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Stabilization by meglumine of an amine compound degraded by formaldehyde in tablets

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ABSTRACT

[3-[(2R)-[[(2R)-(3-Chlorophenyl)-2-hydroxyethyl]amino]proryl]-1*H*-indol-7-yloxy]acetic acid (AJ-9677), which was being developed as an antidiabetic, was observed to degrade in tablet preparations. The main degradation product in tablets, AD-9889, was a carbon adduct of the drug substance. When the drug substance was exposed to formaldehyde in aqueous solutions, a correlation was found between the level of formaldehyde and the quantity of AD-9889 formed during storage. Comprehensive one- and two-dimensional NMR analysis of the reaction product identified the location of the carbon atom which originated from formaldehyde, thus proving that AD-9889 was produced by a reaction with formaldehyde. Since it was demonstrated in our previous report that meglumine is an amine that can react with, and reduce amounts of formaldehyde, its stabilizing effect on AJ-9677 was examined. The results showed that in a solution system containing AJ-9677, formaldehyde and meglumine, meglumine reduced formaldehyde levels and suppressed degradation. Addition of meglumine into the tablet formulation of AJ-9677 was also effective in suppressing degradation and successfully stabilized the drug substance. This effect was almost certainly due to meglumine absorbing formaldehyde from around the drug substance and we believe that meglumine can be used with many other drug substances degraded by formaldehyde.

1. Introduction

[3-[(2R)-[[(2R)-(3-Chlorophenyl)-2-hydroxyethyl]amino]proryl]-1*H*-indol-7-yloxy]acetic acid (AJ-9677, molecular formula: $C_{21}H_{23}ClN_2O_4$, molecular weight: 402.88, Fig. 1A) is a selective β_3-adrenergic receptor agonist that was newly synthesized at Dainippon Sumitomo Pharma Co., Ltd. (Osaka, Japan), and was targeted for use in the treatment of type II (non-insulin dependent) diabetes mellitus and obesity (Kato et al., 2001; Francke, 2002; Sakura, 2002; Sakura et al., 2002; Harada et al., 2005). It was demonstrated that this compound had antidiabetic and antiobesity effects associated with improvement of insulin resistance in the diabetic obese mouse model (Kato et al., 2001; Francke, 2002; Sakura, 2002; Sakura et al., 2002; Harada et al., 2005).

The drug substance was expected to show efficacy with low dose strengths by oral administration based on the results of animal experiments. However drug products with low dose strengths generally have poor chemical stability, because the higher the dilution ratio of excipients to drug substance, the more easily the drug substance can be degraded (Badawy et al., 1999). It is critical to assure sufficient quality and safety for pharmaceutical products and an essential requirement is to suppress the degradation of drug substances to meet regulatory criteria (ICH Q3B, 2006). Although AJ-9677 was intended to be developed in tablet form for ease of use, its chemical stability in tablet preparation was insufficient, and the main degradation product (AD-9889, see Fig. 1B) was thought to be produced by a reaction with formaldehyde.

The objective of this study was to demonstrate that the generation of AD-9889 was due to formaldehyde and to stabilize AJ-9677 in tablets by limiting the formaldehyde reaction.

2. Materials and methods

2.1. Materials

AJ-9677 was synthesized at Dainippon Sumitomo Pharma Co., Ltd., Osaka, Japan.

Pharmaceutical excipients: Japanese Pharmacopoeia (fifteenth edition) grade excipients were used. Lactose (Pharmatose® 200M) (DMV International, Veghel, The Netherlands), D-mannitol (Mitsubishi Shoji Foodtech Co., Ltd., Tokyo, Japan), low-substituted hydroxypropylcellulose (L-HPC, LH-21) (Shin-Etsu Chemical Industry Co., Ltd., Tokyo, Japan), hydroxypropylcellulose low-viscosity type (HPC L) (Nippon Soda Co., Ltd., Tokyo, Japan), MCC (Ceolus®

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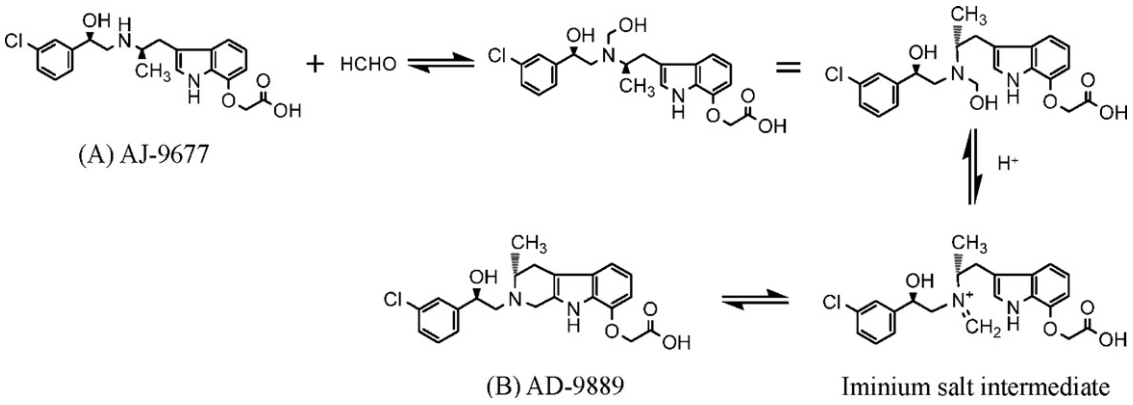


Fig. 1. Proposed formation mechanism of AD-9889 from AJ-9677. (A) [3-[(2R)-[[(2R)-(3-Chlorophenyl)-2-hydroxyethyl]amino]proryl]-1*H*-indol-7-yloxy]acetic acid (AJ-9677, molecular formula: $C_{21}H_{23}ClN_2O_4$, molecular weight: 402.88) and (B) [[(2R)-(3-chlorophenyl)-2-hydroxyethyl]-(3R)-methyl-1,2,3,4-tetrahydro-β-carboline-8-yloxy]acetic acid (AD-9889, molecular formula: $C_{22}H_{23}ClN_2O_4$, molecular weight: 414.89).

PH-101) (Asahi Kasei Corp., Tokyo, Japan), magnesium stearate (Mg-St, vegetable origin) (Taihei Chemical Industrial Co., Ltd., Osaka, Japan) and light anhydrous silicic acid (LASA, Aerosil® 200) (Nippon Aerosil Co., Ltd., Tokyo, Japan) were used. Meglumine was purchased from Merck KgaA, Darmstadt, Germany.

Reagents: Acetonitrile of HPLC grade was obtained from Kanto Chemical Co., Inc., Tokyo, Japan. All other reagents were of analytical grade and purchased from Wako Pure Chemical Industries, Ltd., Osaka, Japan.

2.2. Preparation and storage of AJ-9677 tablets

The AJ-9677 tablet formulation contains the drug substance 0.1 mg, D-mannitol 70.9, 70.4 or 69.9 mg, L-HPC 10 mg, HPC L 2.5 mg, meglumine 0, 0.5 or 1 mg (components for the drug granules), MCC 15 mg, magnesium stearate 1 mg and LASA 0.5 mg per tablet (total weight: 100 mg per tablet). Drug granules for the tablets were prepared by a wet granulation method using a planetary mixer (5DM, Shinagawa Machinery Works Co., Ltd., Nara, Japan), followed by mixing with external addition components. Then the mixtures were compressed using a single punch tablet press (2B, Kikusui Seisakusho Ltd., Kyoto, Japan) fitted with 6.5 mm diameter punches to obtain tablets. The tablets were stored in a closed high density polyethylene bottle (100 tablets/container) at 40 °C and 60 °C for 14 days for stability testing.

2.3. Preparation and storage of binary and ternary aqueous solutions

AJ-9677, formalin and purified water were mixed to make three aqueous binary solutions, where the AJ-9677 concentration was 0.1 μmol/ml and the formaldehyde concentration was 0.1, 1 and 10 μmol/ml. In these binary solutions, mole ratios of AJ-9677 to formaldehyde were 1:1, 1:10 and 1:100, respectively.

AJ-9677, formalin, meglumine and purified water were mixed to make four aqueous ternary solutions, where AJ-9677 and formaldehyde concentrations were 0.1 and 1 μmol/ml, respectively and meglumine was 0.1, 1, 10 and 100 μmol/ml. In these ternary solutions, mole ratios of AJ-9677:formaldehyde:meglumine were 1:10:1, 1:10:10, 1:10:100 and 1:10:1000, respectively.

For the stability test, 100 ml of each mixed solution was stored in a glass flask with a glass closure at 25 °C for 3 days.

2.4. HPLC analysis of the main degradation product of AJ-9677

Analysis of the main degradation product of AJ-9677 was performed using an HPLC system consisting of an LC-10AS pump, a CTO-10A column oven, an SPD-10A UV-visible detector, an SCL-10AVP system controller, an SIL-10AXL auto injector and a CLASS-VP data system (Shimadzu Corp., Kyoto, Japan). A 150 mm × 4.6 mm internal diameter column packed with octadecyl silica gel (ODS) of 5 μm particle size, Develosil ODS-5 (Nomura Chemical Co., Ltd., Aichi, Japan) was used at 40 °C. The mobile phase consisted of 75% 0.01 mol/l citrate buffer (pH 2.5) and 25% acetonitrile and the flow rate was set at about 1.0 ml/min. AJ-9677 and its degradation products were extracted from 10 tablets with 5 ml of the mobile phase and 10 μl of the extract was injected. For analysis of the binary and ternary solutions, 50 μl of sample solutions was directly injected. The drug substance and its degradation products were detected at a wavelength of 220 nm and the amount of the main degradation product was expressed as a peak area percentage to the total of the drug substance and its degradation products.

2.5. Assay of formaldehyde by HPLC

Formaldehyde in the sample solution was quantified by modifying the method reported by Benassi et al. (1989) as previously described by the authors (Fujita et al., 2009). Briefly, a sample or standard solution was diluted appropriately with purified water, mixed with acetonitrile and then derivatized by adding DNPH reagent. The solution was analyzed by HPLC method at a wavelength of 345 nm after stabilization with phosphate buffer (pH 6.8) and sodium hydroxide solution. The amount of formaldehyde in each sample was calculated from the peak area ratio to the standard solution and expressed as a percentage to a theoretical amount in the initial solution.

2.6. Fractionation of the reaction product between AJ-9677 and formaldehyde

An aqueous methanol solution of approximately 99% methanol containing about 1.5 μmol/ml of AJ-9677 and formaldehyde was stored at 80 °C for 1 day to fractionate their reaction product. The fractionation used a preparative HPLC system similar to that used for the analysis of the AJ-9677 degradation product described above. The method used a 250 mm × 20 mm internal diameter column packed with ODS of 10 μm particle size, YMC-Pack ODS-AM (YMC Co., Ltd., Kyoto, Japan) at 40 °C. The mobile phase was a mixture of 0.01% trifluoroacetic acid and acetonitrile (65:35) and was delivered at a flow rate of 9.99 ml/min. The detector was set at a wavelength of 268 nm and each injection volume was 400 μl. The fractions where the retention time was approximately between 9.5 and 11.0 min were collected and freeze-dried using an FD-1 freeze dryer (Tokyo Rikakikai Co., Ltd., Tokyo, Japan) to obtain around

Table 1
Stability of AJ-9677 in tablets without meglumine.

Storage condition	Generated amount of AD-9889 (%)	Theoretical amount of formaldehyde required for the reaction (μg/100 tablets)
Initial	0.05 ± 0.01	0.4 ± 0.1
After 14 days at 40 °C	0.28 ± 0.00	2.1 ± 0.0
After 14 days at 60 °C	0.59 ± 0.00	4.4 ± 0.0

The data are expressed as means ± standard deviations, $n = 3$.

17 mg of powder. Powder of the reaction product between AJ-9677 and ^{13}C-formaldehyde was also prepared by the same procedure.

2.7. ^{1}H and ^{13}C NMR spectroscopy

Samples were dissolved in deuterated dimethylsulfoxide before measurement. Proton and ^{13}C NMR spectra were acquired using a Mercury vx-400 Spectrometer (Varian, Inc., CA, USA) at 40 °C with an operating frequency of 400 and 100 MHz, respectively. Proton and ^{13}C chemical shift assignments were referenced internally to the tetramethylsilane peak. Two-dimensional NMR experiments of HSQC (Heteronuclear Single Quantum Correlation spectroscopy) and HMBC (Heteronuclear Multiple Bond Correlation spectroscopy) were also conducted for structure determination.

3. Results and discussion

3.1. Degradation of AJ-9677 in tablet preparation

After storage of AJ-9677 tablets without meglumine at 40 °C and 60 °C, the amount of the degradation product AD-9889 increased with rising temperature as presented in Table 1. AD-9889 is [[(2R)-(3-chlorophenyl)-2-hydroxyethyl]-(3R)-methyl-1,2,3,4-tetrahydro-β-carboline-8-yloxy]acetic acid (molecular formula: $C_{22}H_{23}ClN_2O_4$, molecular weight: 414.89, Fig. 1B) and is the main degradation product of AJ-9677 in tablet preparations. Whereas AD-9889 was present at levels of only 0.05% at the initial time point, these increased to 0.28% and 0.59% after storage for 14 days at 40 °C and 60 °C, respectively. In medicinal products, amounts of individual degradation products are generally expected not to exceed 1.0% at 40 °C for 6 months for quality assurance (ICH Q3B, 2006), which means that the quality of AJ-9677 tablets probably cannot be ensured. In order to stabilize AJ-9677 in tablets, it was important to suppress the generation of AD-9889. Since AD-9889 is a carbon adduct of AJ-9677, the degradation product was assumed to be produced by a Mannich reaction of the parent compound with formaldehyde (Pine et al., 1982), which is a similar reaction to the formation of tetrahydro-β-carbolines from L-tryptophan, etc. (Brossi et al., 1973; Narasimhan et al., 1983; Herraiz and Papavergou, 2004). The formation mechanism of AD-9889 from AJ-9677 is proposed as shown in Fig. 1, where the degradation product is produced via an iminium salt intermediate, which is a Schiff base formed between AJ-9677 and formaldehyde. In this mechanism, theoretical amounts of formaldehyde required for the reaction are 0.4, 2.1 and 4.4 μg per 100 tablets or 10 g of ingredients stored under the above three conditions as shown in Table 1. The authors have reported in the previous report that after storage of AJ-9677 tablets at 80 °C for 14 days, 0.3–1.3 μg of formaldehyde was detected per gram of each of the excipients (D-mannitol, L-HPC, HPC L, MCC, magnesium stearate and LASA) (Fujita et al., 2009). During storage for long periods like 6 months at 40 °C, it is almost certain that more formaldehyde will be generated, probably enough to degrade the AJ-9677 tablets as described above.

3.2. Influence of formaldehyde on stability of AJ-9677 in solutions

In order to confirm the hypothesis that formaldehyde contributes to the formation of AD-9889, the stability of AJ-9677 was examined in binary aqueous solutions containing three controlled levels of formaldehyde. Fig. 2A shows that the amount of AD-9889 generated during storage increased with concentration of formaldehyde, and this phenomenon confirms that formaldehyde causes formation of the degradation product. In this experiment, there was little change in the amount of formaldehyde before and after storage (Fig. 2B). From the detected amounts of AD-9889, the theoretical amounts of formaldehyde consumed for the reaction with AJ-9677 are estimated at only 0.3%, approximately, of initial values in all solutions with three concentrations of formaldehyde and the results in Fig. 2B correspond to this estimation.

3.3. Identification of the carbon atom originating from formaldehyde in AD-9889

The reaction product between AJ-9677 and formaldehyde was fractionated and freeze-dried and then analyzed by ^{1}H NMR, ^{13}C NMR, HSQC and HMBC to determine its structure. The ^{1}H and ^{13}C NMR spectra of the product are shown in Fig. 3A and B, respectively. Direct bindings between protons and carbon atoms were determined from the HSQC spectrum (data not shown). Finally, the ^{1}H and ^{13}C chemical shift assignments of the product were determined as shown in Table 2 from comprehensive one- and two-dimensional NMR analysis by reference to AJ-9677 assign-

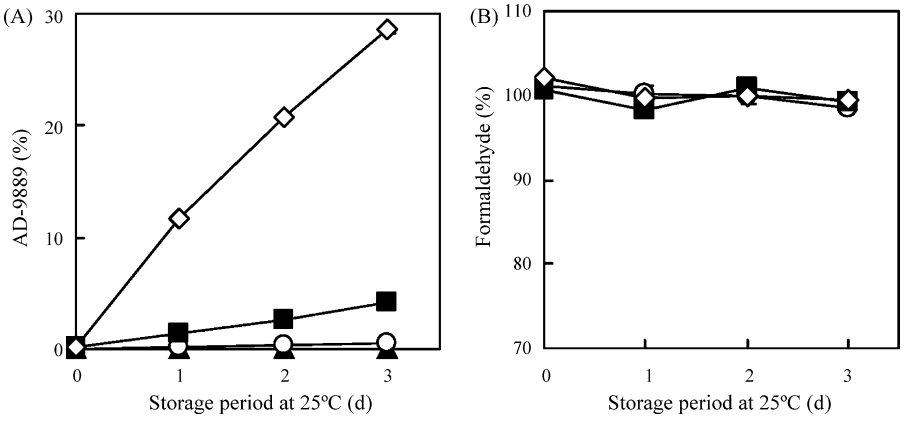


Fig. 2. (A) Generation percentages of AD-9889 and (B) residual percentages of formaldehyde in AJ-9677 aqueous solutions containing various levels of formaldehyde. ▲, AJ-9677 only; ○, AJ-9677 + formaldehyde (1:1*); ■, AJ-9677 + formaldehyde (1:10*); ◇, AJ-9677 + formaldehyde (1:100*) (*mole ratio). Mixed aqueous solutions of AJ-9677 and various levels of formaldehyde were stored at 25 °C for 3 days. The data are expressed as means ± standard deviations, $n = 3$.

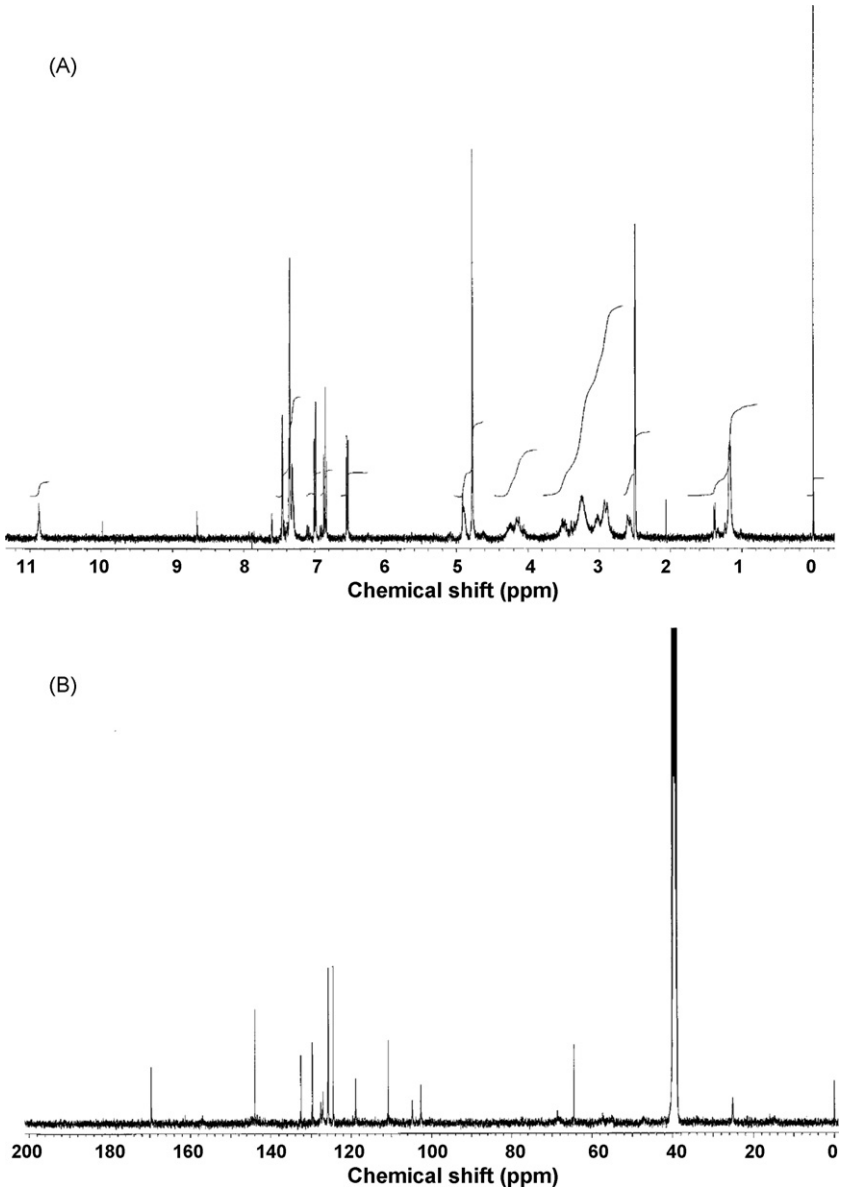


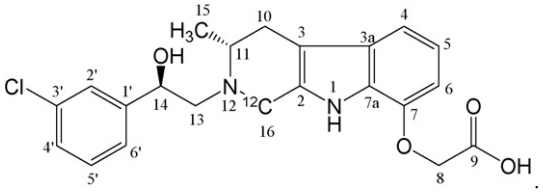
Fig. 3. (A) ^{1}H NMR and (B) ^{13}C NMR spectra of the reaction product between AJ-9677 and formaldehyde.

ments, and the reaction product was confirmed to be AD-9889. The long-range connectivity between a carbon atom at 104.80 ppm and protons at 4.10–4.31 ppm observed in the HMBC spectrum (data not shown) is consistent with the partial structure of a quaternary carbon atom at position 3 and methylene protons at position 16 as shown in Table 2. In order to clarify the location of the formaldehyde-originating carbon atom in AD-9889, the reaction product between AJ-9677 and ^{13}C-formaldehyde was analyzed by ^{13}C NMR. The result identified the carbon atom as position 16, because a signal of ^{13}C was observed at 47.00 ppm in the spectrum. This identification bore out the proposed formation mechanism shown in Fig. 1.

3.4. Stabilizing effect of meglumine on AJ-9677 in solutions

Since meglumine was revealed to be effective in reducing formaldehyde levels as previously described by the authors (Fujita et al., 2009), its ability to prevent the formation of AD-9889 was examined in ternary aqueous solutions containing AJ-9677, formaldehyde and four controlled levels of meglumine. Fig. 4A shows that the amounts of AD-9889 generated in these solutions decreased with increasing amounts of meglumine. However, the decrease in AD-9889 was not linear, and a complete reduction in formaldehyde levels combined with a complete prevention of degradation was not possible, even with large amounts of meglumine. For example, when 10 and 100 times as much meglumine as formaldehyde was added, reduction in generation of AD-9889 was only 20% and 67%, respectively, compared to its generation in the control solution. As for residual formaldehyde, when 10 and 100 times as much meglumine as formaldehyde was added, a decrease of only around 5% and 10%, respectively of the initial amount occurred, as presented in Fig. 4B. Taking into consideration the fact that very little formaldehyde was consumed for the reaction with AJ-9677 as shown in the above result (Fig. 2B), this suggests that most of the decrease of formaldehyde in this study was due to meglumine. These results demonstrate that meglumine has the ability to reduce formaldehyde amounts and prevent the formation of AD-9889. Though much more meglumine than formaldehyde is required, the addition of an adequate amount of meglumine into drug products is considered feasible because the

Table 2
^{1}H and ^{13}C NMR assignments of the reaction product between AJ-9677 and formaldehyde



Position	Chemical shifts (δ_C, ppm)	Chemical shifts (δ_H, ppm), multiplicity, J (Hz), integration
1	–	10.86, bs, 1H
2	129.85	–
3	104.80	–
3a	127.63	–
4	110.78	6.98, d, 7.9, 1H
5	118.83	6.82, t, 7.9, 1H
6	102.70	6.53, d, 7.9, 1H
7	143.94	–
7a	125.88	–
8	64.55	4.56, s, 2H
9	169.67	–
10a	25.16	3.01, m, 1H
10b		2.58, m, 1H
11	55.15	3.51, m, 1H
13	57.34	2.91, m, 2H
14	68.61	4.78, m, 1H
15	14.73	1.20, d, 6.2, 3H
16	47.09	4.10-4.31, m, 2H
1′	144.59	–
2′	125.73	7.45, bs, 1H
3′	132.58	–
4′	127.14	7.30-7.39, m, 1H
5′	129.72	7.30-7.39, m, 1H
6′	124.52	7.30-7.39, m, 1H

Abbreviations: bs = broad singlet; d = doublet; m = multiplet; s = singlet; t = triplet. The assignments for '*a*' and '*b*' on position 10 are interchangeable.

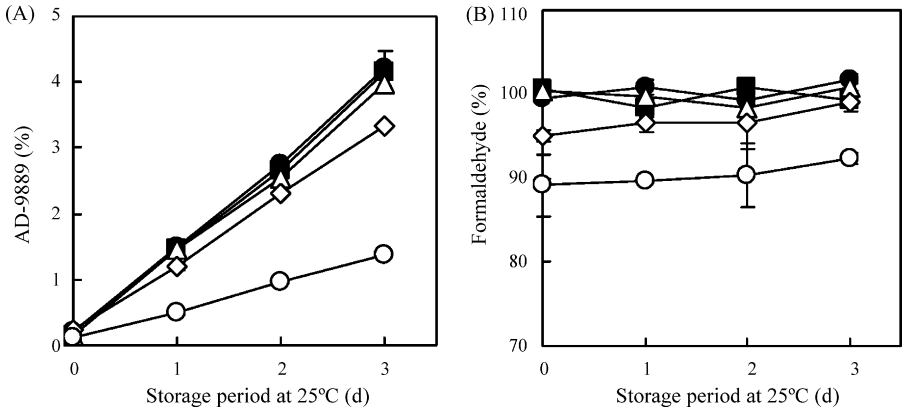


Fig. 4. (A) Generation percentages of AD-9889 and (B) residual percentages of formaldehyde in AJ-9677 aqueous solutions containing formaldehyde and various levels of meglumine. ■, AJ-9677 + formaldehyde (1:10*); ●, AJ-9677 + formaldehyde + meglumine (1:10:1*); △, AJ-9677 + formaldehyde + meglumine (1:10:10*); ◇, AJ-9677 + formaldehyde + meglumine (1:10:100*); ○, AJ-9677 + formaldehyde + meglumine (1:10:1000*) (*mole ratio). Mixed aqueous solutions of AJ-9677, formaldehyde and various levels of meglumine were stored at 25 °C for 3 days. The data are expressed as means ± standard deviations, $n = 3$.

Table 3
Effect of meglumine on stability of AJ-9677 in tablets.

Meglumine (%)	Generated amount of AD-9889 (%)		
	Initial	After 14 days at 40 °C	After 14 days at 60 °C
0.0	0.05 ± 0.01	0.28 ± 0.00	0.59 ± 0.00
0.5	0.01 ± 0.01	0.07 ± 0.00	0.35 ± 0.01
1.0	0.01 ± 0.01	0.01 ± 0.01	0.27 ± 0.02

The data are expressed as means ± standard deviations, $n = 3$.

amounts of formaldehyde around drug products are generally so small.

3.5. Stabilizing effect of meglumine on AJ-9677 in tablets

AJ-9677 tablets containing 0.0, 0.5 and 1.0% meglumine were prepared to examine the stabilizing effect of meglumine on AJ-9677 in tablets. The stability test results of these tablets are described in Table 3. Generation of AD-9889 during storage was apparently reduced by increasing the meglumine content in the tablets. After storage at 40 °C for 14 days, 0.28% of the drug substance was degraded in tablets without meglumine, whereas the degradation product level was only 0.07% and 0.01% in tablets containing 0.5% and 1.0% meglumine, showing the tablets containing 1.0% meglumine have sufficient stability. At 60 °C a similar result was obtained and generation of AD-9889 was decreased from 0.59% to 0.27% by adding 1.0% meglumine. These results demonstrated that the addition of meglumine into the tablet formulation is effective in suppressing degradation and successfully stabilizes the drug substance. This effect is thought to be due to meglumine absorbing formaldehyde from around the drug substance.

4. Conclusion

The main degradation product of AJ-9677 in tablet preparation was a carbon adduct of the drug substance, called AD-9889. When the drug substance was exposed to formaldehyde in aqueous solutions, a correlation was found between the level of formaldehyde and the quantity of AD-9889 formed during storage, which suggested that AD-9889 was formed by the drug substance reacting with formaldehyde. Comprehensive one- and two-dimensional NMR analysis of the reaction product identified the location of the formaldehyde-originating carbon atom in AD-9889, which proved that it was produced by the reaction with formaldehyde. In the solution system containing AJ-9677, formaldehyde and meglumine, meglumine reduced formaldehyde levels and suppressed degradation. Addition of meglumine into the tablet formulation of AJ-9677 was effective in suppressing degradation and successfully stabilized the drug substance. This effect is thought to be due to meglumine absorbing formaldehyde from around the drug substance. It is hoped that meglumine can be similarly used with many other drug substances degraded by formaldehyde.

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