Solvents Inducing Oxidation of Hydroxylamines

Shizuyo HORIYAMA,*[,a] Kiyoko SUWA,[a] Masae YAMAKI,[a] Hiromi KATAOKA,[a] Toyoshi KATAGI,[a] Mitsuo TAKAYAMA,[b] and Takae TAKEUCHI[c]

[a] *Faculty of Pharmaceutical Sciences, Mukogawa Women's University; 11–68 Koshien Kyuban-cho, Nishinomiya 663–8179, Japan:* [b] *Graduate School of Sciences, Yokohama City University; 22–2 Seto, Kanazawa-ku, Yokohama 236–0027, Japan: and* [c] *Department of Chemistry, Faculty of Science, Nara Women's University; Kitauoyanishi-machi, Nara 630–8506, Japan.* Received February 18, 2002; accepted April 2, 2002

Hydroxylamines gradually undergo oxidation to their oximes on being dissolved in organic solvent (*e.g.* methanol). This phenomenon was followed by ^{1}H-NMR and liquid chromatography-mass spectrometry (LC-MS). The oxidation rate was estimated from the peak area observed on the mass chromatogram at the protonated molecule or fragment ion on LC-atmospheric pressure chemical ionization (APCI)-MS. The results showed that the oxidation rate of hydroxylamines depended on the solvent type.

Key words hydroxylamine; oxidation; LC-MS; NMR

Oxime derivatives are well known as having antiallergic, anti-inflammatory, antipyretic, fungicidal and parasiticidal activities.[1,2] Recently, Kramer *et al.* reported that some oximes and hydroxylamines, which were prepared as a wide range of di-*tert*-butylphenol containing compounds, were inhibitors of cyclooxygenase (COX) and 5-lipoxygenase (5-LO).[3] We also have synthesized *O*-acyl oximes, free oximes and hydroxylamines for the purpose of developing a series of nonsteroidal anti-inflammatory drugs having dual inhibitors of COX[4] and 5-LO, and reported their anti-inflammatory activity. We then have found that some *O*-acyl oximes, depending on the measuring conditions of their mass spectra, showed thermal degradation occurring at characteristic sites[5] and reductive degradation by a matrix containing a thiol group.[6] In the present work,we examined the properties of hydroxylamines and found that they were unstable in some organic solvents.

Hydroxylamines have amphetamine-like activity, inhibit monoamine oxidase and other activities (*e.g.* spontaneous motor activity and blood pressure effects),[7] and also are important metabolic products of *N*-oxidation of aliphatic primary and secondary amines, for example, amphetamine and phentamine. In the 1970s, several studies were devoted to examining the instability of hydroxylamines. Beckett *et al.*[8,9] reported that *N*-hydroxyamphetamine (2-hydroxylamino-1-phenylpropane) was readily oxidized to the corresponding oxime in nonacidic buffered or non-buffered aqueous solutions by aerial oxygen, with the oxidation being dependent on pH and time as well as being enhanced by the presence of trace heavy metal ions. They suggested that extraction with organic solvents such as ether, benzene or chloroform is effective for protecting hydoxylamines from the aqueous environment. Lindeke and Anderson.[10] reported that the oxidation takes place in aerated toluene, in which, *N*-hydroxyamphetamine gave only the nitrosodimer.

In this paper, we report that some hydroxylamines were gradually oxidized to the corresponding oximes in some organic solvents (*e.g.* methanol) with no aeration. The change occurring when they were left standing was followed by measuring ^{1}H-NMR and liquid chromatography-mass spectrometry (LC-MS) spectra of the mixture of hydroxylamine and the product. The oxidized products were identified by comparing their ^{1}H-NMR and LC-MS spectra with those of authentic samples, 4′-piperidinoacetophenone oxime (**2**), 4′-morpholinoacetophenone oxime (**4**) and 4′-methoxybenzyl methyl ketone oxime (**6**).

Results and Discussion

All compounds **1**—**6** (Fig. 1) involved in this study were prepared from the corresponding methylketones in the usual manner,[11,12] and carefully purified by crystallization. Their purities were checked by ^{1}H-NMR spectrum and LC-MS chromatogram. Table 1 shows the ^{1}H-NMR data of compound **1**—**6** as the standard. Table 2 shows the retention times of each compound on the liquid chromatogram, and their protonated molecule and characteristic fragment ions in the mass spectra of each peak on LC-atmospheric pressure chemical ionization (APCI)-MS spectra.

Hydroxylamines **1**, **3** and **5** were dissolved in methanol and chloroform for LC-MS and in methanol-d_4 and chloroform-*d* for NMR, which were chosen as representative of protic and aprotic solvents, respectively. The change on standing was followed by measuring ^{1}H-NMR and LC-MS spectra. After 24 h, new signals appeared in the ^{1}H-NMR spectra of each methanolic solution, and their intensity gradually increased with the elapse of time. In the case of **1**, new signals appeared at δ 2.18 (s), 3.20 (m) and 7.50 (d), corresponding to 2-CH_3, 2″,6″-CH_2 and aromatic protons (2′,6′-H) of oxime **2**, as shown in Fig. 2 (Asterisks indicate new signals). In the case of **3**, signals appeared at δ 2.19 (s), at δ 3.17 (m) and 7.54 (d), matching with those for 2-CH_3, 2″,6″-CH_2 and aromatic protons (2′,6′-H) of oxime **4**. In the case of **5**, the signals agreed with 3-CH_3 and 1-CH_2 of oxime **6**

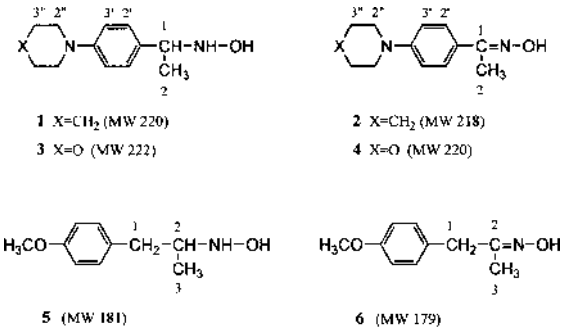

Fig. 1. Structures of Compounds Examined in This Study

* To whom correspondence should be addressed. e-mail: horiyama@mwu.mukogawa-u.ac.jp

Table 1. ^{1}H-NMR Spectral Data of Hydroxylamines **1**, **3** and **5**, and Oximes **2**, **4** and **6**

H	**1**	**2**	**3**	**4**	H	**5**	**6**
1	3.96 q (6.41)	—	3.97 q (6.59)	—	1	2.45 dd (13.55, 7.69)	3.39 s
2	1.33 d (6.41)	2.18 s	1.33 d (6.59)	2.19 s		2.85 dd (13.55, 5.86)	
2′,6′	7.23 d (8.55)	7.50 d (8.98)	7.24 d (8.42)	7.54 d (8.98)	2	3.07 m	—
3′,5′	6.94 d (8.55)	6.93 d (8.98)	6.93 d (8.79)	6.94 d (8.98)	3	1.00 d (6.22)	1.72 s
2″,6″	3.10 m	3.20 m	3.11 m	3.17 m	2′,6′	7.11 d (8.42)	7.12 d (8.55)
4″	1.58 m	1.61 m	—	—	3′,5′	6.84 d (8.79)	6.85 d (8.55)
3″,5″	1.71 m	1.70 m	3.82 m	3.82 m	OCH_3	3.76 s	3.77 s
OH	N.D.	N.D.	N.D.	N.D.	OH	N.D.	N.D.
NH	N.D.	—	N.D.	—	NH	N.D.	—

* Coupling constants (J in Hz) are given in parentheses. N.D.; not detected.

Table 2. Retention Time (t_R), $[M+H]^+$ and Fragment Ion of Hydroxylamines **1**, **3** and **5**, Oximes **2**, **4** and **6** on LC-MS

	t_R (min)	$[M+H]^+$	$[M-17]^+$	$[M-15]^+$	$[M-33]^+$
1	5.4	221 (38)	203 (100)	—	188 (88)
2	6.3	219 (100)	—	203 (11)	—
3	4.2	223 (4)	205 (100)	—	190 (92)
4	4.8	221 (100)	—	205 (26)	—
5	5.9	182 (100)	164 (11)	—	149 (9)
6	5.5	180 (100)	—	164 (4)	—

∗ % Intensity is given in parentheses.

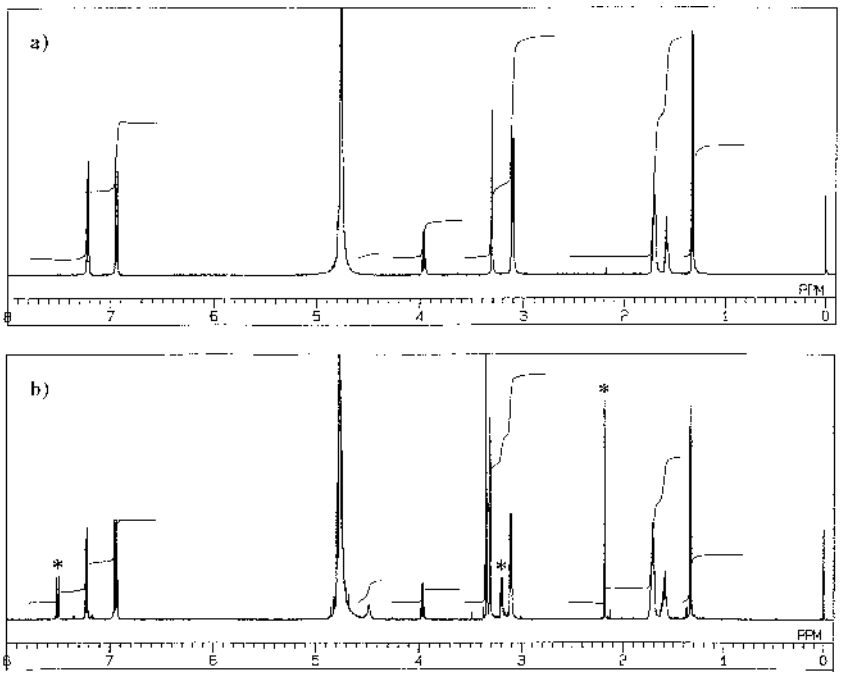

Fig. 2. ^{1}H-NMR Spectra of **1** at (a) Soon After and (b) After 24 h Dissolution in Methanol-d_4

being observed at δ 1.72 (s), 3.38 (s). Also, slightly smaller signals appeared at δ 1.71 (s, 3-CH_3) and 3.65 (s, 1-CH_2), suggesting the presence of a *syn*-isomer.[13] In the ^{1}H-NMR spectra of each chloroform-*d* solution, no changes were observed.

LC-MS spectra of each methanolic solution showed a new peak after 24 h, with the peak area increaseing with time. The retention time of the new peak was 6.3 min for **1**, 4.8 for **3** and 5.5 min for **5**, which agreed with the retention time of the corresponding oximes, respectively. In spectra of **5**, a small peak appeared at 5.3 min, which seemed to be dependent on the presence of *syn*-isomer from $[M+H]^+$ *m*/*z* 180 in its mass spectrum, agreeing with the result observed for the ^{1}H-NMR spectrum. These findings, confirmed that hydroxylamines **1**, **3** and **5** gradually changed to oxime **2**, **4** and **6** only on being dissolved in methanol; no change was seen in chloroform solution.

Next, we tried to calculate the oxidation rates from mass chromatograms, using the peak area of $[M+H]^+$ *m*/*z* 221 for **1**, while fragment ion *m*/*z* 190 was used for **3** and *m*/*z* 149 for **5** because the retention time of each oxidizing compound was very close to the original peak. The peak area of

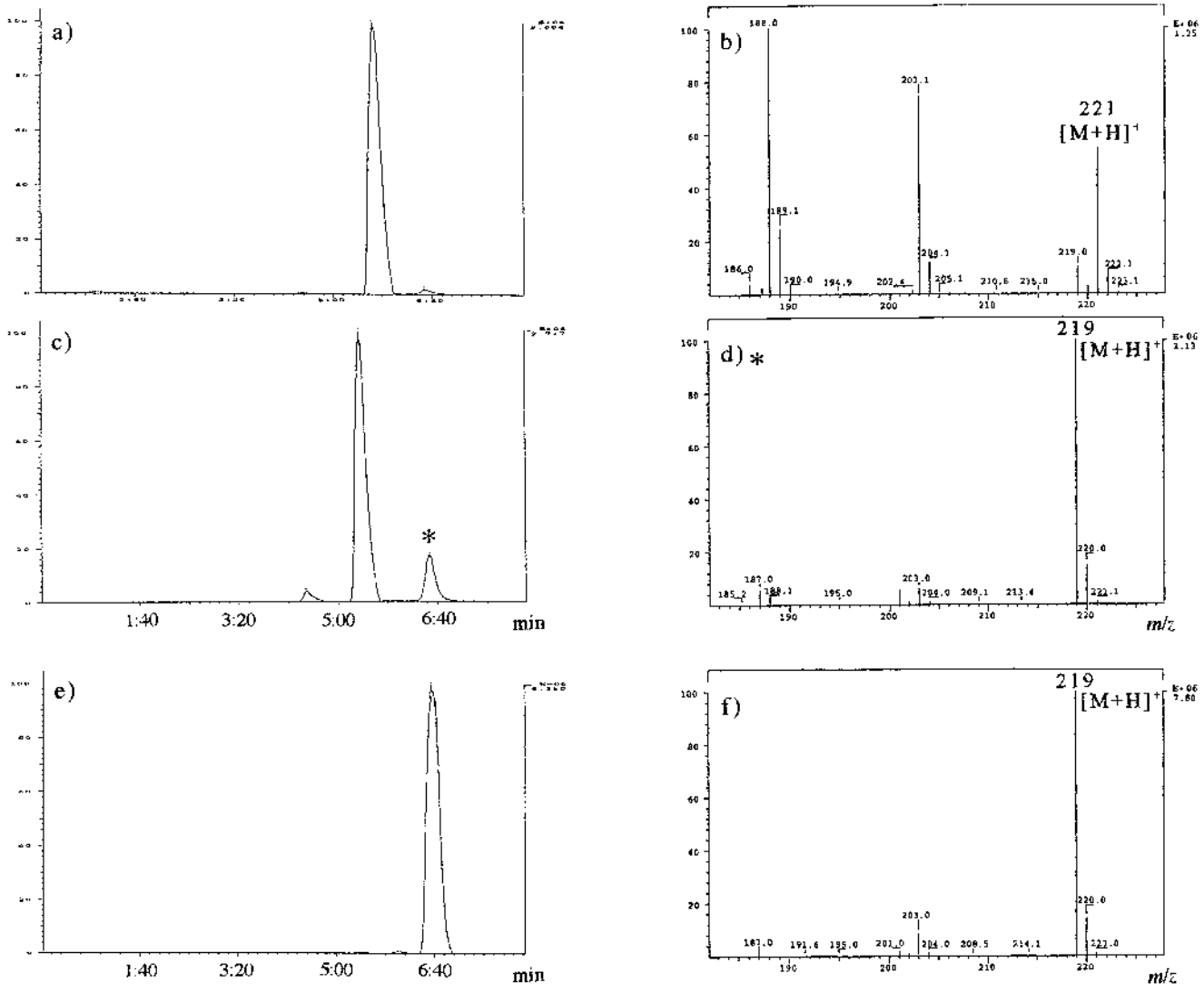

Fig. 3. LC-APCI-MS Chromatograms (a, c, e) and Spectra (b, d, f) of **1** and **2** in Methanol

(a), (b): Immediately after dissolution of **1**. (c), (d): 24 h after dissolution of **1**. (e), (f): **2**.

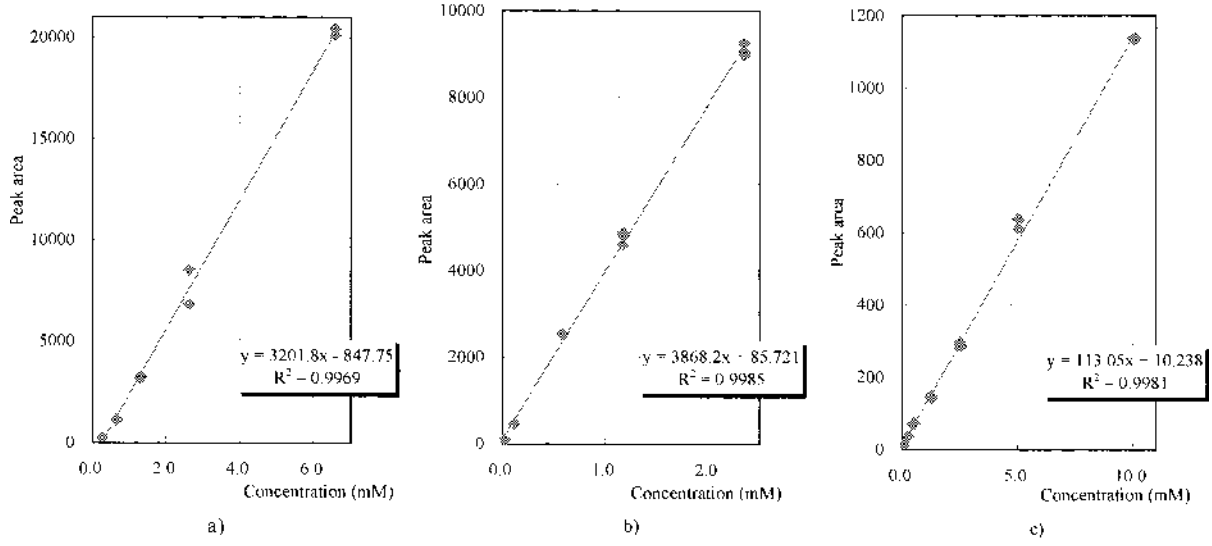

Fig. 4. Calibration Curve of (a) **1** (*m*/*z* 221), (b) **3** (*m*/*z* 190) and (c) **5** (*m*/*z* 149)

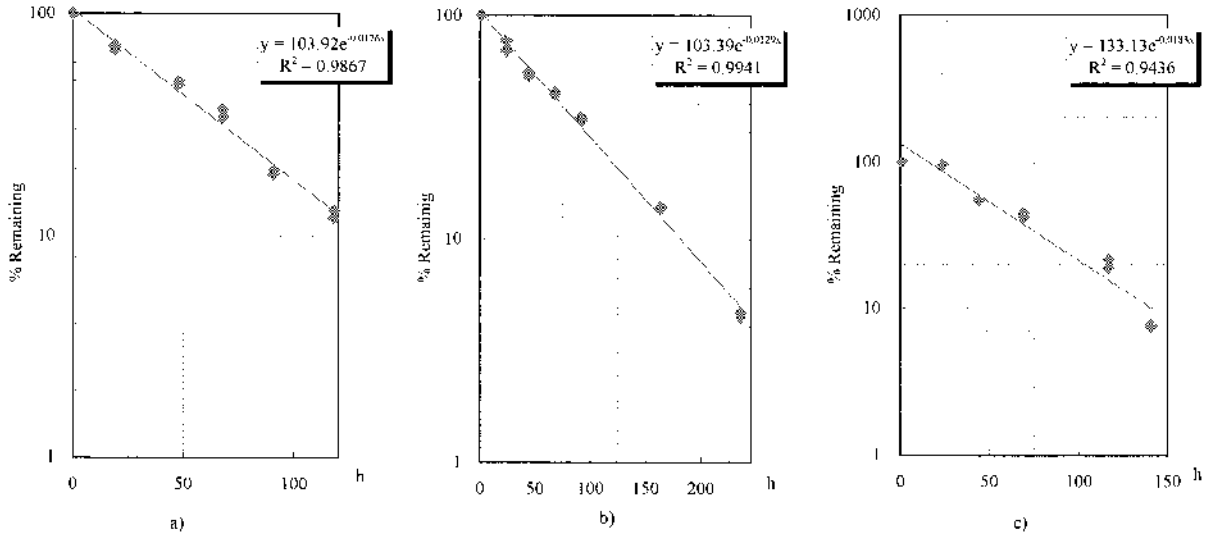

Fig. 5. First-order Plots for the Oxidation of (a) **1**, (b) **3** and (c) **5** in Methanol

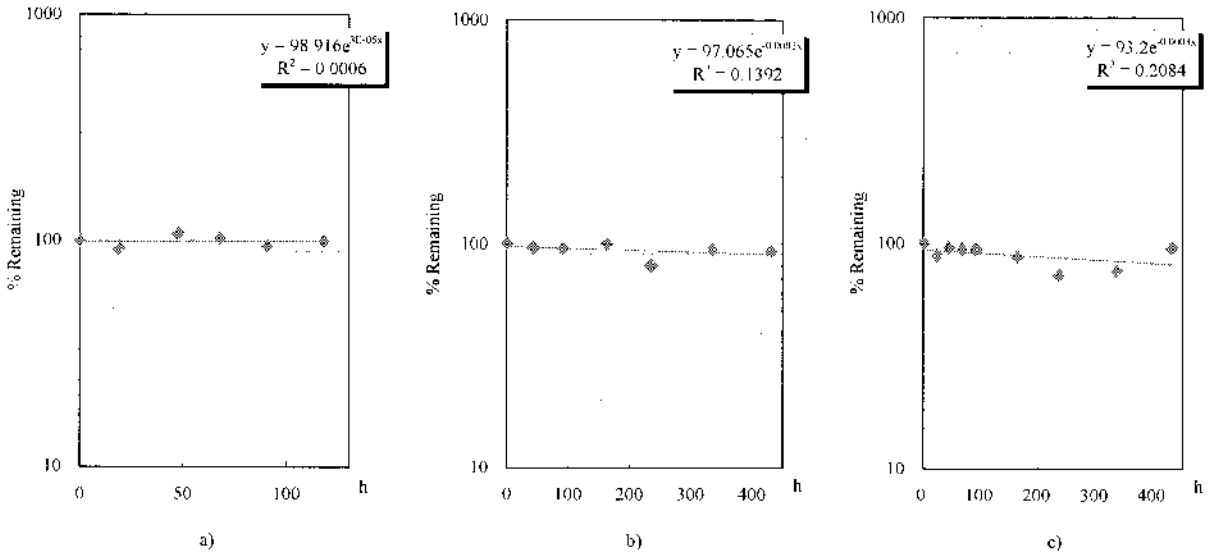

Fig. 6. First-order Plots for the Oxidation of (a) **1**, (b) **3** and (c) **5** in Chloroform

$[M+H]^+$ may be affected by the isotope peak of $[M+H]^+$ from oxidizing compounds. The calibration graphs (Fig. 4) for **1**, **3** and **5** were generated from the peak areas at *m*/*z* 221, *m*/*z* 190 and *m*/*z* 149 of increasing amounts of standard sample of **1**, **3** and **5** and the external standard was 2.5 mM methanol solution of **2**. A linear calibration curve was constructed using the least-squares method of quantities *versus* peak area. The linearity was good in the range of 0.26 to 6.59 mM (r^2=0.9969) for **1**, of 0.02 to 2.35 mM (r^2=0.9985) for **3** and 0.13 to 10.07 mM (r^2=0.9981) for **5**. Using the remaining percentages of these peak areas, we determined the pseudo first-order rate constants ($-k$) for the oxidation of hydroxylamines. Figures 5 and 6 show pseudo first-order plots for the oxidation of **1**, **3** and **5** in methanol and chloroform, with the percentage of $\log(A_t/A_0)$ being plotted against time (h). *k*-Values (−h) of **1**, **3** and **5** in methanol were 0.018, 0.013 and 0.018 and in chloroform were 3×10^{-5}, 2×10^{-4} and 3×10^{-4}, respectively. These results indicated that the oxidation phenomenon of hydroxylamines depended on the solvent.

We also examined the stability of hydroxylamine **1** in various solvents, ethanol, pyridine, methylamine, benzene and toluene, and calculated their *k*-values, which were 0.009, 0.017, 0.401, 0.002 and 0.003, respectively. These results showed that the oxidation proceeded very rapidly in methylamine, slowly in the ethanol and pyridine, and almost not at all in benzene and toluene. It seems that the oxidation proceeded in protic solvents more than in aprotic solvents, but the data did not indicate a dependence on the basicity (pK_a)[14] of the solvents, which was 15.5 for methanol, 16.0 for ethanol, 5.1 for pyridine and 10.6 for methylamine.

Beckett and Lindeke *et al.* reported that aerial oxygen, heavy metal and pH cause the oxidation of hydroxylamine, but in our case, the oxidation occurred only on dissolution in solvent. We confirmed the oxidation phenomena of hydroxylamines **1**, **3** and **5** to oxime **2**, **4** and **6** in methanol solution by using ^{1}H-NMR and LC-APCI-MS techniques. Further study is needed to clarify the oxidation mechanism.

Experimental

General All melting points were measured with a Yanaco MP-S3 apparatus and are uncorrected. Nuclear magnetic resonance spectra were obtained at 500 MHz by means of a JEOL GSX-500 instrument (JEOL, Tokyo, Japan). Solutions were prepared in chloroform-*d* or methanol-d_4 and tetramethylsilane (TMS) serves as the internal reference. Mass spectrometric experiments were performed using a TSQ-7000 triple stage quadruple mass spectrometer (Thermo Quest, San Jose, CA, U.S.A.) operating with APCI, connected with an HP series 1050 HPCL (Hewlett-Packard, Palo Alto, CA, U.S.A.). Chromatography was conducted at ambient temperature on an Excelpack SIL-C18/5 reverse-phase C18 column (5 μm, 5×150 mm) (Yokogawa, Tokyo, Japan) and Capcell Pack C_{18} reverse-phase C18 column (5 μm, 4.6×150 mm) (Shiseido, Tokyo, Japan) operated at flow-rate of 0.6 ml/min with eluent CH_3OH–H_2O (80 : 20) for **1** and CH_3OH–H_2O (70 : 30) for **3** and **5**. The mass spectrometer was operated in the positive ion mode with the vaporizer at 500 °C and the capillary at 175 °C. Nitrogen was used as the sheath gas (70 psi) to assist neblization. The electron multiplier was set at 1000 V.

Calibration **1**, **3** and **5** were dissolved in chloroform to 10 mM (stock solution) and further calibration levels were prepared by diluting the stock solution.

Sample Preparation for LC-MS **1**, **3** and **5** dissolved in He-degassed methanol, chloroform, ethanol, pyridine, methylamine, benzene and toluene to 1 mM, and 3 μl was injected at set intervals of about 24 h or more.

Synthesis Hydroxylamines **1**,[6] **3** and **5** were prepared from **2**, **4** and **6** by modification of Borch's[11] method using $NaBH_3CN$. Oximes **2**, **4** and **6** were prepared from the corresponding methylketone with hydroxylamine in the usual way.[12]

1-Hydroxylamino-1-(4′-piperidinophenyl) Ethane (**1**)[6]: ^{1}H-NMR (methanol-d_4): Table 1.

1-Hydroxylamino-1-(4′-morpholinophenyl) Ethane (**2**): mp 111.5—113 °C (from ethyl acetate). *Anal.* Calcd for $C_{12}H_{18}N_2O_2$: C, 64.84; H, 8.16; N, 12.60. Found: C, 64.69; H, 8.41; N, 12.59. APCI-MS: *m/z* 223 $[M+H]^+$. ^{1}H-NMR (methanol-d_4): Table 1.

2-Hydroxylamino-1-(4′-mothoxyphenyl) Propane (**3**): mp 65—67 °C (from petroleum ether). *Anal.* Calcd for $C_{10}H_{15}NO_2$: C, 66.27; H, 8.34; N, 7.73. Found: C, 66.18; H, 8.63; N, 7.77. APCI-MS: *m/z* 182 $[M+H]^+$. ^{1}H-NMR (methanol-d_4): Table 1.

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