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Determination of α- and β-Hydroxycarbonyls and Dicarbonyls in Snow and Rain Samples by GC/FID and GC/MS Employing Benzyl Hydroxyl Oxime Derivatization

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A flame ionization detector (FID) combined with capillary gas chromatography (GC/FID) and gas chromatography/ mass spectrometry (GC/MS) has been used to identify multifunctional carbonyls in wet precipitation samples. The carbonyl groups were first derivatized to *O*-benzylhydroxyloxime (BH oxime) by using *O*-benzylhydroxylamine. The BH oxime derivatives were then treated with *N,O*-bis(trimethylsilyl)acetamide for the hydroxyl group to derive their TMS ethers. The BH oxime/TMS derivatives were measured using GC/FID as well as GC/MS on positive EI and CI (isobutane was used as CI gas) modes. Three groups of carbonyl compounds (monoaldehydes, dicarbonyls, hydroxycarbonyls) were identified in the samples by using this method. We have identified, for the first time, a group of α- and β-hydroxycarbonyls, glycolaldehyde, hydroxyacetone, and 4-hydroxy-2-butanone, in wet precipitation samples. Concentrations of hydroxycarbonyls ranged from 0.9 to 53.8 μg/L in the precipitation samples. Their concentration level is similar to that of low molecular weight dicarboxylic acids, which have been reported as major water-soluble organic compounds in rain.

Rainwater and snow samples abundantly contain polar organic compounds which are largely scavenged from the atmosphere.[1−6] These organic compounds frequently have functional groups, such as carbonyl, hydroxyl, and carboxyl groups, that are thought to be derived by the atmospheric oxidation of organic compounds such as anthropogenic and/or biogenic hydrocarbons, fatty acids, and other organic compounds. Especially, the multifunctional (MF) carbonyls are potentially important intermediates in the chain reactions of organic compounds with atmospheric ozone, OH, and NO_x.[7−12] The carbonyls are also considered as potential precursors to produce ozone in the lower troposphere. Because the MF carbonyls are polar and frequently water soluble, they are thought to act as cloud condensation nuclei (CCN); hence, they may potentially act as a radiative force on the climate.[13−16] The carbonyls containing an OH group are the likely intermediates in the atmospheric oxidation reactions of biogenic hydrocarbons such as terpenoids and isoprene as well as anthropogenic hydrocarbons such as alkylbenzenes[17,18] in the atmosphere.

Gas chromatography/electron capture detector (GC/ECD) and GC/mass spectrometry (GC/MS) analysis of MF carbonyl compounds have been developed for biological blood and ozonized wastewater samples by using derivatization with *O*-(2,3,4,5,6-pentafluorobenzyl)hydroxylamine (PFBHA).[19,20] All of these methods for MF carbonyls were previously based on chemical derivatization with PFBHA[19−23] or 2,4-dinitrophenylhydrazine (DNPH)[24−28] for the carbonyl functional group. However, the

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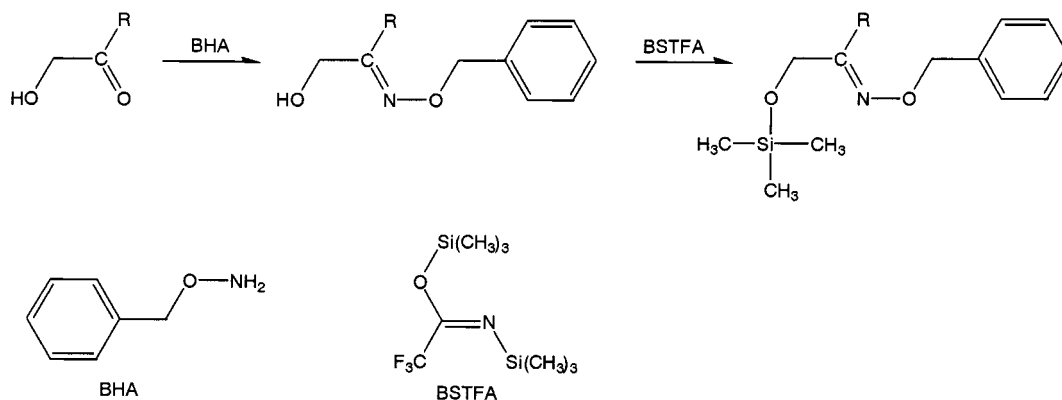(28) Grosjean, E.; Grosjean, D. *Int. J. Environ. Anal. Chem.* **1995**, *61,* 47−64.

Figure 1. Analytical procedure for two-step derivatization. The MF carbonyls in snow and rainwater samples are derivatized to BH oxime in the aqueous solution. If the oxime has an additional hydroxy group (OH) then it is further derivatized to TMS ether.

derivatives are not suitable for a flame ionization detector (FID), which is widely used as a GC detector, because they contain halogen atoms with very high electronegativity that extremely reduce the FID response. Therefore, a mass spectrometer or an electron capture detector has been used for the analysis of the carbonyl derivatives. However, ECD generally does not give a linearity with wide dynamic range.

In the course of methodology development for MF carbonyls in environmental samples, we discovered a group of α- and β-hydroxycarbonyls (C_2-C_6) in the rain and snow samples employing *O*-benzylhydroxyloxime (BH oxime) derivatization. Here, we report the newly modified method for the determination of hydroxyaldehydes, hydroxyketones, and dicarbonyls in wet precipitation samples using capillary GC/FID and GC/MS.

EXPERIMENTAL SECTION

Sample Collection. A rainwater sample was collected on August 6, 1999, on the roof of the Institute of Low Temperature Science (ILTS, suburban area) ~15 m above the ground level. A snow sample was collected on March 4, 1999, at an experimental farm of Hokkaido University (adjacent to ILTS). To avoid microbial degradation during sample storage, snow and rainwater were treated with $HgCl_2$ and stored in an 800-mL glass bottle at 4 °C prior to analysis.

Purification of *O*-Benzylhydroxylamine (BHA). *O*-Benzylhydroxylamine chloride) (BHA·HCl) was purchased from Wako Pure Chemical Industries (Osaka, Japan). Approximately 1 g of BHA·HCl was dissolved in pure water (~5 mL), to which 10 KOH pellets (flushed by methanol) were added to remove potential contaminations. Free benzyl hydroxylamine solution was extracted with methylene chloride. An 8 M HCl solution (preextracted with *n*-hexane) was added to the free amine solution, to precipitate BHA·HCl. The crystalline BHA·HCl was filtered on quartz fiber filter and dissolved in pure water. The BHA·HCl solution was used as a reagent for BH oxime derivatization.

Other Chemicals. BSTFA containing 1% trimethylchlorosilane (served as catalyst) was purchased from Supelco (Bellefonte, PA). Methanol, methylene chloride, *n*-hexane, HCl, and KOH (all were special grade) were purchased from Wako Pure Chemical Industries. Organic solvents were redistilled in the laboratory before using. The following authentic standards were also purchased: glyoxal 3-hydroxy-2-butanone (Tokyo Kasei, Tokyo, Japan), methylglyoxal (Sigma, St. Louis, MO), glycolaldehyde

(Aldrich, Milwaukee, WI), hydroxyacetone, 4-hydroxy-2-butanone, 4-chlorobutanal, and *n*-octanal (Wako).

Sample Treatment. Rainwater and snow samples (50−150 mL) were filtered on a quartz fiber filter and transferred to a round-bottom flask (200 mL) to which the purified BHA·HCl reagent (~0.5 mL) was added. The BH oxime derivatization at room temperature (25 °C) generally needed 5 h. To remove the excess BHA, 1 mL of 8 M HCl was added to the sample solution, and then, the BH oxime derivatives were extracted with ethyl acetate in a separatory funnel. The extracts were concentrated by rotary evaporator (30 °C, 90 rpm) and transferred to a 1.5-mL glass vial, where BH oxime derivatives were concentrated nearly to dryness using a nitrogen blowdown system. Finally, 50 μL of *N,O*-bis-(trimethylsilyl)acetamide (BSTFA) was added to the vial for the silylation of the OH group contained in the BH oxime derivatives. The BSTFA silylation needs 1 h at room temperarture. During these treatments, carbonyl group ($>C=O$) is derivatized to BH oxime whereas hydroxy (OH) and carboxyl (COOH) groups are derivatized to TMS ethers (see Figure 1). The silylated BH oxime derivatives were concentrated nearly to dryness under a nitrogen flow and dissolved in 100 μL of *n*-hexane. Two microliters of the *n*-hexane solution was injected into GC/FID and GC/MS.

GC/FID and GC/MS Analysis. A capillary GC (Carlo Erba GC6000) equipped with a cold on-column injector, fused-silica DB-5 capillary column (0.32 mm i.d. × 30 m length, 0.5 μm), and a FID detector (320 °C) was used for the analysis of MF carbonyl derivatives. The column oven temperature was programed from 70 (1 s) to 75 °C at 40 °C/mim, then to 120 °C at 10 °C/min, and to 320 °C at 5 °C/min (10 min.). Mass spectra of the derivatives were obtained with a capillary GC/MS (ThermoQuest Voyager and JEOL JMS-700). Ionization current, ionization energy, and ion source temperature for the CI+ mode (JMS-700) were 600 μA, 70 eV, and 200 °C, respectively. Those of the EI+ mode (Voyager and JMS-700) were 400 μA, 70 eV, and 180 °C, respectively.

RESULTS AND DISCUSSION

Examination of Reaction Time for BH Oxime Derivatization. The BH oxime derivatization time was examined based on the FID response of the MF carbonyl derivatives of authentic standards as a function of time. The results of most standards used show that the FID response reached a plateau within 30 min. However, methylglyoxal required 5 h, probably because it has steric hindrance in the carbonyl structure (efficiency of the
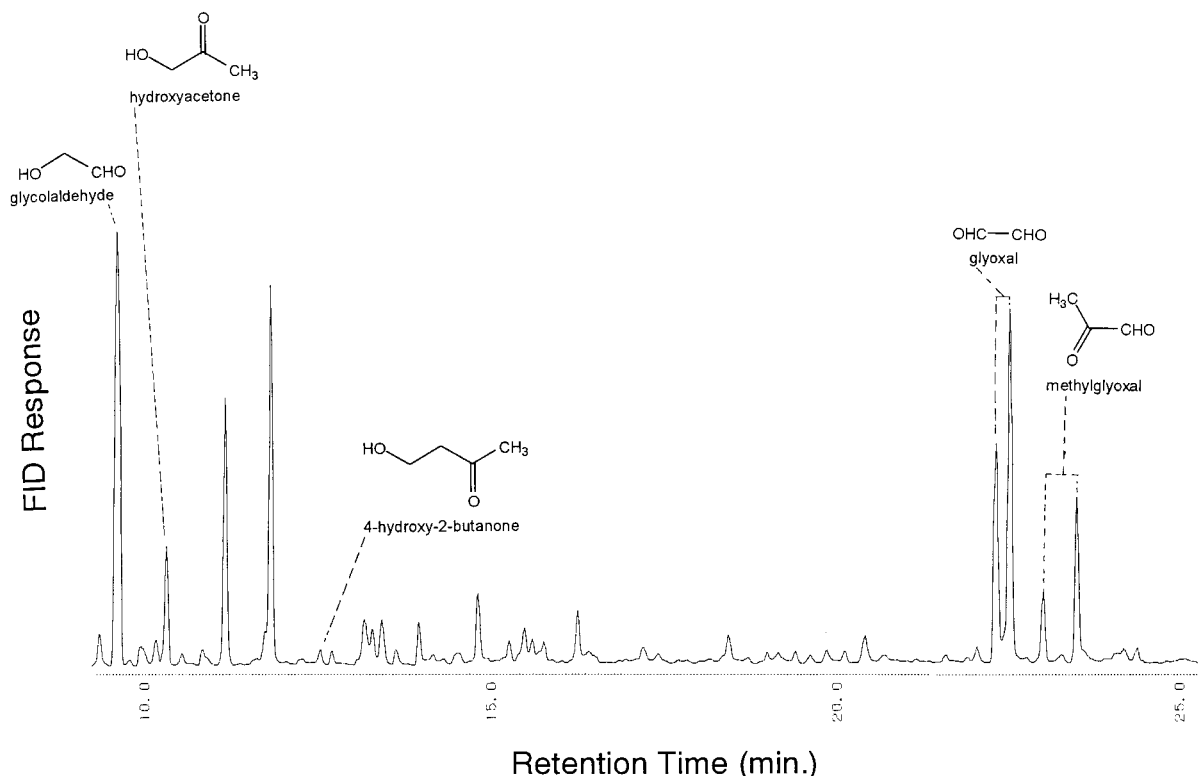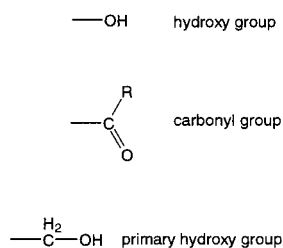
Figure 2. Capillary gas chromatogram of BH oxime/TMS ether derivatives from snow samples (collected on March 4, 1999). Some derivatives of MF carbonyls such as glyoxal showed double peaks because of the formation of geometrical isomers.
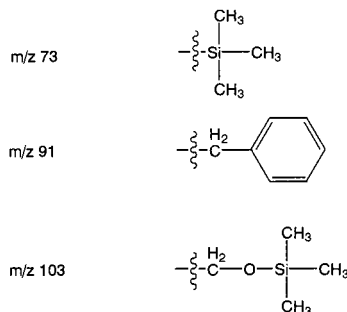

Figure 3. Characteristic mass fragment ions of from MF carbonyl BH oxime derivatives.

derivatization was ~70% when the derivatization time was 30 min). The FID response of the derivatives of the MF carbonyls did not change when derivatization time was longer than 5 h. Hence, derivatization time was determined to be 5 h.

Identification of MF Carbonyls. Figure 2 shows the gas chromatogram of MF carbonyls as BH oxime derivatives isolated from the wet precipitation sample. Glyoxal, methylglyoxal, and three hydroxycarbonyls (glycolaldehyde, hydroxyacetone, 4-hydroxy-2-butanone) were identified by comparing their retention times and mass spectra with those of authentic standards. Blank levels of each MF carbonyls in this analytical procedure were less than 1% of the actual samples.

Structural Elucidation of Methylglyoxal and C_3-C_6 Hydroxycarbonyls. EI+ mass spectra of the carbonyl BH oxime derivatives gave a characteristic fragment ion at m/z 91. The derivatives containing the O-TMS group should give a characteristic fragment ion at m/z 73. In fact, some GC peaks gave a mass fragment ion at m/z 103, which suggests the presence of primary

hydroxy group. On the basis of this mass spectral information, glycolaldehyde ($C_{2\alpha}$ hydroxycarbonyl) was postulated to exist in the atmospheric samples.

Isobutane CI+ mass spectra of the glycolaldehyde BH oxime/TMS ether derivative gave the $[M + H]^+$ ion at m/z 238 (see Figure 3). The comparison of the CI+ spectra and GC retention time with those of the actual sample proved that the presence of glycolaldehyde was identified in the wet precipitation samples. Similarly, CI+ mass spectra on the GC chromatogram (see Figure 2) gave characteristic $[M + H]^+$ ions at m/z 238, 252, 266, 280, and 294, suggesting the structures of C_2-C_6 hydroxycarbonyls. Using authentic standards, hydroxyacetone and 4-hydroxy-2-butanone were identified in the samples as well as methylglyoxal (see Figure 4 for the mass spectra). C_5 and C_6 hydroxycarbonyl derivatives provided $[M + H]^+$ ions at m/z 280 and 294. However, $[M + H]^+$ ions at m/z 308 (294 + 14) and others (308 + 14n, n = 1−5) were not detected in the mass spectra for the samples analyzed.
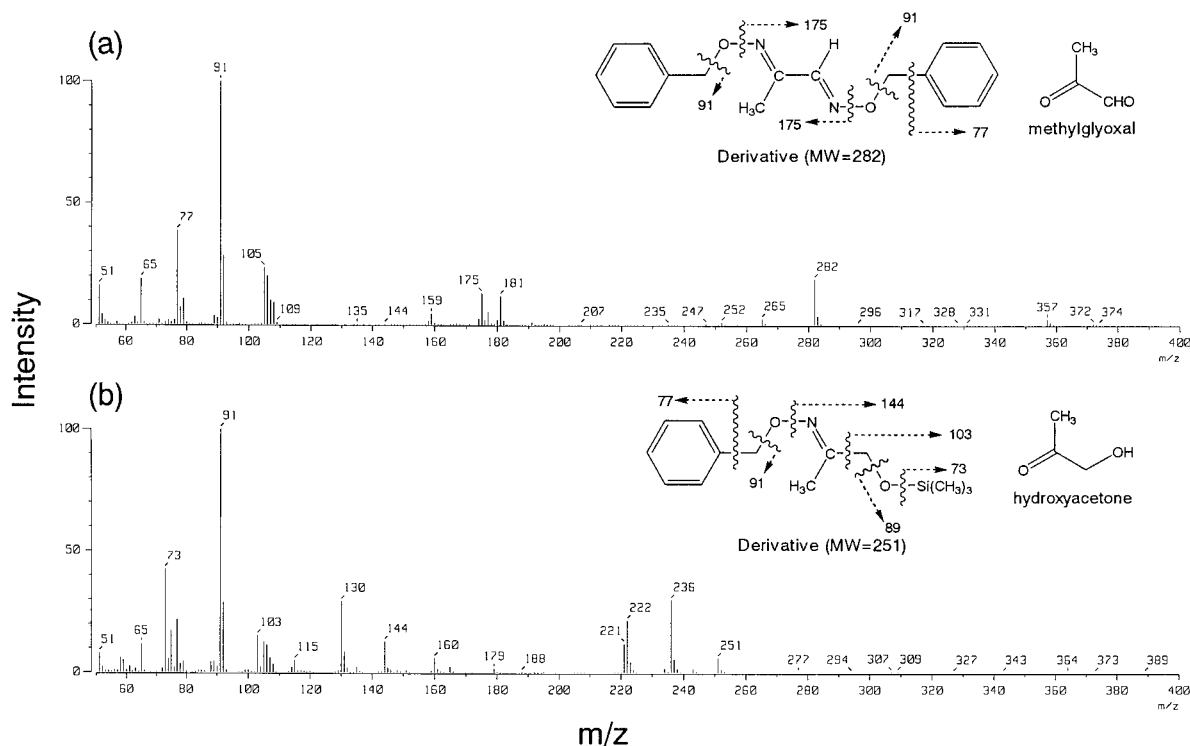
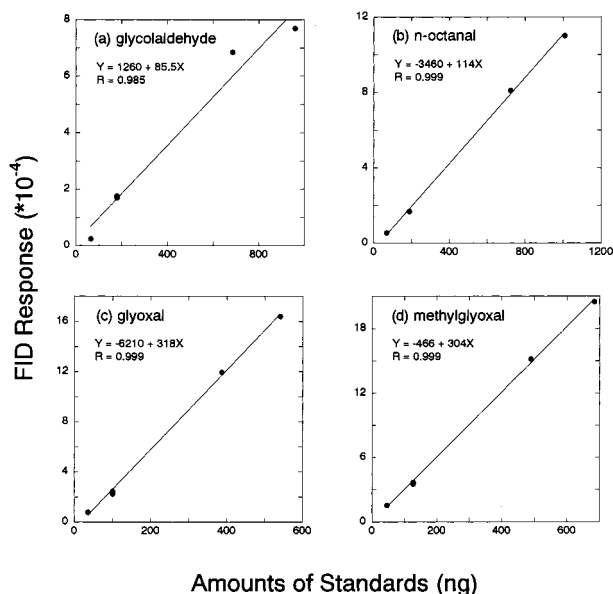**Figure 4.** EI+ mass spectra of the BH oxime/TMS ether derivatives of (a) methylglyoxal and (b) hydroxyacetone.

Figure 5. Calibration curves for MF carbonyls.

Figure 6. Results of spiked experiments. The authentic standards (a−d) were spiked to melted snow sample (collected on March 4, 1999).

Linearity of Derivatization of MF Carbonyls. We examined the linearity of the FID response of the derivatives to their amounts using four types of authentic carbonyl standards: glycolaldehyde, *n*-octanal, glyoxal, and methylglyoxal. Figure 5 shows the calibration curves for these carbonyl types. Each calibration curve shows a wide range of linearity and sensitivity that is considered to be enough for the quantification of these MF carbonyls in environmental samples such as rainwater and snow. The FID response for the glycolaldehyde derivative deviates a maximum 14% from the regression line obtained by a least-squares fit. The accuracy of hydroxycarbonyl seems to be less than those of other compound types (see Figure 5).
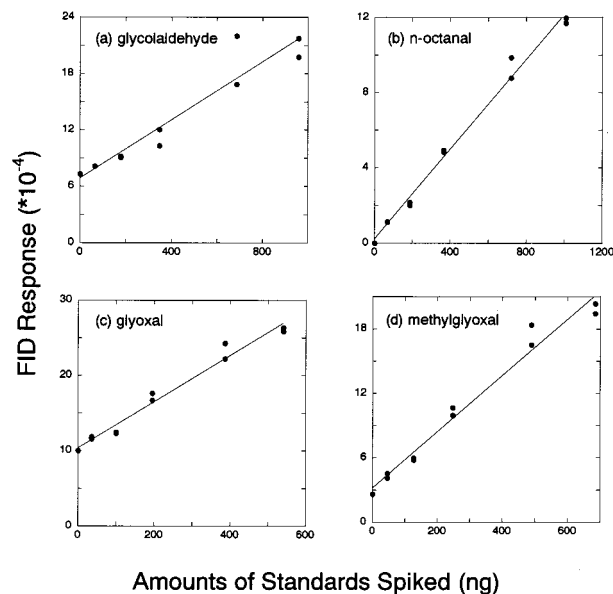
Spiked Experiment of Standards to the Environmental Samples. Figure 6 shows an FID response of the derivatives of MF carbonyls as a function of the amounts of authentic standards (glycolaldehyde, *n*-octanal, glyoxal, methylglyoxal) that were spiked to the melted snow samples (collected on March 4, 1999). This spiked experiment showed sufficient linearity between the FID response of derivatives and amounts of standard solution spiked, indicating that BH oxime derivatization was not influenced by other chemical species present in the environmental sample.

Determination of MF Carbonyls and Reproducibility. The concentrations of the MF carbonyls were calculated based on the

Table 1. Concentrations (μg/L) of MF Carbonyls in Snow and Rain Samples

	snow sample (Mar 4, '99)	rain sample (Aug 6, '99)	rain sample (Nov 1, '99)
Dicarbonyls			
glyoxal (C_2)	12.6	13.9	16.2
methylglyoxal (C_3)	17.1	3.7	6.8
Hydroxyaldehydes			
glycoaldehyde (C_2)	34.8	36.2	53.8
Ketoalcohols			
hydroxyacetone (C_3)	25.4	2.6	8.0
4-hydroxy-2-butanone (C_4)	1.4	1.7	0.9
Dicarboxylic Acids			
oxalic acid (C_2 diacid)	49.3	56.6	59.5
malonic acid (C_3 diacid)	7.7	21.7	11.3
succinic acid (C_4 diacid)	11.2	17.2	9.5

FID response and their calibration curves. The concentration of hydroxyacetone was calculated based on the calibration curve of glycolaldehyde because the structure of its BH oxime/TMS ether derivatives is similar to that of glycolaldehyde which has one CHO and one OH. The FID responses of the derivatives was corrected according to their carbon numbers. Duplicate analyses of the rainwater sample showed that standard deviation of the concentrations of MF carbonyls ranged from 1.0 to 14%. These results indicate a sufficient reproducibility of this method for the quantitative determination of MF carbonyls in the wet precipitation samples.

Distributions of MF Carbonyls in Rainwater and Snow Samples. Table 1 presents concentrations of MF carbonyls in snow and rainwater samples. Their concentration levels were found to be equivalent to those of low molecular weight dicarboxylic acids (LMW diacids; oxalic acid is the dominant species), which have been widely reported in wet precipitation samples.[2,4] LMW diacids are thought to be one of the end product groups in the oxidative reactions of various organic molecules in the atmosphere.[1-5] MF carbonyls identified in the wet precipitation samples are probably the intermediate products in the atmospheric chemical reactions, which may be further oxidized to LMW diacids in the atmosphere. Hence, the distributions of MF carbonyls in the meteoric waters may provide important information on the atmospheric chemical reactions,[23] which should be responsible for the formation of water-soluble organic carbon in the atmosphere.

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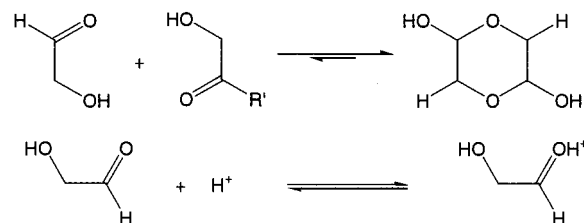
Figure 7. Equilibrium between glycolaldehyde and its cyclic hemiacetal and a protonation of glycolaldehyde in acidic solution.

Stability of α-Hydroxyaldehyde. Glycolaldehyde was found as a dominant MF carbonyl in the wet precipitaion samples. α-Hydroxyaldehyde is known to form a cyclic hemiacetal dimer in an aqueous solution (see Figure 7).[29] The equilibrium is known to largely lie toward hemiacetal. Further, cyclic hemiacetal is much more refractory to oxidation than the hydroxyaldehyde. Therefore, it is reasonable that glycolaldehyde is more abundant than other MF carbonyls. It is of interest to note that glycolaldehyde is further stabilized in the precipitation samples by protonation (see Figure 7).[30]

CONCLUSION

The GC/FID and GC/MS method employing BH oxime/TMS ether derivatization allowed us to determine five MF carbonyls including glycolaldehyde, hydroxyacetone, 4-hydroxy-2-butanone, glyoxal, and methylglyoxal in snow and rain samples. C_2−C_4 α- and β-hydroxycarbonyls (glycolaldehyde, hydroxyacetone, 4-hydroxy-2-butanone) were identified for the first time in the wet precipitation samples. This study demonstrates that MF carbonyls are abundant in the atmospheric wet samples. In addition, this method is also applicable for determination of MF carbonyls that can be extracted from other types of environmental samples (e.g., gas, particles) collected by using aerosol sampler, annular denuder, etc.