***Regular Article***

# Degradation Pathway of a Taxane Derivative DS80100717 Drug Substance and Drug Product

Kousuke Tamura,*[,a] Makoto Ono,[b] Takefumi Kawabe,[a] Motomu Ohara,[a] and Etsuo Yonemochi*[,c]

[a] *Analytical and Quality Evaluation Research Laboratories, Daiichi Sankyo Co., Ltd.; 1–2–58 Hiromachi, Shinagawa-ku, Tokyo 140–8710, Japan:* [b] *Quality Assurance Department, Daiichi Sankyo Co., Ltd.; 3–5–1 Nihonbashi Honcho, Chuo-ku, Tokyo 103–8426, Japan: and* [c] *Graduate School of Pharmaceutical Sciences, Hoshi University; 2–4–41 Ebara, Shinagawa-ku, Tokyo 142–8501, Japan.*

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**The degradation pathway of a taxane derivative and anticancer agent, DS80100717, was investigated. Several degradants were generated under acidic, basic, and oxidative stress conditions in solution. The chemical structures of eight degradants of DS80100717 were elucidated using MS and NMR. The major degradant of the DS80100717 drug substance derived by heating in solid-state was the *N*-oxide form *via* oxidation and C2′-epimer of the side chain *via* acid hydrolysis. We proposed previously unreported degradation pathways of DS80100717 with taxane derivatives such as paclitaxel, docetaxel, and cabazitaxel.**

**Key words** forced degradation; chemical stability; structure elucidation; impurity; epimerization

## Introduction

Taxane derivatives, including paclitaxel, docetaxel, and cabazitaxel, are anticancer agents that bind along the interior surface of the microtubules and are well known for their remarkable antitumor activities.[1,2] Currently, these agents are utilized and evaluated in lung, breast, ovarian, and other cancers. Various taxane derivatives are being researched and developed with the aim of improving the diverse medicinal properties and therapeutic effects including as a prodrug,[3] albumin-bound,[4] and encapsulated with liposome.[5]

As a part of the pharmaceutical development, the stability of the drug and the identification of degradation products are strictly required as quality control and safety considerations based on the International Conference on Harmonization (ICH) guidelines.[6–9] Stress studies are used to support the development of stability-indicating analytical methods, to understand the intrinsic stability of the drug substances and products, and to estimate the degradation pathways.[10] The studies are aligned with the ICH guidelines and exposed to various conditions in both the solid-state and solution state.[11] Stress studies on taxane derivatives, including paclitaxel,[12] docetaxel,[13] and cabazitaxel,[14] have been reported and used for pharmaceutical drug development and quality control.

DS80100717 ((1*S*,2*S*,3*R*,4*S*,5*R*,8*R*,9*S*,10*R*,13*S*)-4-acetoxy-2-benzoyloxy-9,10-[(1*S*)-2-(dimethylamino)ethylidenedioxy]-5,20-epoxy-1-hydroxytax-11-en-13-yl (2*R*,3*S*)-3-(*tert*-butoxycarbonylamino)-2-hydroxy-3-(3-methyloxetan-3-yl)propionate) was designed as a taxane derivative compound and was developed as an anticancer agent for injection.[15] The chemical structure of DS80100717 is shown in Fig. 1. This study aimed to clarify the chemical stability and degradation pathway of DS80100717, both drug substance and drug product (lyophilized injection formulation), under various stress conditions. The degradation products were characterized by using LC-MS, LC-MS/MS, online hydrogen-deuterium (H/D) exchange LC-MS, and NMR spectral data.

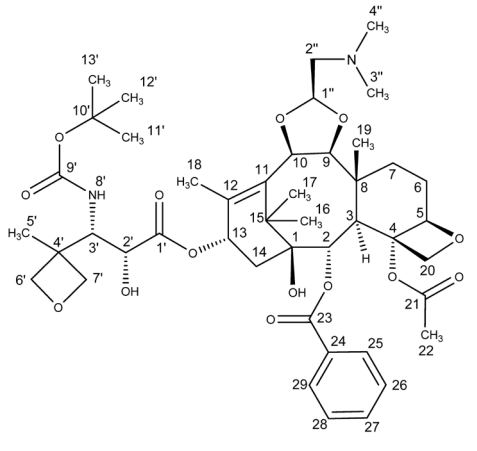

Fig. 1. Chemical Structure of DS80100717

## Experimental

**Materials** DS80100717 drug substance was obtained from Daiichi Sankyo Co., Ltd. (Tokyo, Japan). Hydrochloric acid (HCl) and sodium hydroxide (NaOH) were as used for volumetric analysis. Lactose monohydrate was of a special grade, and hydrogen peroxide ($H_2O_2$) was of a special grade from FUJIFILM Wako Pure Chemical Corporation (Osaka, Japan). Lactic acid was of a special grade from Thermo Fisher Scientific (Waltham, MA, U.S.A.). Britton-Robinson buffer solutions (pH 2.0 to 12.0, ionic strength: 1.0) were of a special grade from Nacalai Tesque, Inc. (Kyoto, Japan). Ammonium formate was of special grade and methanol was of HPLC grade from FUJIFILM Wako Pure Chemical Corporation. Acetonitrile of HPLC grade was purchased from Kanto Chemical Co., Inc. (Tokyo, Japan). Acetonitrile-$d_3$, methanol-$d_4$, and deuterium oxide were purchased from Cambridge Isotope Labs (Andover, MA, U.S.A.). Purified water for LC/MS was purchased from FUJIFILM Wako Pure Chemical Corporation. All other chemicals and reagents were commercial products of analytical grade.

* To whom correspondence should be addressed. e-mail: tamura.kousuke.aa@daiichisankyo.co.jp; e-yonemochi@hoshi.ac.jp

Table 1. Components and Composition of DS80100717 Drug Product

| Functions | Component | Recipe |
|---|---|---|
| Active pharmaceutical ingredients | DS80100717 | 10 mg |
| Bulking agent | Lactose monohydrate | 50 mg |
| pH adjusting agent | Lactic acid | 2.1 mg |
| pH adjusting agent | Hydrochloric acid | q.s.[a)] |
| pH adjusting agent | Sodium hydroxide | q.s.[a)] |
| pH | | 4.0 |
| Total volume | | 1.0 mL, q.s.[a)] |

*a*) Quantum sufficit.

**Preparation of DS80100717 Lyophilized Samples** The components and composition of the DS80100717 drug product are described in Table 1. The samples were prepared as follows. The DS80100717 drug substance and additives (lactose monohydrate, lactic acid) were dissolved in purified water. The pH of the solution was adjusted to 4.0 with 1 mol/L hydrochloric acid solution or 1 mol/L sodium hydroxide solution. The total volume of the solutions was adjusted to the target volume with purified water and then filtered with a 0.2 $\mu$m membrane filter (Millex-LG Hydrophilic polytetrafluoroethylene, Merck KGaA, Darmstadt, Germany). The bulk solution was filled into vials, and then the filled vials were half-stopped with rubber stoppers. The samples were lyophilized using a freeze dryer (VirTis AdVantage Plus, SP Scientific, Stone Ridge, NY, U.S.A.). The lyophilization program was as follows (the chamber pressure was not controlled during the lyophilization process): Freezing: −40°C, 2 h; Primary drying: −20°C, 12 h, Secondary drying: −5°C, 5 h, Tertiary drying: 5°C, 3 h. After lyophilization, the samples were vacuum released with nitrogen. Next, the rubber stoppers were fully inserted into the vials with caps.

**Stress Study of DS80100717 Drug Substance in Solution** The DS80100717 drug substance was subjected to various stress conditions in solution at a DS80100717 concentration of 1 mg/mL. Stress studies were performed under hydrolytic conditions as follows: for acidic hydrolysis, a sample solution was mixed with a 0.1 mol/L hydrochloric acid solution and exposed to 40°C for 1 h; for basic hydrolysis, a sample solution was mixed with the Britton-Robinson buffer solution (pH 10.0, ionic strength: 0.2) and exposed as such. A stress study under an oxidative condition was performed as follows: A sample solution was mixed with 0.3% hydrogen peroxide solution and exposed to 25°C for 1 h.

**Stress Study of DS80100717 Drug Substance in Solid-State** The DS80100717 drug substance was subjected to the various stress conditions in solid-state. A stress study was performed under a heated condition: DS80100717 drug substance (100 mg) in an open dish was stored in an oven at 60°C for 4 weeks. Stress study under a humidity condition: DS80100717 drug substance (100 mg) in an open dish was stored under 75% relative humidity (RH) at 40°C for 4 weeks. Stress study under a photo-irradiation condition: DS80100717 drug substance (100 mg) in an open dish was stored as per ICH requirement (ICH Q1B option 1, D65 light source, 2000 lx) at room temperature for 4 weeks (1344000 lx·h).

**Stress Study of DS80100717 Drug Product under Heat Condition** The DS80100717 drug products were exposed to degrade at 25 and 40°C for 4 weeks, and at 60°C for 2 weeks.

**Sample Preparation for HPLC Analysis** The DS80100717 drug substances, drug products, and its stressed samples were separately prepared by mixing with water and acetonitrile (4 : 6 (v/v)) at each concentration (0.5 mg/mL).

## Measurement

**HPLC Analysis** The Shimadzu Nexera X2 LC system (Shimadzu Corporation, Kyoto, Japan) equipped with a photodiode array (PDA) detector was used for HPLC analysis. A YMC Triart C18 column (2.0 mm i.d. × 75 mm, 1.9 $\mu$m particle size, YMC, Kyoto, Japan) was used as an analytical column. The temperature of the column oven was set at 40°C. UV detection was performed at 229 nm, and the online PDA spectra were collected between 200 and 400 nm. The mobile phase A and B were 20 mmol/L ammonium formate in water and acetonitrile/methanol (57 : 43 (v/v)), respectively. The flow rate was set at 0.5 mL/min. Gradient elution conducted as follows: the step gradient by changing the percentage of mobile phase B at different times, $T$ (min)/% mobile phase B = 0/35, 2.3/50, 4.1/66, 5.1/90, 10/90, 10.1/35, 14/35. The injection volume was 2 mL. Empower 3 software (Waters Corporation, Milford, MA, U.S.A.) was used for data acquisition.

**Preparative LC Conditions** Isolation of the degradation products (Ac-5 and Ox-1) was undertaken using the Shimadzu Nexera X2 LC system (Shimadzu Corporation) and an Explorer-220 solvent evaporator (Thermo Fisher Scientific). A Cadenza CD-C18 column (6.0 mm i.d. × 100 mm, 3 $\mu$m particle size, Imtakt Corporation, Kyoto, Japan) was used for preparative isolation works. The mobile phase, the temperature of the column oven, and UV detection were the same as the analytical HPLC conditions described previously. Isocratic elution was conducted as the mobile phase A/B (40 : 60, (v/v)), and the flow rate was 2 mL/min. The injection volume was 50 mL and the run time was 12 min. The degradation product fractions were collected, and the solvent was removed using a solvent evaporator to obtain Ac-5 (HPLC purity: 94.2%) and Ox-1 (HPLC purity: 94.0%) as a solid.

**LC-MS and MS/MS Analyses** LC-MS and LC-MS/MS analyses were undertaken using a Shimadzu Nexera X2 LC system equipped with an LTQ Orbitrap XL mass spectrometer (Thermo Fisher Scientific). The HPLC conditions were the same as the analytical HPLC conditions previously described. For online H/D exchange LC-MS analysis, deuterium oxide and methanol-$d_4$ were used as solvents for the preparation of mobile phase A and B. Electrospray ionization (ESI) was used in the positive ion mode. The spray voltage was set at 4.0 kV. The capillary temperature was set at 275°C. The sheath gas flow was set at 60 arb and the aux gas flow was set as 10 arb. The MS/MS measurement was performed by collision-induced dissociation with a collision energy of 35%.

**NMR Analysis** NMR experiments were conducted using an Avance 400 MHz NMR spectrometer (Bruker BioSpin Corporation, Billerica, MA, U.S.A.) equipped with a broadband observe 5 mm probe. All spectra acquired from the isolated samples (Ac-5 and Ox-1) were dissolved in acetonitrile-$d_3$ and deuterium oxide (9 : 1 (v/v)). NMR experiments were performed at 25°C. The chemical shifts were referenced to residual solvent peaks for acetonitrile-$d_3$ ($\delta$: 1.94 ppm for proton and 1.32 ppm for carbon). The number of accumulations was 32 to 4096. NMR experiments (1D ($^1$H-NMR, $^{13}$C-NMR) and 2D ($^1$H–$^1$H correlation spectroscopy, distortionless enhance-

ment by polarization transfer, $^{1}H–^{13}C$ heteronuclear multiple quantum correlation spectroscopy, $^{1}H–^{13}C$ heteronuclear multiple bond quantum correlation spectroscopy, and $^{1}H–^{1}H$ nuclear Overhauser effect spectroscopy (NOESY))) were acquired in order to fully elucidate the isolated samples.

## Results and Discussion

**Stability Study of DS80100717 Drug Substance and Drug Product** DS80100717 decomposed under acidic, basic, and oxidative conditions in solution. The representative HPLC chromatograms of the solution stability study of DS80100717 drug substances are shown in Fig. 2. We identified 8 degradation products (Ac-1, -2, -3, -4, -5, Ba-1, -2, and Ox-1). These potential degradation products were defined at levels greater than 10% of the total degradation and were more than 25% of the largest individual degradant.[16] The actual degradation products during long-term stability, as defined in the ICH Q3A and Q3B guidelines, were reported as a subset of the potential degradation products observed during stress studies.[17] The chemical structures and degradation pathways of these degradation products are shown in Fig. 3.

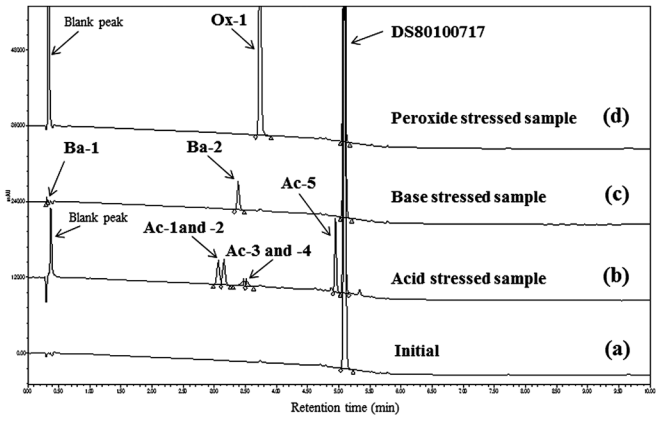


Fig. 2. Representative HPLC Chromatograms of the Solution Stability Studies of DS80100717 Drug Substance

(a) Initial, (b) 0.1 mol/L HCl at 40°C for 1 h, (c) Britton–Robinson Buffer (ionic Strength: 0.2, pH 10.0) at 40°C for 1 h, (d) 0.3% $H_2O_2$ at 25°C for 1 h.

On exposure to heat in the solid-state, the DS80100717 drug substance degraded *via* oxidation to form Ox-1 (0.32 → 0.48%) and *via* acid hydrolysis to form Ac-5 (0.03 → 0.09%). Alternatively, the DS80100717 drug substance was stable under humidity and photo-irradiation (D65 light 2000 lx). The results of the solid-state stability of DS80100717 drug substances under various conditions are shown in Table S1, Supplementary Materials.

The representative HPLC chromatograms of the solid-state stability of the DS80100717 drug products under heat conditions are shown in Fig. 4. When exposed to heat, DS80100717 degraded *via* acid hydrolysis to form Ac-5 (0.10 → 2.39%) at 40°C for 4 weeks. On the other hand, the oxidation product (Ox-1) was not increased under a heated condition owing to the nitrogen atmosphere in the vial. The stability results of the DS80100717 drug products under heat conditions are shown in Table S2, Supplementary Materials.

**Structure Elucidation of Degradation Products**

Degradation Products in Acidic Condition

LC-MS analysis data of all degradation products are summarized in Table 2. The ESI mass spectra of both Ac-1 and -2 generated a protonated molecular ion at *m/z* 801, which is 56 Da less than that of DS80100717 $[M+H]^+$ at *m/z* 857. The accurate mass spectra of Ac-1 and -2 showed a protonated molecule $[M+H]^+$ at *m/z* 801.3800 and 801.3804 (Calcd for $C_{41}H_{57}O_{14}N_2$: 801.3810), respectively. Characteristic fragment ions were observed at *m/z* 582, 313, and 295. These fragment ions indicated that Ac-1 and -2 retained a baccatin III analogous moiety. Online H/D exchange LC-MS analysis of Ac-1 and -2 indicated the presence of four labile hydrogen

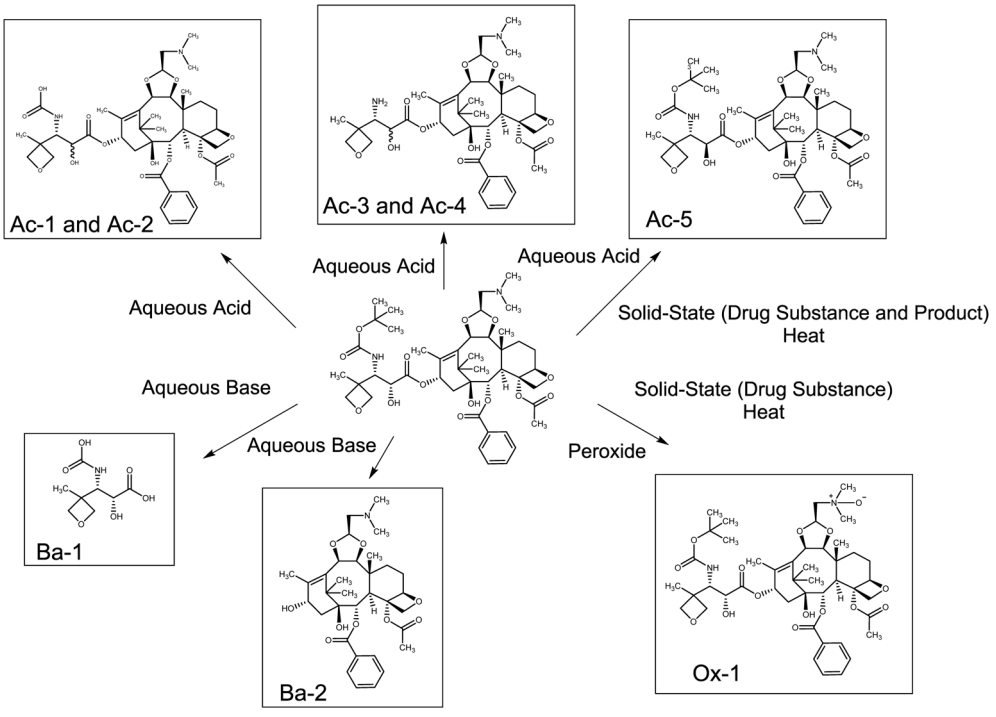


Fig. 3. Chemical Structure of DS80100717 and Degradation Products

atoms, which are one mass larger than the molecular mass of DS80100717. Based on these results, Ac-1 and -2 were identified as a C2′-epimer forms (2*R*, 3*S* and 2*S*, 3*S*), hydrolyzed at the *tert*-butoxy moiety of DS80100717.

The ESI mass spectra of Ac-3 and -4 demonstrated a protonated molecular ion at *m/z* 757, which is 100 Da less than that of DS80100717 $[M+H]^+$ at *m/z* 857. The accurate mass spectra of Ac-3 and -4 showed a protonated molecule $[M+H]^+$ at *m/z* 757.3903 and 757.3898 (Calcd for $C_{40}H_{57}O_{12}N_2$: 757.3912), respectively. Characteristic fragment ions were observed at *m/z* 582, 313, and 295. These fragment ions indicated that Ac-3 and -4 retained the baccatin III analogous moiety. Online H/D exchange LC-MS analysis of Ac-3 and -4 indicated the presence of four labile hydrogen atoms, one mass larger than the molecular mass of DS80100717. Based on these results, Ac-3 and -4 were identified as C2′-epimer forms (2*R*, 3*S* and 2*S*, 3*S*), hydrolyzed at the *tert*-butoxycarbonyl moiety of DS80100717.

The ESI mass spectrum of Ac-5 demonstrated a protonated molecular ion at *m/z* 857, which is the same as DS80100717 $[M+H]^+$ at *m/z* 857. The accurate mass spectrum of Ac-5 showed a protonated molecule $[M+H]^+$ at *m/z* 857.4422 (Calcd for $C_{45}H_{65}O_{14}N_2$: 857.4436). The characteristic fragment ions were *m/z* 839, 801, and 783. These fragment ions confirmed the same as DS80100717. Online H/D exchange LC-MS analysis of Ac-5 and DS80100717 indicated no difference in the number of labile hydrogen atoms in each compound. Figure 5 shows the $^1$H-NMR spectrum of the isolated Ac-5 sample. Compared to the $^1$H-NMR spectrum of DS80100717, the signals of Ac-5 at H2′, H3′, and H5′ in the side chain moiety were significantly shifted to a low magnetic field. In addition, the signals of Ac-5 at H22, H6′, and H7′ were significantly shifted to a high magnetic field. The same trend was observed and confirmed with the signals of $^{13}$C-NMR spectrum at C22, C2′, C3′, C4′, C5′, C6′, and C7.′ These shifts suggested that the change in the stereochemistry at the C2′ position occurred (Tables S3 and S4, Supplementary Materials). The $^1$H-$^1$H NOESY experiment indicated that the signals of H2′, H3′, H6′, and H7′ correlated with the signal of H22, located in closer positions as observed by the conformation of the paclitaxel molecule.[18] In the $^1$H-NMR spectrum, the most important observation was that the vicinal coupling constant of the signals of Ac-5 at $\delta_{H2'}$ 4.76 ppm (1H, d, $J_{(2'\text{-}3')} = 2.1$ Hz) were clearly different compared to DS80100717 at $\delta_{H2'}$ 4.51 ppm (1H, d, $J_{(2'\text{-}3')} = 6.0$ Hz) (Tables S3 and S4, Supplementary Materials). This data suggested that the conformation between H2′ and H3′ of Ac-5 demonstrated anti-conformation, in accordance with the Karplus equation.[19] Based on these results, Ac-5 was characterized as the C2′-epimer (2*S*, 3*S*) of the side chain.

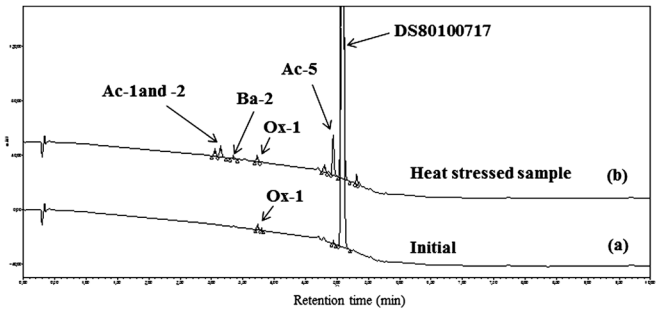


Fig. 4. Representative HPLC Chromatograms of DS80100717 Drug Products

(a) Initial, (b) 40°C for 4 Weeks.

Degradation Product under Basic and Oxidative Conditions

The ESI mass spectrum of Ba-1 demonstrated a protonated molecular ion at *m/z* 220, which is 637 Da less than that of DS80100717 $[M+H]^+$ at *m/z* 857. The accurate mass spectrum of Ba-1 showed a protonated molecule $[M+H]^+$ at *m/z* 220.0819 (Calcd for $C_8H_{14}O_6N$: 220.0821). Characteristic fragment ions were observed at *m/z* 176, 174, 130, and 100. These fragment ions were in accordance with the side chain of DS80100717 at the C13 position. Based on these results, Ba-1 was identified as the side chain which was cleaved at the C13 position of DS80100717.

The ESI mass spectrum of Ba-2 generated a protonated molecular ion at *m/z* 600, which is 257 Da less than that of DS80100717 $[M+H]^+$ at *m/z* 857. The accurate mass spectrum of Ba-2 showed a protonated molecule $[M+H]^+$ at *m/z* 600.3162 (Calcd for $C_{33}H_{46}O_9N$: 600.3173). Characteristic fragment ions were observed at *m/z* 313 and 295. These fragment ions indicated that Ba-2 retained the baccatin III analogous moiety. Online H/D exchange LC-MS analysis of Ba-2 indicated the presence of two labile hydrogen atoms, one mass less than the molecular mass of DS80100717. Based on these results, Ba-2 was identified as a baccatin III analogous moiety.

The ESI mass spectrum of Ox-1 demonstrated a protonated molecular ion at *m/z* 873, which is 16 Da more than that of DS80100717 $[M+H]^+$ at *m/z* 857. The accurate mass spectrum of Ox-1 showed a protonated molecule $[M+H]^+$ at *m/z*

Table 2. Results of LC-MS Analysis

| Target | Relative retention time (RRT) by LC-MS analysis | Mass data (*m/z*) | Proposed molecular formula ($[M+H]^+$) | Error (mDa)[a)] | Product ions by MS/MS analysis (*m/z*) | Number of labile hydrogen atoms |
|---|---|---|---|---|---|---|
| DS80100717 | 1.00 | 857.4425 | $C_{45}H_{65}O_{14}N_2$ | −1.08 | 839, 801, 783, 661, 313, 295 | 3 |
| Ac-1 | 0.62 | 801.3800 | $C_{41}H_{57}O_{14}N_2$ | −0.95 | 783, 582, 564, 522, 400, 313, 295 277 | 4 |
| Ac-2 | 0.64 | 801.3804 | $C_{41}H_{57}O_{14}N_2$ | −0.63 | 783, 741, 582, 522, 400, 313, 295, 277 | 4 |
| Ac-3 | 0.70 | 757.3903 | $C_{40}H_{57}O_{12}N_2$ | −0.87 | 739, 670, 582, 313, 295 | 4 |
| Ac-4 | 0.71 | 757.3898 | $C_{40}H_{57}O_{12}N_2$ | −1.35 | 739, 670, 582, 313, 295 | 4 |
| Ac-5 | 0.97 | 857.4422 | $C_{45}H_{65}O_{14}N_2$ | −1.34 | 839, 801, 783 | 3 |
| Ba-1 | 0.07 | 220.0819 | $C_8H_{14}O_6N$ | −0.24 | 202, 176, 174, 158, 156, 130, 100 | 6 |
| Ba-2 | 0.67 | 600.3162 | $C_{33}H_{46}O_9N$ | −1.03 | 582, 564, 540, 522, 460, 331, 313, 295 | 2 |
| Ox-1 | 0.77 | 873.4361 | $C_{45}H_{65}O_{15}N_2$ | −2.37 | 817, 773 | 3 |

*a*) Error (mDa) = (observed mass − calculated mass) × 1000

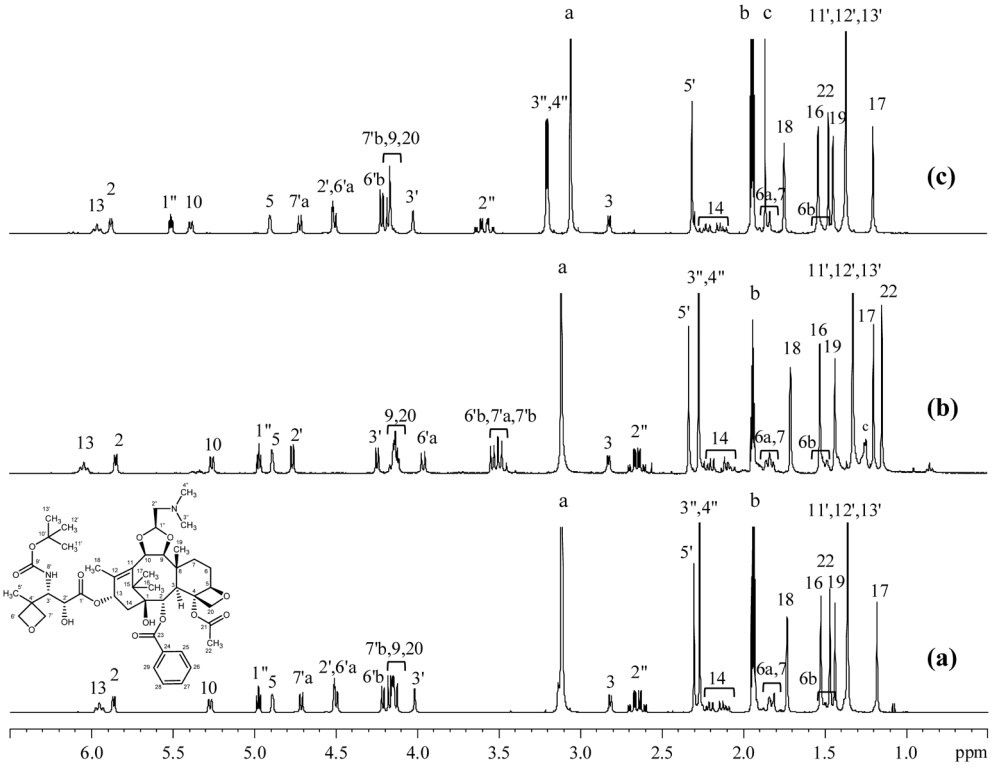


Fig. 5. Overlay of $^{1}$H-NMR Spectra of (a) DS80100717, (b) Ac-5, (c) Ox-1

[a] Residual solvent (water), [b] Residual solvent (acetonitrile), [c] Contaminants.

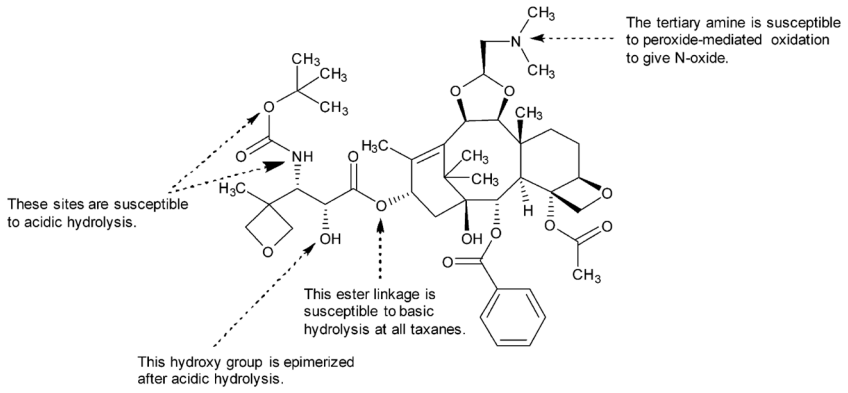


Fig. 6. Summary of the Reactive Sites of DS80100717

873.4361 (Calcd for $C_{45}H_{65}O_{15}N_2$: 873.4385). The characteristic fragment ion was *m*/*z* 817. This fragment ion was assigned as an oxygen atom adducted to DS80100717. Online H/D exchange LC-MS analysis of Ox-1 and DS80100717 indicated no difference in the number of labile hydrogen atoms in each compound. The $^{1}$H-NMR spectrum of the isolated Ox-1 sample is shown in Fig. 5. Compared to the $^{1}$H-NMR spectrum of DS80100717, the signals of Ox-1 at H1″, H2″, H3″, and H4″ in the baccatin III analogous moiety were significantly shifted to a low magnetic field. The same trend was observed and confirmed with the signals of the $^{13}$C-NMR spectrum at C1″, C2″, C3″, and C4″, which would be consistent with a change in the third amine position in the baccatin III analogous moiety (Table S5, Supplementary Materials). Based on these results, Ox-1 was characterized as an *N*-oxide form of DS80100717.

**Degradation Pathway of DS80100717** As the results of the stress testing of DS80100717, many degradation products formed under various conditions. Chemical structures of the degradants and estimated degradation pathways are shown in Fig. 3. Based on the elucidated chemical structures of the degradants, the reactive sites within the DS80100717 molecule demonstrating susceptibility to degradation were estimated, as shown in Fig. 6. DS80100717 was susceptible to hydrolysis (both acid and base) and peroxide-mediated oxidation in both solid-state and solution. The C13 side chain of taxane derivatives was commonly eliminated under basic conditions.[12–14] Although taxane derivatives reportedly oxidize at the C10[13] and C3–C11 bridge,[12,14] DS80100717 formed *N*-oxide at other positions as the major degradant. Furthermore, $\beta$-OH at C7 of paclitaxel and docetaxel was easily epimerized into $\alpha$-OH as the result of the retro-aldol reaction.[12,13] On the other hand, the C2′ side chain of DS80100717 was easily epimerized. The oxetane group on the side chain of DS80100717 was assumed to undergo epimerization at the C2′ position since it has less

steric hindrance than the benzyl group on the side chain of other taxanes. The C2′-epimer at the side chain of docetaxel was reported as a minor process impurity rather than as a degradation product.[20] *N*-Oxide and C2′-epimer were previously unreported as degradation products of taxane derivatives.

## Conclusion

The DS80100717 drug substance and drug product were subjected to stress studies, and the degradation products were evaluated. Eight degradation products were identified, isolated, and characterized by various spectroscopic techniques including MS and NMR. *N*-Oxide and C2′-epimer were observed even in the solid-state. These degradation products are likely to be formed in actual long-term stability studies. We were able to examine these degradation products with appropriate stress studies. These degradation products are important in pharmaceutical drug development and quality control of the drug substance and drug product.

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**Conflict of Interest** Kousuke Tamura, Makoto Ono, Takefumi Kawabe, and Motomu Ohara are currently employees of Daiichi Sankyo Co., Ltd., respectively.

**Supplementary Materials** The online version of this article contains supplementary materials.

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