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

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

METHODS: The method of chemical derivatization followed by gas chromatography-flame ionization detection (GC-FID), gas chromatography/quadruple mass spectrometry (GC/QMS) and gas chromatography/time-of-flight mass spectrometry (GC/TOFMS) analysis was used to identify hydroxy- and keto-diacids in remote marine aerosols. Atmospheric samples were collected at Chichijima Island in the western North Pacific and extracted with organic free ultrapure water for diacids and related compounds. A two-step derivatization technique were used in this study using 14% BF₃/n-butanol for the butylation of carboxyl groups and acidic ketones followed by N,O-bis(trimethylsilyl)trifluoroacetamide (BSTFA) for the trimethylsilylation of hydroxyl groups.

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

CONCLUSIONS: Utilizing GC-FID, GC/QMS and GC/TOFMS, hydroxy- and keto-diacids were identified in the remote marine aerosols. A complete structural characterization was achieved with extensive mass spectral analysis. Molecular distributions of hydroxydiacids generally showed the predominance of malic acid followed by tartronic acid. We consider that these hydroxydiacids are important intermediates in the atmospheric oxidation of organic aerosols to result in smaller diacids.

Keywords: remote marine aerosol, ketodicarboxylic acids, hydroxydicarboxylic acids, oxaloacetic acid, keto-enol tautomerization, GC/TOFMS.
Organic aerosols are present not only in the continental atmosphere, but also in the marine atmosphere. They can significantly contribute to the global budget of atmospheric particles and thus have an impact on the Earth’s albedo, climate, atmospheric chemistry, and biogeochemical cycles of carbon and nitrogen. The fate of hydrophilic organics in the marine atmosphere is highly uncertain. Organic compounds in marine aerosols may act as important precursors and a dominant sink for OH radicals, leading to the formation of various low-molecular-weight diacids by their degradation. However, water-soluble organic carbon in remote marine aerosols has not been well characterized at the molecular level.

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

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

Trifunctional hydroxy- and keto-diacids can give rise to stable homo- and hetero-molecular clusters through hydrogen bonding, being similar to the clusters proposed for
bifunctional compounds\textsuperscript{[20]}, and may as such participate in new particle formation and
growth.\textsuperscript{[21]} Since they are highly oxygenated, it is expected that studies of keto- and
hydroxy-diacids provide useful information to better understand the oxidative reaction
mechanisms of organic matter in the marine atmosphere during long-range transport.
Identification and quantification of these compounds will thus help identify the principle
mechanisms for the chemical aging of organic aerosols in the marine atmosphere.

In this study, many unknown peaks were detected in the gas chromatogram after
trimethylsilyl (TMS) derivatization of the dibutyl ester fraction of remote marine aerosols.
Mass spectral examination of the unknown peaks suggests that they belong to a
homologous series of hydroxydiacids (hC\textsubscript{3}dihC\textsubscript{6}d), including tartronic acid, malic acid,
3-hydroxyglutaric acid, 2-hydroxyglutaric acid, 2-hydroxyadipic acid, 3-hydroxyadipic
acid, and tartaric acid. Some of these compounds are individually detected in laboratory
photo-oxidations\textsuperscript{[22]} and ambient aerosol samples\textsuperscript{[23]}, but there is no report on the
identification of the homologous series of hydroxyl diacids formed in marine aerosols.
Many isomeric ketodicarboxylic acids have been identified in atmospheric aerosol
particles.\textsuperscript{[24]} Oxaloacetic acid was suggested to exist in aerosols by the photo-oxidation of
succinic acid\textsuperscript{[25]}, but has not been detected in ambient aerosols previously. Here,
oxaloacetic acid was successfully identified as isomeric forms in marine aerosols.
Tentative fragmentation pathways are proposed for the derivatized unknown
hydroxydiacids and oxaloacetic acid in marine aerosols.

**EXPERIMENTAL**

**Reagents and chemicals**

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

**Aerosol sampling**

Total suspended particles (TSP) were collected on weekly basis from 1 December
2010 to 30 November 2011 (n = 53) using pre-combusted (450 °C) quartz fiber filters and
a high volume air sampler at a flow rate of 1.0 m$^3$ min$^{-1}$ at the Ogasawara Downrange Station of the Japan Aerospace Exploration Agency (JAXA) at Chichijima Island (27°04′N; 142°13′E) in the western North Pacific, an outflow region of the Asian dust and polluted air masses from China and other neighboring countries. Sampling periods were generally five days. After sampling, the filters were placed in precombusted glass jars with a Teflon-lined screw cap, sent to Hokkaido University in Sapporo, and stored in a freezer room at -20 °C prior to analysis.

**Preparation of dibutyl esters**

Filter aliquots were extracted three times with 10 mL organic-free pure water under ultrasonication for 10 min. To remove insoluble particles and filter debris, the extracts were passed through a glass column (Pasteur pipette) packed with quartz wool into a pear-shape flask. The water extracts were pH-adjusted to 8.5-9.0 with 0.05 M aqueous KOH solution and concentrated almost to dryness using a rotary evaporator under a vacuum. A 14% BF$_3$/n-butanol was added to the concentrates in the flask to allow esterification at 100 °C for 1 h. During the reaction, carboxyl groups are derivatized to their butyl esters, and keto groups to dibutoxy acetals. The derivatives were extracted with 10 mL of n-hexane after adding 10 mL of organic free-water and 0.5 mL of acetonitrile. Acetonitrile transfers the excess n-butanol into the aqueous phase efficiently. The hexane layer was further washed with organic free-water (2 x 10 mL). Using a rotary evaporator under a vacuum and a nitrogen blow-down system, extracts were concentrated and then dissolved in 100 µL of n-hexane.

**BSTFA derivatization**

An aliquot of the dibutyl ester fraction containing hydroxyl diacids was dried under a gentle stream of nitrogen, and hydroxyl diacid dibutyl esters were derivatized to corresponding TMS ethers with 50 µL of N,O-bis(trimethylsilyl)trifluoroacetamide (BSTFA) at 80 °C for 30 min. The derivatives were dried under a nitrogen stream, dissolved in n-hexane (50 µL) and analyzed using gas chromatography/quadruple mass spectrometry (GC/QMS) and gas chromatography/time of flight-mass spectrometry (GC/TOFMS). Their concentrations were quantified using gas chromatography-flame ionization detection (GC-FID). Known amounts of authentic standards (tartronic, malic, 3-hydroxyglutaric, tartaric, and oxaloacetic acids) were spiked on a pre-combusted quartz fiber filter and derivatized to butyl esters-TMS ethers by the procedure described above. We found that the recoveries of hydroxyl diacids are more than 80%. The analytical errors
in duplicate analysis of the aerosol sample (QFF3769) for hydroxy- and keto-diacids are about 10%.

**Instrumentation**

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

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

**RESULTS AND DISCUSSION**

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

In order to distinguish dicarboxylic acids with and without hydroxyl groups, the water extracts of marine aerosols were subjected to a two-step derivatization procedure, i.e., butylation followed by trimethylsilylation, by which carboxyl groups are converted to butyl esters whereas hydroxyl groups are converted to TMS ethers. Methylation TMS-derivatization has been often used for hydroxydicarboxylic acids[24] but in this study the butylation TMS method was used because butyl ester derivatization is highly selective for bifunctional compounds[26] and the recoveries are high even for small diacids. [16] On the other hand, methyl esters of diacids are sometimes highly volatile and thus are lost by evaporation during the analytical procedure. [27]
Figure 1(a) and 1(b) shows reconstructed total ion chromatogram of butylated-TMS derivatives from Chichijima Island in the western North Pacific (QFF3769) and a blank filter chromatogram. In the gas chromatogram (Fig. 1(a)), nine new peaks were observed after TMS derivatization of the diacid butyl ester fraction. They are unknown structures and thus named as compound U1 to U9, which are considered to contain additional hydroxyl- and keto- groups in their diacid structures. The detailed interpretation and characterization for unknown compounds U1 to U9 are presented below.

**Tartronic acid**

The EI mass spectra for the butyl ester TMS-derivative of unknown compound U1 allow us to infer the molecular weight (MW) of the underivatized compound as 120 (retention time (RT): 16.5 min; MW of 304 for the butyl ester TMS-derivative; [M - CH₃]⁺ ion at $m/z$ 289; C₁₃H₂₅O₄Si; measured mass: 289.14569, error: -5 ppm). These results suggest that U1 is tartronic acid (2-hydroxymalonic acid). Tartronic acid has been postulated to exist in marine aerosols via the photooxidation of malonic acid [13], but no evidence has been provided for its presence in ambient aerosols samples. In the present study, we successfully identified tartronic acid (Fig. 5(a)) in remote marine aerosols.

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

To our knowledge, this is the first identification of tartronic acid in ambient aerosols. We found that the measured concentration of tartronic acid ranged from 0.01 to 5.27 ng m⁻³ in total suspended particles collected at Chichijima Island during the period of December 2010 to 30 November 2011 (n = 53). This wide range is due to seasonal changes in the air masses. The sampling site is strongly influenced by westerly winds in winter to spring, oceanic clean air masses in summer, and both oceanic and continental air...
masses in autumn \[^7\], affecting the concentration of tartronic acid in remote marine aerosols.

**Malic acid**

The EI mass spectral data for the butyl ester TMS-derivative of unknown compound U2 allow us to infer that the MW of the underivatized compound is 134 (RT: 18.2 min; MW of 318 for butyl ester TMS-derivative; [M - CH\(_3\)]\(^+\) ion at m/z 303; C\(_{14}\)H\(_{25}\)O\(_3\)Si; measured mass: 303.16563, error: 9 ppm). These results suggest that compound U2 is malic acid (2-hydroxysuccinic acid) (Fig. 5 (b)). Malic acid has been proposed to result from the hydroxylation of succinic acid in marine aerosols using a 14% BF\(_3\)/n-butanol as derivatizing agent.\[13\] We found that it is the most abundant hydroxyl diacid in marine aerosols. Because the hydroxyl group was underivatized in the previous technique for diacid dibutyl ester derivatization, malic acid dibutyl ester often showed peak tailing in the chromatogram, which may lead to underestimate the concentrations of this compound.\[30\]

Here, we addressed this problem by derivatizing hydroxyl groups to TMS ethers with BSTFA. The malic acid concentration in all the measured samples (n = 53) ranged from 0.03 to 14.7 ng m\(^{-3}\), which is ca. 3 times higher than those reported in previous studies without TMS derivatization.

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

**3- and 2-hydroxyglutaric acids**

The EI mass spectra for the butyl ester TMS-derivatives of both unknown compounds U4 and U5 suggest that the MW of the underivatized compounds is 148 with a MW of 332 for butyl ester TMS-derivatives and m/z 317 for [M - CH\(_3\)]\(^+\) ion. The RT of U4 and U5 are 19.1 min and 20.03 min, and the measured m/z values of C\(_{15}\)H\(_{29}\)O\(_3\)Si are at 317.17713 and 317.17765 with errors of -4 ppm and -2 ppm, respectively. These results suggest U4 and
U5 are positional isomers, which are assigned to 3-hydroxyglutaric and 2-hydroxyglutaric acids (Figs. 5(c) and 5(d)). Based on the mass spectral fragmentation patterns we could distinguish both isomers. 3-hydroxy and 2-hydroxyglutaric acids have been reported in fine PM$_{2.5}$ aerosols from rural K-puszta, Hungary as photo-oxidation products of $\alpha$-pinene. [23].

Compound U4 butyl ester TMS-derivative shows fragment ions at m/z 317, 261, 259, 243, 217, 205, 187, 169, 145, 113, 101, 73, 57, and 41 (Fig. 2 (e)). Structurally informative ions include m/z 317 [M-15]$^+$, and m/z 261 [M-15-56]$^+$, while the peak at m/z 259 [M-73]$^+$, may be due to the loss of a butoxy radical (OC$_4$H$_9$) from the molecular ion by $\alpha$-cleavage. The m/z 217 [M-115]$^+$ could be explained by loss of a butyl ethanoate radical [CH$_2$COOC$_4$H$_9$] from the molecular ion by $\beta$-cleavage, suggesting that the position of hydroxyl group is at the third carbon. [31] The 3-hydroxyglutaric acid standard TOFMS spectrum was obtained (Fig. 2 (f)) and compared with that of U4 (Fig. 2 (e)). The results showed a good agreement and similar retention time allowing the confirmation of U4 as 3-hydroxyglutaric acid. Proposed fragmentation pathways that are structurally informative for the butyl ester TMS-derivative of 3-hydroxyglutaric acid are summarized in Scheme S3 (see Supporting Information). The atmospheric concentration of 3-hydroxyglutaric acid in all measured samples ($n$ = 53) ranged from 0.01 to 4.98 ng m$^{-3}$.

Compound U5 butyl ester TMS-derivative shows fragment ions at m/z 317, 259, 231, 203, 187, 175, 159, 129, 85, 73, 57, and 41 (Figure 3(a)). Structurally informative ions include m/z 317 [M-15]$^+$, m/z 259 [M-73]$^-$. The peak at m/z 231 [M-101]$^+$ or via [M-73-28]$^-$ could be due to a loss of neutral molecule of carbon monoxide (CO, 28 u) from the [M-73]$^+$ ion, and m/z 203 could be explained by the elimination of neutral molecule of ethene (C$_2$H$_4$, 28 u) from the ion of m/z 231. Tentative fragmentation pathways that are structurally informative for the butyl ester TMS-derivative of unknown U5 (2-hydroxyglutaric acid) are summarized in Scheme 1. The fragment ion of m/z 231 [M-101]$^+$ suggests that the position of hydroxyl group is at the second carbon and hence, helping us to distinguish 2-hydroxyglutaric acid from 3-hydroxyglutaric acid. Based on these experimental observations, U5 is assigned to 2-hydroxyglutaric acid. The atmospheric concentration of 2-hydroxyglutaric acid in all the measured samples ($n$ = 53) ranged from 0.03 to 4.79 ng m$^{-3}$.

2-hydroxyadipic and 3-hydroxyadipic acids

The EI mass spectra for the butyl ester TMS-derivatives of both U7 and U8 inferred
that the MW of the underivatized compounds is 162 with a MW of 346 for butyl ester TMS-derivatives and with a [M - CH₃]⁺ ion at m/z 331. The RT of U7 and U8 are 21.7 and 21.8 min and the measured m/z values of C₁₆H₃₁O₈Si are at 331.19289 and 331.19226 with errors of -4 ppm and -5 ppm, respectively. Thus, the mass spectral information indicates that compounds U7 and U8 are positional isomers, which are attributed to 2-hydroxyadipic and 3-hydroxyadipic acids, respectively (Fig. 5(e) and 5(f)). Based on the mass spectral fragmentation patterns we could distinguish the two isomers. 2-
Hydroxyadipic and 3-hydroxyadipic acids have not been identified in marine aerosols. On the other hand, 3-hydroxyadipic acid has been identified as a reaction product during laboratory photo-oxidation of cyclohexane.\(^{[32]}\)

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

The butyl ester TMS-derivative of compound U8 shows fragment ions at m/z 331, 273, 231, 217, 201, 157, 127, 85, 73, 57, and 41 (Fig. 3(c)). Structurally informative ions include m/z 331 [M-15]⁺ and 273 [M-73]⁺. The peak at m/z 217 may be due to a neutral loss of carbon monoxide (CO, 28 u) and ethene (C₂H₄, 28 u) from the [M-73]⁺ ion whereas m/z 231 [M-115]⁺ could be explained by loss of a butyl ethanoate radical [CH₃COOC₄H₆] from the molecular ion by β-cleavage, suggesting that the position of the hydroxyl group is at the third carbon. Tentative fragmentation pathways that are structurally informative for the butyl ester TMS-derivative of unknown U8 (3-hydroxyadipic acid) are summarized in Scheme 3. The fragment ions at m/z 245 [M-101]⁺ and m/z 231 [M-115]⁺ in U7 and U8 indicates the location of hydroxyl group.\(^{[31]}\) Based on these experimental observations U7 and U8 are assigned to 2-hydroxyadipic and 3-hydroxyadipic acids, respectively. The concentrations of 2-hydroxyadipic and 3-hydroxyadipic acids in all the measured samples (n=53) ranged from 0.04 to 0.73 ng m⁻³ and 0.19 to 0.82 ng m⁻³, respectively.

**Tartaric acid**

The EI mass spectra for the butyl ester TMS-derivative of unknown compound U6 suggested that the MW of the underivatized compound is 150 (RT; 20.7 min) and the MW is 406 for the butyl ester TMS-derivative with [M - CH₃]⁺ ion of m/z 391. The measured
320 $m/z$ value of C$_{17}$H$_{35}$O$_{6}$Si$_{2}$ is 391.19572 with an error of -4 ppm. This information suggests that compound U6 is tartaric acid (2,3-dihydroxyssuccinic acid) (Fig. 5(g)). Tartaric acid has been reported in continental aerosols, [30] however, there is no report in marine aerosols. This is the first report on the presence of tartaric acid in continentally influenced remote marine aerosols.

325 The mass spectrum of the butyl ester TMS-derivative of tartaric acid presents characteristic fragment ions at $m/z$ 391, 335, 305, 276, 233, 189, 147, and 73 (Fig. 2(g)). Structurally informative ions include $m/z$ 391 [M-15]$^+$, $m/z$ 335 [M-15-56]$^+$ and $m/z$ 305 [M-101]$^+$. The peak at $m/z$ 233 could be caused by the combined loss of a butoxycarbonyl ((C=O)OC$_3$H$_6$) and a hydrogen radical by $\alpha$-cleavage from the ion of $m/z$ 335. The abundant fragment ions at $m/z$ 147 and 73 are commonly seen in ditrimethylsilyl ethers. [33,34]. Tartaric acid standard TOFMS spectrum was obtained (Fig. 2(h)) and compared with that of U6 (Fig. 2(g)), showing a good agreement and similar retention time allowing the confirmation of U6 as tartaric acid. Proposed fragmentation pathways that are structurally informative for the butyl ester TMS-derivatives of tartaric acid are summarized in Scheme S4 (see Supporting Information). The concentration of tartaric 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 atmospheric particles with their sources and formation mechanisms. [24] However, oxaloacetic acid (3-oxodicarboxylic acid) has not been detected in atmospheric aerosols, possibly due to its rapid decarboxylation. In the present study, we successfully identified unknown compounds U3 and U9 as isomers of oxaloacetic acid in the remote marine aerosol samples. The EI mass spectra for the butyl ester TMS-derivatives of both unknown compounds U3 and U9 suggest that the MW of undervatized compounds is 132. The RT of U3 and U9 are 19.5 and 24.0 min, respectively. The derivative of U3 provides a fragment ion at $m/z$ 301 [M - CH$_3$]$^+$ with measured $m/z$ value of C$_{14}$H$_{23}$O$_5$Si at 301.14764 with an error of 2 ppm, whereas U9 provides a fragment ion at $m/z$ 273 [M-101]$^+$ with a measured $m/z$ value of C$_{13}$H$_{29}$O$_4$ at 273.20609 with an error of -2 ppm. This mass spectral information indicates that compounds U3 and U9 are isomers of oxaloacetic acid; U3 is the enolic form of oxaloacetic acid whereas U9 is the keto form of oxaloacetic acid (Figs. 5(h) and 5(i)).

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

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

The derivative of the keto form of oxaloacetic acid (U9) shows fragment ions at \(m/z\) 273, 245, 217, 189, 161, 105, 57, and 41(Fig. 4(c)). Structurally informative ions include \(m/z\) 273 [\(M-101\)]\(^{+}\). The peak at \(m/z\) 245 [\(M-73-56\)]\(^{+}\) could be explained by the loss of a butoxy radical (\(C_{4}H_{9}O\)) and butene (\(C_{4}H_{8}, 56 u\)) from the molecular ion by α-cleavage followed by a McLafferty rearrangement. The fragment ion of \(m/z\) 217 was derived by the loss of carbon monoxide from the ion of \(m/z\) 245. Proposed fragmentation pathways for the butyl esters derivative of unknown U5 (keto-oxaloacetic acid) are summarized in Scheme S6 (see Supporting Information). The proposed structure was subsequently confirmed by comparison of its gas chromatographic and mass spectral data (Fig. 4 (c)) with those of an authentic standard (Fig. 4(d)). This is the first report on the existence of two forms of oxaloacetic acid in marine aerosols. Oxaloacetic acid may be the intermediate in the oxidation of dicarboxylic acids such as succinic acid to result in
pyruvic acid, which is further oxidized to oxalic acid. The concentrations of the enol and keto form of oxaloacetic acid in all the samples ($n = 53$) ranged from 0.01 to 1.52 ng m$^{-3}$ and 0.01 to 0.51 ng m$^{-3}$, respectively.

**SUMMARY AND CONCLUSIONS**

Unknown intermediates of diacid formation have been successfully identified in remote marine aerosols as tartronic acid, malic acid, 3-hydroxyglutaric acid, 2-hydroxyglutaric acid, 2-hydroxyadipic acid, 3-hydroxyadipic acid, tartaric acid and the enol and keto form of oxaloacetic acid by detailed analysis of chromatographic and mass spectral data. All the identified compounds were further quantified, indicating that malic acid is the most abundant hydroxydiacid followed by tartronic acid in the remote marine atmosphere. This is the first report to identify and quantify homologous series of hydroxydiacids (hC$_3$di-hC$_6$di) including tartaric acid. We also demonstrate that oxaloacetic acid is present as two forms and is in part converted to pyruvic acid in remote marine aerosol samples. A further study is required to better understand the seasonal variations of hydroxydiacids in remote marine aerosols and their sources and transformation pathways.

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

(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
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.