Degradation pathways of 4-methylmethcathinone in alkaline solution and stability of methcathinone analogs in various pH solutions

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1. Introduction

Methcathinone (MC, Fig. 1) is a psychoactive stimulant with a structure similar to methamphetamine. It was used for a short time in the treatment of depression in the former Soviet Union in the late 1930s and 1940s [1]. Starting in the 1970s and 1980s, the abuse of MC has been reported in Russia, the United States, and the Republic of South Africa [1,2]. In Japan, MC was classified as a narcotic by the Narcotic and Psychotropic Control Law in 1995.

MC has been reported to be unstable with respect to heating [2] and in neutral-to-basic aqueous solutions [3]. Noggle et al. reported that MC was thermally oxidized during gas chromatographic analysis, and proposed the oxidized product was a 2,3-enamine [2]. Togawa et al. reported that MC and cathinone, an N-demethylated analog of MC, were unstable in neutral-to-basic (pH 7–9) solutions but stable in acidic (pH 5) solutions [3]. The degradation products in the neutral-to-basic solution were not reported.

During the last several years, MC analogs such as 4-methylmethcathinone (4-MMC), 4-fluoromethcathinone (4-FMC), 3-fluoromethcathinone (3-FMC), 2-fluoromethcathinone (2-FMC), 4-methoxymethcathinone (4-MeOMC), N-ethylcathinone (EtCAT), and N,N-dimethylcathinone (DMC) have been abused as a "legal high" all over the world (Fig. 1) [4–6]. Except for EtCAT and DMC, these compounds all have substituents on the benzene ring.

Because urinary metabolites of most MC analogs are undefined (reported only as 4-MMC [7]), their unchanged forms are usually targeted during the toxicological analysis. Liquid–liquid extraction of basified samples is the standard method to determine the unchanged form of these drugs in biological samples. However, as MC itself is unstable, it is suspected that its analogs are also unstable in alkaline solution.

MC analogs have the same partial structure as a so-called Amadori product (an α-aminocarboxyl compound), an intermediate in the Maillard reaction. The presence of oxygen or reactive oxygen species is important in this reaction [8,9]. Considering the similarity of the chemical structures, oxidants may also be involved in the decomposition of MC analogs.

Herein, we report: (i) the identification of the degradation products of 4-MMC, a typical MC analog, in alkaline solution and its
degradation pathways, (ii) effects of antioxidants on the stability of 4-MMC in alkaline solution, and (iii) the stability of seven MC analogs (4-MMC, 4-FMC, 3-FMC, 2-FMC, 4-MeOMC, EtCAT, and DMC) in various pH solutions.

2. Materials and methods

2.1. Chemicals

Seven MC analogs (4-MMC, 4-FMC, 3-FMC, 2-FMC, 4-MeOMC, EtCAT, and DMC) were previously synthesized as the HCl salt in our laboratory by the methods of Archer [10] with slight modifications. 1-(4-Methylphenyl)-1,2-propanedione (MPPD), N,4-dimethylbenzamide (DMBA), and N-acetyl-4-MMC (N-Ac-4-MMC) (Fig. 2) were synthesized in our laboratory. These synthetic methods are described in detail below. 4-Methylbenzoic acid (MBA, Fig. 2) was obtained from Tokyo Chemical Industry (Tokyo, Japan). All other reagents used were of analytical grade.

Buffer solutions used in this study were 0.01 mol/l ammonium acetate buffer (pH 4.0, pH was adjusted with acetic acid), 0.1 mol/l sodium phosphate buffer (pH 7.0), 0.1 mol/l sodium carbonate buffer (pH 10.0), and 0.1 mol/l sodium monohydrogen phosphate – 0.1 mol/l NaOH buffer (pH 12.0).

2.2. Synthetic procedures for the 4-MMC degradates

1H and 13C nuclear magnetic resonance (NMR) and gas chromatography–mass spectrometry (GC–MS) were used to characterize the products. NMR spectra were measured on a JEOI JNM-EC600 spectrometer (Akishima, Japan). Tetramethylsilane was used as an internal standard. GC–MS analysis was performed in electron ionization (EI) mode under the described conditions.

2.2.1. MPPD

MPPD was synthesized by the method described by Riley et al. [11]. Selenium dioxide (890 mg, 8 mmol) was added to 4'-methylpropiophenone (1 g, 6.7 mmol) in 10 ml of 95% ethanol, and allowed to sit at room temperature for 4 days. Purification was performed first by silica gel column chromatography using hexane–ethyl acetate (6:1) as an eluent, then by forming the bisulfite adduct and decomposing it in NaOH solution [12], and finally by the aforementioned column chromatography. After purification, 2 mg of a yellow oily product was obtained. 1H NMR (CDCl3, 600 MHz): δ 7.91 (2H, d, J = 8.1, 1.8 Hz, Ar), 7.30 (2H, d, J = 8.1 Hz, Ar), 2.52 (3H, s, Ar–CH3), 2.44 (3H, s, COCH3). 13C NMR (CDCl3, 150 MHz): δ 200.9 (Ar–COCH3), 151.2 (Ar–COCOCH3), 145.9 (Ar), 132.5 (Ar), 130.4 (Ar), 129.6 (Ar), 26.5 (Ar–CH3), 21.9 (COCH3). EI-MS m/z: 162 ([M]+, 2), 119 (100), 91 (72), 65 (23), 43 (10).

2.2.2. DMBA

Thionyl chloride (3 g, 25.2 mmol) was added to MBA (2 g, 14.7 mmol) in dimethylformamide (4 g, 28 mmol) and heated at 80 °C for 1.5 h. The excess thionyl chloride was evaporated to give crude 4-methylbenzoyl chloride, and this intermediate was dissolved in aceton. A solution of 40% methanol in water (4 ml, 10 mmol/ml, 40 mmol) was slowly added to the acetone solution at 0 °C. After several minutes, the solution was evaporated and the residue dissolved in ethyl acetate. The ethyl acetate solution was washed with 1 mol/l NaOH solution, and evaporated to yield a white solid. The crude solid was recrystallized from chloroform/hexane to give DMBA (196 mg) as a white solid. 1H NMR (CD3OD, 600 MHz): δ 7.69 (2H, d, J = 8.2, 1.9 Hz, Ar), 7.26 (2H, d, J = 8.2 Hz, Ar), 2.90 (3H, s, NH–CH3), 2.38 (3H, s, Ar–CH3). 13C NMR (CD3OD, 150 MHz): δ 169.4 (Ar–CONHCH3), 141.9 (Ar), 131.4 (Ar), 128.8 (Ar), 126.5 (Ar), 125.5 (Ar–COCH3), 20.0 (Ar–CH3). EI-MS m/z: 149 ([M]+, 33), 119 (100), 91 (59), 148 (31), 63 (25), 120 (10).

2.2.3. N-Ac-4-MMC

Acetic anhydride (80 µl) and pyridine (20 µl) were added to 20 µg of 4-MMC HCl. The mixture was heated at 45 °C for 30 min. Excess reagents were evaporated under a nitrogen stream. EI-MS m/z: 219 ([M]+, 2), 58 (100), 100 (50), 43 (12), 91 (10).

2.3. GC–MS analysis

The degradation products of 4-MMC were identified by GC–MS. GC–MS analysis was performed on a GCMS-QP5050A (Shimadzu, Kyoto, Japan). The temperature of the injector and the interface was 250 °C. Helium was used as the carrier gas in constant pressure mode (initial carrier gas flow: 1.0 ml/min). Chromatographic
separation was performed on a J&W DB-5ms capillary column (30 m length, 0.25 mm i.d., and 0.25 μm film thickness), and the oven temperature was held at 80 °C for 1 min, then raised to 320 °C at 15 °C/min. The mass spectrometer was operated under the El mode at an ionization energy of 70 eV. The analysis was performed in scan mode (mass range: m/z 40–500).

2.4. HPLC analysis

HPLC with UV detection was used to determine the percent remaining of the initial concentration. The HPLC system consisted of a Waters Alliance 2690 series liquid chromatograph (Milford, MA, USA) equipped with a Waters 996 diode array detector. The chromatographic separation was performed on a Mightysil RP-18 column (150 mm × 2.0 mm, 3 μm, Kanto Chemical, Tokyo, Japan) maintained at 40 °C. The mobile phase was a gradient of a mixture of 10 mM ammonium acetate (pH 4.0) and acetonitrile with a constant flow rate of 0.2 ml/min. The acetonitrile percentage was as follows: 0–15 min, linear increase from 10% to 90%; 15–15.1 min, from 90% to 10%; 15.1–28 min, 10% (re-equilibration step). The detection wavelength was set at 280 nm for 4-MeOMC and at 250 nm for all other analytes.

2.5. Identification of the 4-MMC degradates in alkaline solution and the involved degradation pathways

The degradation products of 4-MMC in alkaline solution were identified by GC-MS. After storing 4-MMC HCl (10 mg/10 ml) in the pH 12 buffer solution at 22 °C, two 1 ml portions of the solution were taken at each sampling time (0, 6, 24, and 48 h). One portion was directly extracted with 0.5 ml of ethyl acetate (sample a). To confirm acidic degradation products, the other portion was extracted in the same manner after adjusting the solution pH to 1 by adding 2 drops of concentrated HCl (sample b). One microliter of each extract was injected into the GC-MS system in split mode (split ratio, 1:10).

To ascertain the degradation pathway, three supplementary experiments were performed. Supplementary study 1 was confirmation of production of MBA from MPPD. After storing MPPD (0.2 mg/ml) in the pH 12 buffer solution at 22 °C for 24 h, the sample b was prepared and analyzed by the above-mentioned procedure. Supplementary study 2 was confirmation of the production of DMBA from a mixture of MBA and methylamine. After storing a mixture of MBA and methylamine HCl (0.5 mg/ml each) in the pH 12 buffer solution at 22 °C for 24 h, sample b was prepared and analyzed by the above-mentioned procedure. The supplementary study 3 involved the comparison of the production amount of N-Ac-4-MMC from 4-MMC with and without acetic acid. After storing 4-MMC HCl (0.5 mg/ml) with and without acetic acid (0.5 mg/ml) in the pH 12 buffer solution at 22 °C for 24 h, sample b was prepared and analyzed by the above-mentioned procedure.

2.6. Effects of antioxidants on the stability of 4-MMC in alkaline solution

Two antioxidants, L-ascorbic acid and sodium sulfite, were used to estimate the involvement of any oxidants, such as dissolved oxygen or reactive oxygen species.

Fig. 3. GC–MS total ion current chromatograms obtained at stated times from ethyl acetate extracts of 4-MMC in the pH 12 buffer. The storage times were 0, 6, 24, and 48 h. Extraction was performed from alkaline (sample a) and acidic (sample b) solutions. The peaks in the chromatograms are as follows: A, MBA; B, MPPD; C, 4-MMC; D, DMBA; E, N-Ac-4-MMC. In addition, unidentified peak 1 was observed in the chromatograms.
in the decomposition of 4-MMC. The appropriate antioxidant was added to the pH 12 buffer to give a final concentration of 0.1% (w/v) for γ-ascorbic acid or 1% (w/v) for sodium sulfite, which did not alter the pH of the buffer. Fifty microliters of the methanol solution of 4-MMC HCl (1 mg/ml) was mixed with 950 μl of the buffer (with and without the antioxidant) in a polypropylene tube. After 0 and 6 h of storage at 22 °C, 5 μl of the solution was injected into the high-performance liquid chromatography (HPLC) system. The stability was evaluated by the percentage of the remaining compound.

The effects of the antioxidants on the degradation pathway of 4-MMC were also examined. After storing 4-MMC HCl (1 mg/ml) in the pH 12 buffer in the presence or absence of the antioxidants (0.1% (w/v) γ-ascorbic acid or 1% (w/v) sodium sulfite) at 22 °C for 6 h, two 1 ml portions of the solutions were taken. Subsequent analytical procedures were same as those described in Section 2.5.

2.7. Evaluation of the degradation rates of the seven MC analogs in various pH buffers

The stability of the seven MC analogs (4-MMC, 4-FMC, 3-FMC, 2-FMC, 4-MeOMC, EtCAT, and DMCA) in various pH (4.0, 7.0, 10.0, and 12.0) buffers was evaluated. Fifty microliters of a methanol solution of each MC analog (1 mg/ml, as HCl salt) was placed in a test tube. At time zero, 950 μl of the buffer was added into the test tube and the solution was shaken by hand. After transferring the sample solution to a polypropylene HPLC vial, a 5 μl aliquot was immediately injected into the HPLC system. The residual solution was stored in an autosampler kept at 22 °C, and injected after the stated intervals. The results were calculated as the percent remaining of the initial concentration, and the first-order decomposition rate constants and half-lives were calculated by non-linear regression (KaleidaGraph, ver. 3.6, Synergy Software, Reading, PA, USA).

3. Results and discussion

3.1. Identification of the degradation products of 4-MMC and its degradation pathways

Fig. 3 shows a series of GC–MS chromatograms obtained from ethyl acetate extracts of 4-MMC in the pH 12 buffer at different times. In these chromatograms, peaks of 4-MMC, MPPD, MBA, DMBA, and N-Ac-4-MMC were identified by comparison with their standards. These mass spectra are shown in Fig. 4(a)–(e). In addition to these peaks, an unidentified peak 1, whose mass spectrum is shown in Fig. 4(f), was also observed. Other cathinone derivatives such as N,N-diethylcathinone (diethylpropion) [13,14] and cathinone [15] gave 1-phenyl-1,2-propanedione as a degradation product. However, propiophenone [14] and diphenylpyr-azine [15], reported as degradation products of other cathinone derivatives, were not detected.

Time courses of the peak areas of 4-MMC and its identified degradation products are shown in Fig. 5. Three patterns were confirmed; pattern 1: continuous decrease (4-MMC); pattern 2: increase at first followed by decrease (MPPD and MBA); pattern 3: continuous increase (N-Ac-4-MMC and DMCA). The second pattern

![Fig. 4. EI mass spectra of the peaks in Fig. 3. (a) MBA; (b) MPPD; (c) 4-MMC; (d) DMBA; (e) N-Ac-4-MMC; (f) unidentified peak 1.](image-url)
indicates the appearance of intermediates followed by their decomposition to other compounds.

The results of the supplementary experiments are summarized as follows. (i) Experiment 1: MBA was produced from MPPD in the pH 12 buffer. (ii) Experiment 2, DMBA was not produced from a mixture of MBA and methylamine in the pH 12 buffer, (iii) Experiment 3: the production amount of N-Ac-4-MMC from 4-MMC increased by addition of acetic acid.

**Fig. 5** and the results of the supplementary experiments showed that the degradation pathways were as follows: (a) 4-MMC → MPPD → MBA → DMBA and (b) 4-MMC → N-Ac-4-MMC (Fig. 6). In pathway (a), the first reaction is the oxidative deamination of 4-MMC to form MPPD (reaction 1 in Fig. 6). Methylamine would be produced at the same time. A similar reaction was observed in the previous study [14], where 1,2-propanedione and diethylamine were identified as the degradation products of N,N-diethylcathinone.

The second reaction forms MBA by oxidative cleavage of MPPD (reaction 2 in Fig. 6). This reaction was supported by the result of supplementary experiment 1. Although no other degradation products except for MBA were identified, the generation of acetic acid was observed in a previous report [16] on the oxidative cleavage of 1-phenyl-1,2-propanedione.

The third reaction was the production of DMBA by amidation of MBA (reaction 3 in Fig. 6). This reaction was supported by the increase of DMBA which correlated with the decrease of MBA over the storage time from 24 h to 48 h in Fig. 5. Unfortunately, the mechanism of the DMBA production was unclear. At least, the supplementary experiment 2 shows DMBA was not generated by the direct reaction of MBA with methylamine.

**Pathway (b)** is the N-acetylation of 4-MMC (reaction 4 in Fig. 6). Addition of acetic acid to the 4-MMC solution enhanced the production of N-Ac-4-MMC in the supplementary experiment 3. This indicated that the acetylation reagent originated directly from acetic acid, which would have been generated in reaction 2 (Fig. 6).

### Table 1

<table>
<thead>
<tr>
<th>Antioxidants</th>
<th>Remaining (%) after 6 h storage</th>
</tr>
</thead>
<tbody>
<tr>
<td>No</td>
<td>69.3 ± 1.8</td>
</tr>
<tr>
<td>l-Ascorbic acid (0.1%, w/v)</td>
<td>84.8 ± 1.2</td>
</tr>
<tr>
<td>Sodium sulfite (1%, w/v)</td>
<td>80.5 ± 1.0</td>
</tr>
</tbody>
</table>

Each value represents the mean ± standard deviation of three determinations. *p < 0.001 compared to no antioxidant.

1.2. The effect of antioxidants on the stability of 4-MMC in the alkaline solution

The percentages of 4-MMC remaining after storage for 6 h in the pH 12 buffer with and without the antioxidants are shown in **Table 1**. There was significantly more 4-MMC remaining in the presence of antioxidants than without them (*p < 0.001*, two-tailed unpaired *t*-test). This result suggested that dissolved oxygen (DO) was involved in the degradation of 4-MMC in

![Fig. 6. Possible 4-MMC degradation pathways. In pathway (a), reactions 1, 2, and 3 are oxidative deamination of 4-MMC, oxidative cleavage of MPPD, and amidation of MBA, respectively. Pathway (b) is the N-acetylation of 4-MMC.](image-url)
alkaline solution, because both of the tested antioxidants are oxygen scavengers in water [17]. The involvement of DO was observed in the decomposition of N,N-diethylcathinone in a similar way [18].

Fig. 7 shows the total ion current chromatograms obtained from 4-MMC after storage for 6 h in the pH 12 buffer with and without the antioxidants. Interestingly, the appearance and disappearance of the degradation products in the presence of l-ascorbic acid differed from those in the presence of sodium sulfite. L-Ascorbic acid caused an obvious decrease in MBA (peak A), DMBA (peak D), and N-Ac-4-MMC (peak E) as well as an increase in a newly observed unidentified peak 2, whose mass spectrum is shown in Fig. 8. Sodium sulfite caused a decrease in N-Ac-4-MMC (peak E) as well as an increase in MPPD (peak B). On the other hand, unlike l-ascorbic acid, sodium sulfite did not obviously change the observed amounts of MBA (peak A) and DMBA (peak D). These results suggested the following: (i) l-ascorbic acid inhibited the oxidative cleavage of MPPD and (ii) sodium sulfite inhibited the acetylation of 4-MMC but not the oxidative cleavage of MPPD. This difference may be attributed to their individual radical scavenging/producing abilities. L-Ascorbic acid acts as a scavenger of the superoxide anion radical [19], which generates the more powerful hydroxyl radical. In contrast, sodium sulfite forms the sulfur trioxide radical ion, and at the same time generates hydroxyl radicals [20].

3.3. The stability of the seven MC analogs in various pH buffers

Considering the possible applications to actual toxicological cases, it was useful to study the stability of MC analogs in biological samples such as blood and urine. However, it was first necessary to study the sample stability in aqueous solution before studying it in biological samples, because it is unclear whether the degradation in biological samples is caused by chemical mechanisms (e.g., pH, dissolved oxygen) or by enzymatic mechanisms.

Half-lives (h) and percent remaining of the seven MC analogs after 12 h storage in various pH buffers (pH 4, 7, 10, and 12) are shown in Table 2. All the MC analogs were stable in the pH 4 solution for at least 12 h, but decomposed in neutral-to-basic solutions (pH 7, 10, and 12). The decomposition rates of the MC analogs calculated by the first-order reaction model had correlation coefficients of more than 0.985 when the percent remaining after 12 h storage was below 80%. Decomposition of the MC analogs accelerated with increasing solution pH except for DMC. This agrees with a previous report on MC [3].

The stability of the analogs in the neutral-to-basic solution depended on the compound. The factors which affected the stability of the MC analogs were: (i) the substituted group on the benzene ring (comparison between 4-MMC, 4-FMC, 3-FMC, 4-MeOMC, and 2-FMC) and ii) the groups attached at the nitrogen atom (comparison between EtCAT and DMC).
The stability of the MC analogs having a group substituted on the benzene ring was assessed based on their half-lives in the pH 12 buffer for simplicity. Four MC analogs (excluding 2-FMC) have a group substituted in the para- or meta-positions. Equation 1 was applied to determine the influence of the functional group X on the reactivity of p- or m-substituents Y in the benzene derivative p- or m-XC6H4Y (Hammett equation) [21].

\[
\log_{10} \left( \frac{k}{k_0} \right) = \log_{10} k - \log_{10} k_0 = \rho \sigma
\]

where \( k \) is the rate constant for the given reaction of p- or m-XC6H4Y; \( k_0 \) refers to the reaction of C6H4Y, i.e., X = H; \( \rho \) is the reaction constant characteristic of the given reaction of Y; \( \sigma \) is the substituent constant characteristic of p- or m-X (Hammett \( \sigma \) constant).

A Hammett plot was constructed by plotting the logarithm of the decomposition rate constant \( k \) (0.693/half-lives) of the four kinds of p- or m-substituted MC analogs (4-MMC, 4-FMC, 3-FMC, and 4-MeOMC) in the pH 12 buffer against their Hammett \( \sigma \) constants taken from the literature [22] (Fig. 9). The logarithm of the \( k \) values against the Hammett \( \sigma \) constants showed a good linear correlation (\( r = 0.9805 \)) with a positive slope (\( \rho = 1.76 \)). This result suggested that negative charge built up in the transition state during the rate determining step [23]. Moreover, the Hammett equation is effective for the prediction of stability of other MC analogs. For example, the \( k \) value of the unsubstituted MC (X = H, Hammett \( \sigma \) constant = 0) was calculated to be 0.102 h\(^{-1} \) (equivalent to a half-life of 6.8 h). This half-life was close to that of EtCAT in the pH 12 buffer (4.99 ± 0.40 h).

2-FMC was the most unstable of the MC analogs tested. It is impossible to explain this instability by the Hammett equation because this equation did not target ortho-substituted isomers. Another reaction may be involved in the decomposition of 2-FMC in addition to the oxidative deamination observed for 4-MMC. In fact, from our GC–MS study on the MC analogs, 2-FMC gave what appeared to be de-hydrofluoride products (the data was not published at the time this article was written).

In terms of nitrogen substitution, the stability of a secondary amine (EtCAT) and a tertiary amine (DMC), both with no additional groups on the benzene ring, were compared. The latter was more stable even in the pH 12 buffer. This difference was assumed to be due to sensitivity to oxidative deamination. This may be supported by the findings from enzymatic metabolism in which tertiary amines generally do not undergo oxidative deamination unlike the secondary aliphatic amines [24].

### 4. Conclusion

The degradation products of 4-MMC in alkaline solution and the responsible decomposition pathways were clarified. Any oxidants such as dissolved oxygen were presumed to be involved in this decomposition due to the suppressive effects generated from the added antioxidants. The decomposition of the MC analogs accelerated with increasing solution pH. The rate of decomposition was dependent on the substituted groups on the benzene ring and on the nitrogen atom. These findings will be very useful for not only forensic analysis but also future pharmacokinetic studies.

### References


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