Characterization of Hydrazine Derivative: Proposed Decomposition Mechanism and Structure Elucidation of Decomposition Compounds

Takayuki Nemoto,* Eliada Lazoura, and Takashi Nomoto

Process R & D, Laboratories for Technology Development, Banyu Pharmaceutical Co., Ltd.; 3–9–1 Kamimutsuna, Okazaki, Aichi 444–0858, Japan.

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Decomposition of protected hydrazine diol (1) hemi-oxalate, a key intermediate of the potent indolocarbazole-based DNA topoisomerase I inhibitor (2), was investigated. Spectroscopic analysis revealed that the main decomposition compounds of the hydrazine derivative were a peroxide (3) and an alcohol derivative (4). The peroxide derivative (3) was proposed to form in the presence of oxygen- and/or H_2O-generated radicals, which was subsequently reduced to the more stable alcohol derivative (4). A plausible decomposition mechanism was proposed and our findings were substantiated by chemical conversion.

Key words hydrazine; radical; decomposition; peroxide; DNA topoisomerase I

DNA topoisomerase I (topo I), presently represents an attractive target for the development of inhibitors for use as anti-bacterial, anti-fungal, and anti-cancer chemotherapeutic agents. Recently our group reported the practical synthesis of a potent indolocarbazole-based topo I inhibitor (**2**), produced by coupling the glucoside (**5**) and protected hydrazine diol (**1**) hemi-oxalate (Chart 1).[1] Often, however, unexpected obstacles are encountered in a scale-up synthesis. In this case, the decomposition of a key intermediate, protected hydrazine diol (**1**) hemi-oxalate, posed problems.

Hydrazine derivatives are known to easily decompose in the presence of radicals.[2,3] Commonly used as rocket fuels, most of them are endothermic compounds.[3] Upon storage, protected hydrazine diol (**1**) hemi-oxalate was found to decompose, even when kept in the dark at 5 °C and under nitrogen.

Here we reports the decomposition of protected hydrazine diol (**1**) hemi-oxalate, structure elucidation of the decomposition compounds and propose a plausible decomposition mechanism.

Experimental

Materials All compounds were prepared by Banyu Pharmaceutical Co. Ltd. (Okazaki, Japan). The purity of protected hydrazine diol (**1**) hemi-oxalate was above 99.9% at the time of storage, in the dark and under nitrogen at 5 °C for 6 months in a polyethylene bag stored in a steel drum. All chemicals and solvent were of analytical reagent grade.

Analytical HPLC The HPLC equipment was as follows : an octyl silica (C8) column (Waters Symmetry C8, 250×4.6 mm i.d.), Waters 2695 separation module coupled to a Waters 2487 dual λ absorbance detector and a Waters 2996 photodiode array detector. Gradient elution was performed from CH_3CN/0.1% H_3PO_4 (10/90) to CH_3CN/0.1% H_3PO_4 (50/50) over 40 min, then to CH_3CN/0.1% H_3PO_4 (90/10) over 20 min and maintained at these conditions for a further 5 min, flow rate 1.0 ml/min. Absorbance was detected at 220 nm.

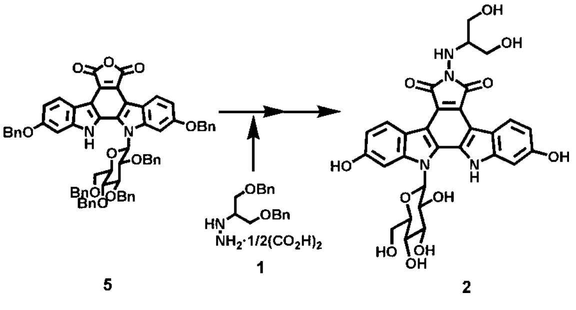

Chart 1. Synthesis of Indolocarbazole Compound (**2**)

Preparative HPLC The HPLC equipment was as follows : an octadecyl silica (ODS) column [Shim-pack PREP-ODS(H) KIT, 250×20 mm i.d.] and Shimadzu VP Series HPLC. Gradient elution was performed from CH_3CN/H_2O (10/90) to CH_3CN/H_2O (50/50) over 40 min, then to CH_3CN/H_2O (90/10) over 20 min and maintained at these conditions for a further 5 min, flow rate 10.0 ml/min. Absorbance was detected at 220 nm. Pure **3** (t_R=56.7 min), and **4** (t_R=54.0 min) were collected for further analysis. While **4** was freeze dried, **3** was extracted from H_2O using benzene to avoid decomposition.

LC/MS Spectrometry (MS) MS spectra were recorded on a JEOL JMS-LC mate and/or a Finnigan Thermoquest Model LC-Q mass spectrometer coupled to an Agilent 1100 Series HPLC. An ODS column (YMC AM-303, 250×4.6 mm i.d.) was used, with gradient condition from CH_3CN/0.04% HCOOH (10/90) to CH_3CN/0.04% HCOOH (50/50) over 30 min and subsequently to CH_3CN/0.04% HCOOH (90/10) for a further 30 min, flow rate 1.0 ml/min (0.2 ml/min split to LC-MS). Absorbance was detected at 220 nm. Mass spectra were recorded in positive electrospray ionization (ESI+) mode.

NMR Spectrometry (NMR) NMR spectra were recorded on a Bruker DRX500 (500 MHz). NMR chemical shifts are reported as δ values in ppm relative to TMS. For LC-NMR of the peroxide derivative **3**, an Agilent 1100 Series HPLC was coupled to the NMR spectrometer. An ODS column (YMC AM-303, 250×4.6 mm i.d.) was used, with gradient condition from CD_3CN/D_2O (10/90) to CD_3CN/D_2O (50/50) in 5 min, then to CD_3CN/D_2O (90/10) for 30 min, and maintained at these conditions for a further 5 min, flow rate 1.0 ml/min. Absorbance was detected at 220 nm. Pure **3** (t_R=17.7 min) was collected for further online analysis.

Results and Discussion

Structure Elucidation of Decomposition Compounds The HPLC profile of decomposed protected hydrazine diol (**1**) hemi-oxalate is shown in Fig. 1. Initial purity was over 99.9%, however, after storage at 5 °C for 6 months under nitrogen in a polyethylene bag and inside a steel drum, the purity decreased to 95.9 % with impurity levels ranging between 0.01—0.96% (Fig. 2). The following decomposition compounds were detected (Fig. 3): a secondary amine (**6**) [*m/z* 272 $(M+H)^+$; 0.11%], formed by homolytical cleavage of the N–N bond by $HO^{\bullet}$;[2,3] 1-benzyloxy-3-benzoyloxy-2-hydrazinopropane (**7**) [*m/z* 301 $(M+H)^+$; 0.06%], formed by oxidation of one of the benzyl oxy groups; and the dimer compound (**8**) [*m/z* 539 $(M+H)^+$; 0.27%], formed by coupling **1** to 1,3-dibenzyloxyketone (**9**), formed by oxidation of

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* To whom correspondence should be addressed. e-mail: nemototy@banyu.co.jp

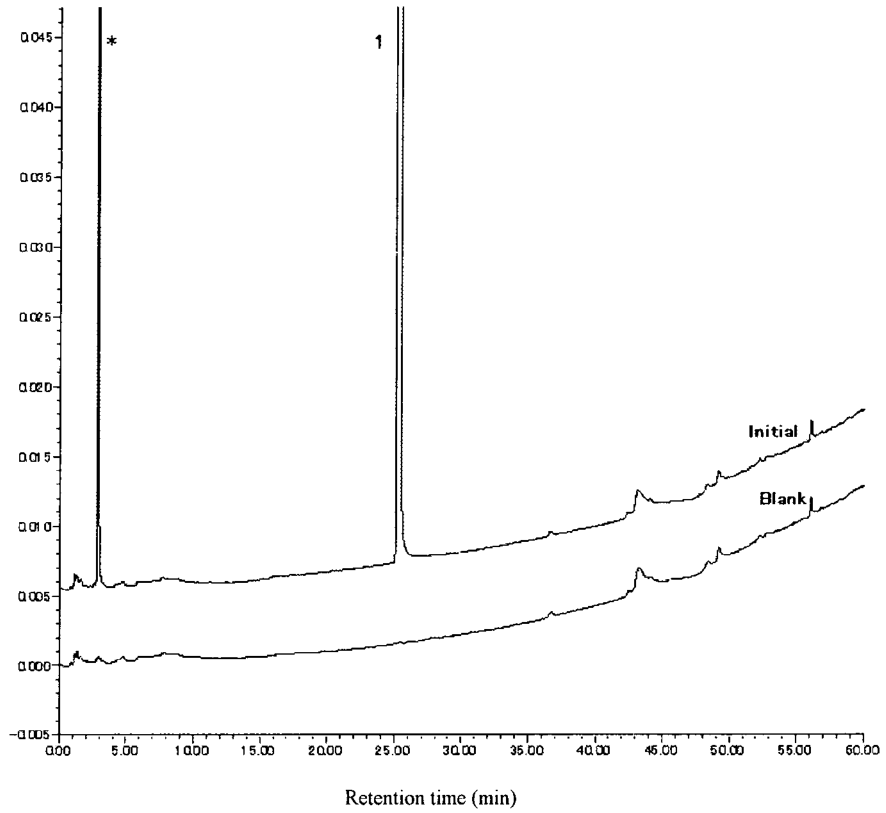

Fig. 1. HPLC Chromatographic Profiles from Hydrazine Derivative (**1**) (Initial)
*Oxalic acid peak.

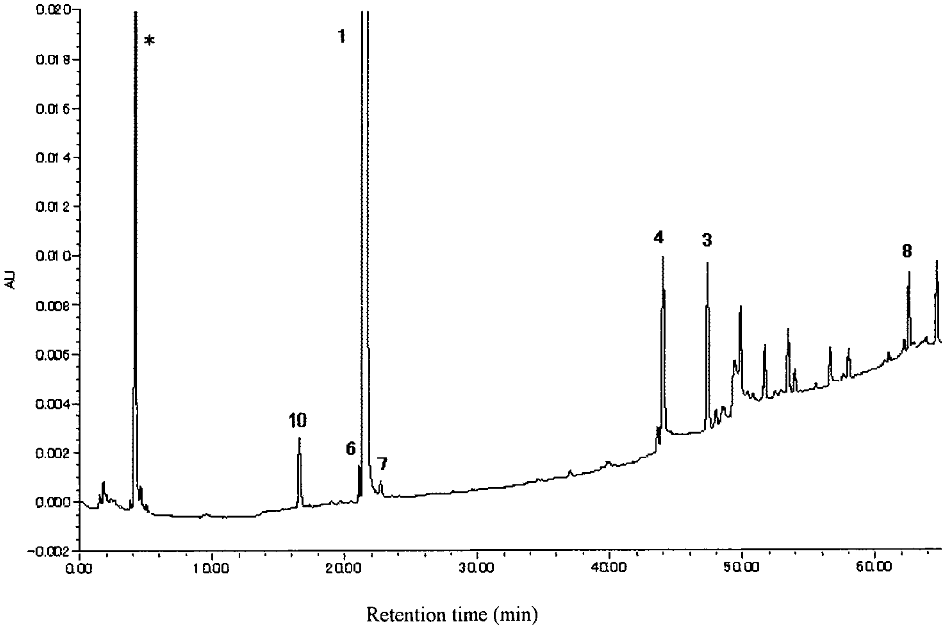

Fig. 2. HPLC Chromatographic Profiles from Hydrazine Derivative (**1**) (Stored at 5 °C for 6 Months under Nitrogen in a Polyethylene Bag)
*Oxalic acid peak.

hydrazine to hydrazone, and subsequent hydrolysis to ketone. To confirm this dimer formation, **1** was mixed with **9** in an $CH_3CN–H_2O$ (1 : 1) solution, since **9** was only detectable under accelerated decomposition conditions (Fig. 5). Furthermore, the benzyl alcohol (**10**) (0.33%), which could not be observed by LC-MS was determined by co-injecting a standard sample.

The main decomposition compounds **3** (0.71%) and **4** (0.96%) could not be elucidated by LC/MS since *m*/*z* peak intensities were too low and complex following chromatographic separation of the crude decomposed hydrazine mixture. Following purification by preparative HPLC, approximately 1 mg of sample was obtained, which was used for NMR studies (Fig. 4). ^{1}H-NMR (C_6D_6) of **4** indicated two methylene groups attached to oxygen [δ 3.43 (d, J=5.4 Hz, 4H)], one oxymethine proton [δ 4.00 (tt, J=5.4, 5.4 Hz, 1H)], and two benzyloxy groups [δ 4.26 (s, 4H), 7.0—7.2 (m, 10H)]. Compound **4** was determined to be the secondary alcohol [m/z 273 $(M+H)^+$], however, **3** decomposed after freeze drying. Therefore, LC-NMR was employed to elucidate the structure of **3** [m/z 289 $(M+H)^+$]. ^{1}H-NMR (CD_3CN–D_2O) of **3**, which indicated two methylene groups attached to oxygen [δ 3.55 (dd, J=11.3, 5.1 Hz, 2H), 3.58 (dd, J=11.3, 4.6 Hz, 2H)], one oxymethine proton [δ 4.12 (tt, 1H, J=5.1, 4.6 Hz)], and two benzyloxy groups [δ 4.46 (s, 4H), 7.1—7.3 (m, 10H)], which were similar to those of alcohol (**4**). Compound **3** was determined to be the peroxide derivartive.[4] Furthermore, the peroxide was extracted with deuterated benzene. This sample was subsequently used for ^{1}H-NMR experiments [δ 3.61 (dd, J=10.7, 4.7 Hz, 2H), 3.64 (dd, J=10.7, 5.6 Hz, 2H), 4.40 (tt, J=5.6, 4.7 Hz, 1H), 4.33 (s, 4H), 7.0—7.2 (m, 10H)].

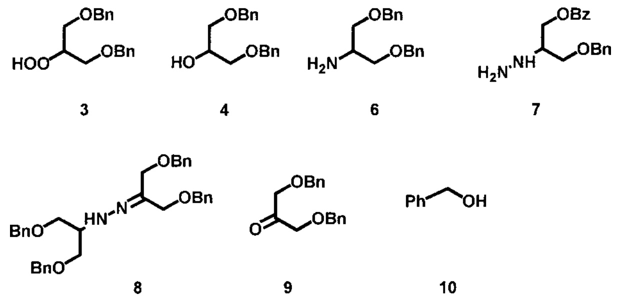

Fig. 3. Structures of Decomposition Compounds

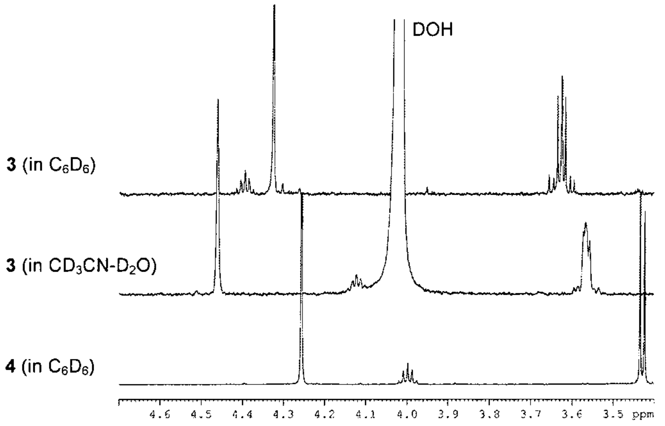

Fig. 4. 500 MHz ^{1}H-NMR Spectra of **3** and **4** in C_6D_6

The LC-NMR ^{1}H-NMR spectrum of **3** in CD_3CN–D_2O is also shown.

Factors Promoting Decomposition The effect of oxygen on the decomposition of the protected hydrazine diol (**1**) hemi-oxalate, which was kept in the dark, in open air and heated at 60 °C for 1 week was examined. As shown in Fig. 5, accelerated decomposition of **1** was observed under these conditions, with increased levels of each decomposition compound (0.01—0.96 % *vs.* 0.01—3.56%). To confirm our findings, **1** was stirred vigorously for several days in $MeOH/H_2O$ (1/1) in open air at 25 °C. As expected, accelerated decomposition of **1** was observed (data not shown).

Chemical Conversion Chemical conversion of the hydrazine derivative (**1**) to the peroxide (**3**) and the peroxide (**3**) to the secondary alcohol (**4**) was performed (Chart 2). The conversion of **1** to **3** (76%) was accomplished by reacting **1** with 30% H_2O_2 in $MeOH/H_2O$ (1/1) for 14 h at 25 °C. Unre-

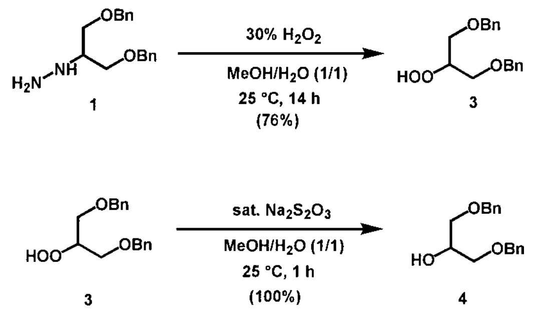

Chart 2. Chemical Conversion of Hydrazine (**1**) and Peroxide (**3**)

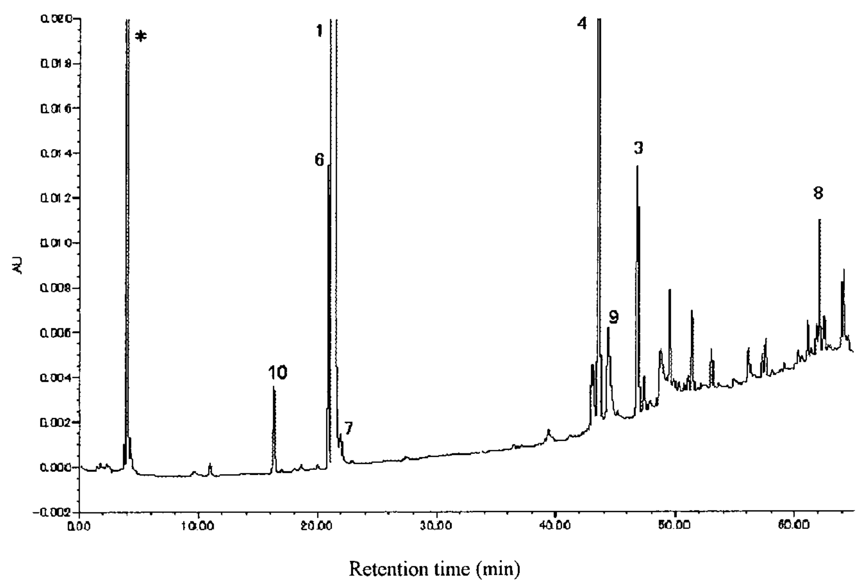

Fig. 5. HPLC Chromatographic Profiles from Hydrazine Derivative (**1**) (Stored at 60 °C for 1 Week under Open Air)

*Oxalic acid peak.

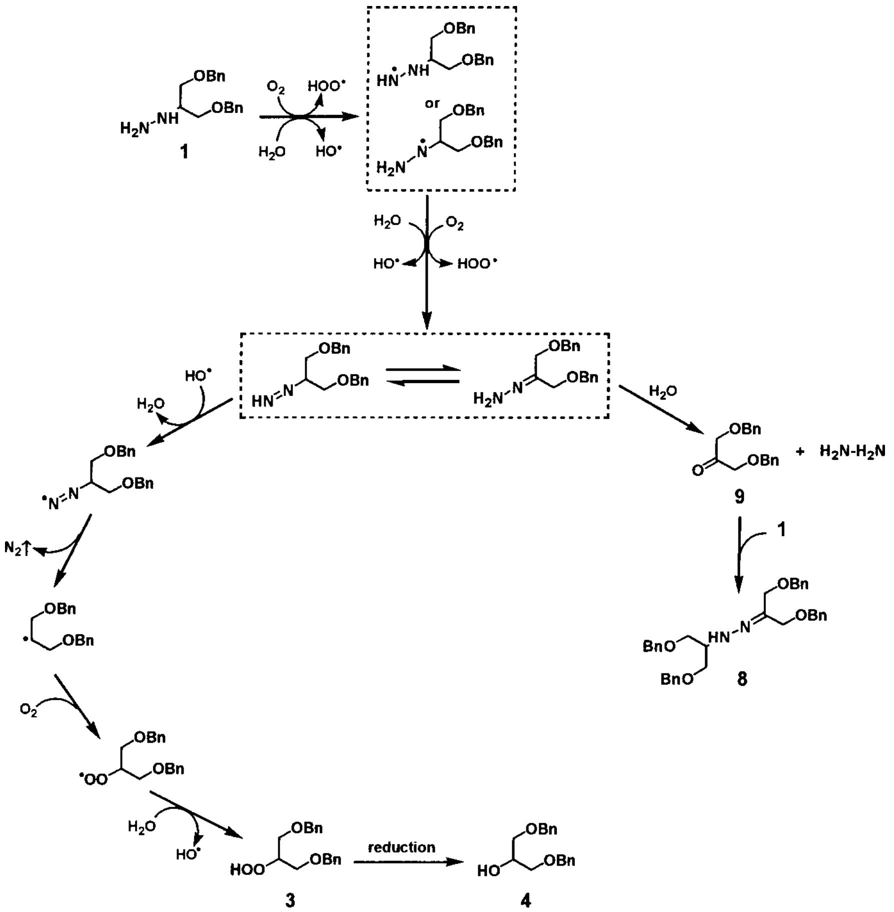

Fig. 6. Proposed Decomposition Mechanism of the Hydrazine Derivative (**1**)

acted hydrazine derivative (**1**) (10%) was recovered and a byproduct (10%), whose structure was not elucidated, were present. The conversion of **3** to **4** (100%) was accomplished by reacting **3** with saturated $Na_2S_2O_3$ in MeOH/H_2O (1/1) for 1 h at 25 °C. The reactions were monitored by LC-MS and/or ^{1}H-NMR.

Radical Formation and Stability of Hydrazines Light, heat and trace amounts of iron, copper, aluminum, platinum and other transition metals reduce molecular oxygen to superoxide ($O_2^{-\bullet}$) and promote hydrogen peroxide radical ($HOO^{\bullet}$) formation and water homolysis, to yield hydroxy radicals ($HO^{\bullet}$).[7] The reaction of hydrazines, which are known to be reactive compounds, with hydroxyl radical ($HO^{\bullet}$), hydrogen peroxide (H_2O_2), O_3 and O_2 gas[2—6] results in the formation of hydrazones, diazenes, NH_3, N_2, alkyl radicals, and/or amines.[2,3,5] In this study, the presence of a small amount of molecular oxygen and/or H_2O initiated a chain reaction with the formation of radical species, although it is not clear which radical species were generated. However, we can assume that the hydrazine derivative (**1**) was decomposed to the peroxide derivative (**3**) by superoxide ($O_2^{-\bullet}$), and/or hydrogen peroxide ($HOO^{\bullet}$) and/or hydroxyl radicals ($HO^{\bullet}$), which also catalyze homolytic cleavage of the N–N bond to give the secondary amine (**6**).[2]

Decomposition Mechanism The proposed decomposition mechanism of **1**, to give the peroxide (**3**) and secondary alcohol (**4**) derivatives as the main decomposition compounds, and a ketone derivative (**9**), hydrazine, and a dimer compound (**8**) as the minor decomposition compounds, is shown in Fig. 6. The removal of all traces of oxygen and/or H_2O from powdered materials is very difficult. Therefore, remaining amounts of oxygen and/or H_2O can be reduced to form numerous radicals [*e.g.* hydrogen peroxide radicals ($HOO^{\bullet}$) and/or hydroxy radicals ($HO^{\bullet}$)] and initiate a decomposition cascade, *i.e.* conversion of **1** to **3** and subsequently to the stable secondary alcohol (**4**), and **1** to **9** and subsequently to the coupling compound (**8**).

Conclusions

Hydrazine, which is known to be an unstable compound was found to decompose when stored at 5 °C for 6 months under nitrogen in a polyethylene bag and inside a steel drum. Our studies revealed that the hydrazine derivative (**1**) easily oxidized in the presence of oxygen and/or H_2O generated radicals to give the peroxide (**3**) and secondary alcohol (**4**) derivatives. A degradation pathway was proposed, which was supported by chemical conversion experiments. As a result of these studies, the protected hydrazine diol (**1**) hemi-oxalate is presently stored at temperatures below −20 °C but not under N_2 gas. To date, after 9 months storage, no decomposition has been observed.

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