacetic acid

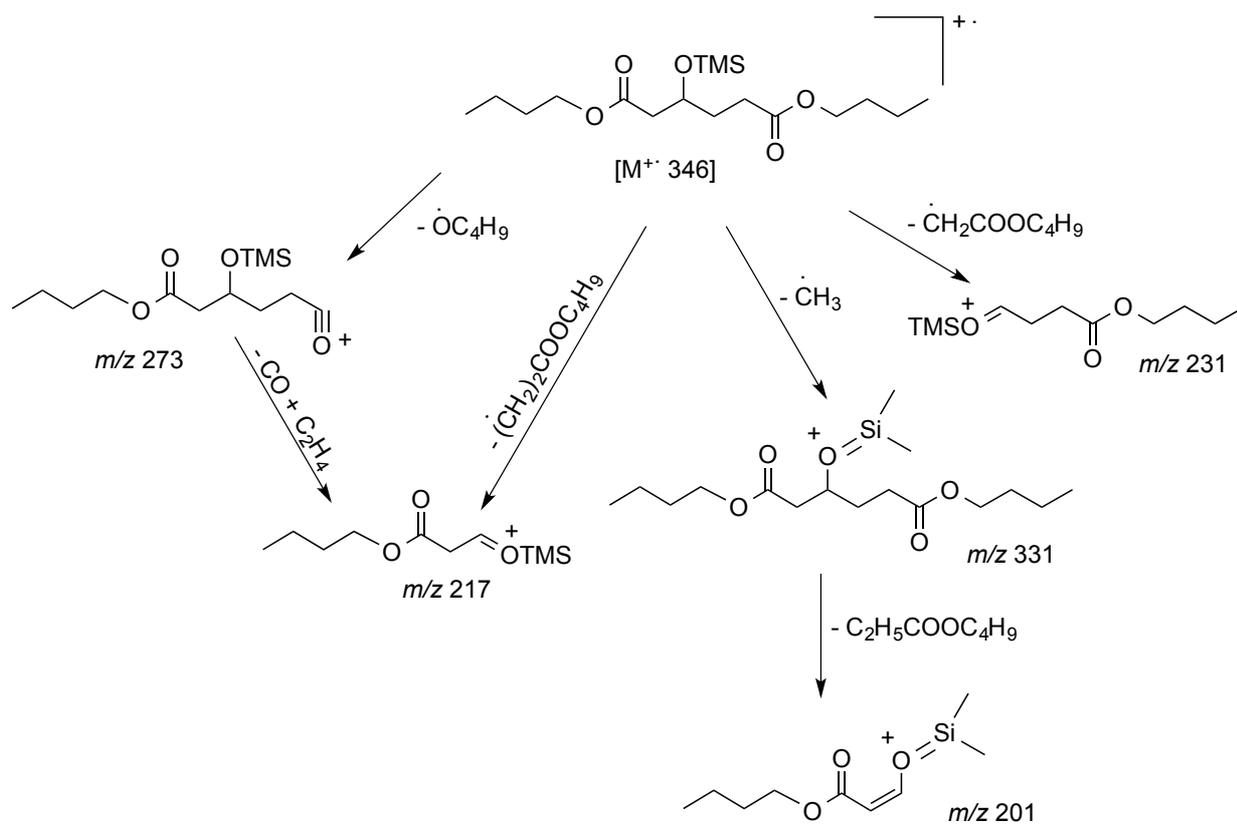
Figure 5. Chemical structures of hydroxy- and keto-dicarboxylic acids identified in remote marine aerosols from the western North Pacific.



Scheme 1: Proposed fragmentation pathways of the butyl ester TMS-derivative of unknown compound U4, which is identified as 2-hydroxyglutaric acid.



Scheme 2: Proposed fragmentation pathways of the butyl ester TMS-derivative of unknown compound U5, which is identified as 2-hydroxyadipic acid.



Scheme 3: Proposed fragmentation pathways of the butyl ester TMS-derivative of unknown compound U6, which is identified as 3-hydroxyadipic acid.