[Chem. Pharm. Bull. 36(11)4593—4596(1988)]

Degradation of β-Lactamase Inhibitor (2*S*,3*R*,5*S*)-3-Methyl-7-oxo-3-(1*H*-1,2,3-triazol-1-yl-methyl)-4-thia-1-azabicyclo[3.2.0]-heptane-2-carboxylic Acid 4,4-Dioxide (YTR-830H)[1] in the Solid State: Structural Elucidation

EIJI MATSUSHIMA, KEN-ICHIRO YOSHIDA, RYOTARO AZUMA, YOSHINORI MINAMI and TERUYOSHI MARUNAKA*

Biological Research Laboratory, Taiho Pharmaceutical Co., Ltd., Hiraishi, Kawauchi-cho, Tokushima 771–01, Japan

(Received April 30, 1988)

(2*S*,3*R*,5*S*)-3-Methyl-7-oxo-3-(1*H*-1,2,3-triazol-1-yl-methyl)-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylic acid 4,4-dioxide (YTR-830H) is a new β-lactamase inhibitor. It was found that the thermal degradation of powdered or lyophilized samples of YTR-830H produced (*Z*)-3-methyl-4-(1*H*-1,2,3-triazol-1-yl)-2-butenoic acid (YTR-830H-IV), 2-amino-3-methyl-3-sulfino-4-(1*H*-1,2,3-triazol-1-yl)-butyric acid (YTR-830H-II) and its related degradation products, and formylacetic acid (YTR-830H-III).

Keywords——(2*S*,3*R*,5*S*)-3-methyl-7-oxo-3-(1*H*-1,2,3-triazol-1-yl-methyl)-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylic acid 4,4-dioxide (YTR-830H); β-lactamase inhibitor; solid-state degradation product; structural elucidation; (*Z*)-3-methyl-4-(1*H*-1,2,3-triazol-1-yl)-2-butenoic acid (YTR-830H-IV)

Introduction

(2*S*,3*R*,5*S*)-3-Methyl-7-oxo-3-(1*H*-1,2,3-triazol-1-yl-methyl)-4-thia-1-azabicyclo[3.2.0]-heptane-2-carboxylic acid 4,4-dioxide, YTR-830H, is a new β-lactamase inhibitor, developed through the co-operation of R. G. Micetich and Taiho Pharm. Co. (Tokushima, Japan).[2]

Combination therapy, utilizing this β-lactamase inhibitor with piperacillin, has been shown to extend the *in vitro* spectrum of β-lactam antibiotics to a number of resistant organisms. It has also been demonstrated to have relatively little intrinsic biological activity.[3]

We have previously reported the stability of YTR-830H in buffer solutions, distilled water, aqueous NaOH solution and NaOH-saturated methanol solution.[1] It was found that YTR-830H is degraded to 2-amino-3-methyl-3-sulfino-4-(1*H*-1,2,3-triazol-1-yl)-butyric acid (YTR-830H-II) and formylacetic acid (YTR-830H-III) through (*E*)-5-methyl-5-sulfino-6-(1*H*-1,2,3-triazol-1-yl)-3-aza-1-heptene-1,4-dicarboxylic acid (YTR-830H-I) as an intermediate, with the YTR-830H-II then proceeding to 1,2,3-triazole and several unidentified products (YTR-830H-IIa, -IIb, -IIc and -IId). The present paper describes the thermal degradation products of YTR-830H.

Experimental

Materials and Reagents——β-Lactamase inhibitor YTR-830H was supplied by our Synthetic Laboratory. Acetonitrile was of liquid chromatographic reagent grade. The CD_3OD used for the measurement of ^{1}H-nuclear magnetic resonance (^{1}H-NMR) spectra was obtained from Merck (Darmstadt, G.F.R). The ion-pair chromatographic reagent, PIC-A® (Low UV), containing tetra-*n*-butyl ammonium salt, was obtained from Waters Assoc. (Milford, MA, U.S.A.). Other chemicals used were all purchased from Wako (Osaka, Japan).

CHP-20P resin (150—300 μm particle size) was obtained from Mitsubishi Chemical Industry (Tokyo, Japan). The PIC-A aqueous solution, used as a mobile phase, was prepared to 5 mM concentration by adding the contents of one vial (*ca.* 20 ml) of commercial PIC reagent to 1.01 of distilled water. The 10 mM sodium dihydrogenphosphate used as a mobile phase was adjusted to pH 3.0 by the addition of phosphoric acid.

Instruments——The infrared (IR) spectra were measured on a Hitachi 260-50 IR spectrophotometer (Tokyo, Japan) by the KBr tablet method. The ultraviolet (UV) spectra were recorded with a Shimadzu UV-265FW spectrophotometer (Kyoto, Japan). ^{1}H-NMR spectra were measured using a JEOL FX-100 NMR spectrometer coupled with an FAFT 70 data system and equipped with a Fourier transformer (Tokyo, Japan), using tetramethylsilane as an internal standard. A JEOL DX-300 mass spectrometer linked to a JMA-DA5100 data system was used for the measurement of electron impact and fast atom bombardment mass spectra (EI- and FAB-MS); the conditions were described in the previous report.[1] A Shimadzu LC-6 high-performance liquid chromatographic system[1] was used for the separation of degradation products.

High-Performance Liquid Chromatography (HPLC)——The separation of the thermal degradation products of YTR-830H formed in the solid state was carried out under the following conditions.

i) HPLC Condition 1: Same as HPLC condition 1 described in the previous report.[1]

ii) HPLC Condition 2: Column, Develosil ODS-5, 150 × 4.6 mm i.d.; column temperature, room termperature; mobile phase, acetonitrile: 10 mM NaH_2PO_4 (pH 3.0) = 5 : 95 (v/v); flow rate, 1.5 ml/min; and detector, UV 220 nm (0.08 a.u.f.s.).

Degradation of Powdered and Lyophilized Samples——The powdered (1 g) and lyophilized (25 mg) samples of YTR-830H in an airtight vial (55 × 25 mm i.d.) containing dried air was stored at 60 °C for six months. Then the preparations were subjected to analysis under the HPLC conditions 1 and 2, and the previously described post-column alkalization[1] to detect the degradation products.

Isolation of YTR-830H-IV——YTR-830H (1 g) which had been stored at 60 °C for six months was dissolved in 1 ml of distilled water. This solution was subjected to CHP-20P column chromatography (150—300 μm particle size, 200 × 20 mm i.d., packed using 0.1% acetic acid solution); the column was washed with 100 ml of 0.1% acetic acid solution and subsequently with 150 ml of 0.1% acetic acid solution containing 30% methanol, and then eluted with 0.1% acetic acid solution containing 50% methanol. The eluate was evaporated to remove methanol and lyophilized to yield 6 mg of YTR-830H-IV as a white powder. FAB-MS *m/z*: 168 $(M+1)^+$. EI-MS *m/z*: 168 $(M+1)^+$, 139 $(M-N_2)$, 122 (M−COOH), 121 $(139-H_2O)$, 94 $(122-N_2)$, 93 (121−CO), 82 $(121-C_2HN)$, 69 $(C_2H_3N_3)$, 53 (C_4H_5). IR (KBr): 1700 ($\nu_{C=O}$), 1660 ($\nu_{C=C}$) cm^{-1}. UV λ_{max}: 208 nm (H_2O), 219 nm (0.25 N NaOH). ^{1}H-NMR (CD_3OD) δ: 1.74 (d, J = 1.5 Hz, 3-CH_3), 5.69 (s, 3-CH_2), 5.96 (q, J = 1.5 Hz, 2-CH=), 7.56 (d, J = 1 Hz, triazole 4-CH=), 8.01 (d, J = 1 Hz, triazole 5-CH=).

Results and Discussion

Degradation of Powdered and Lyophilized Samples

Figure 1 shows the chromatograms obtained by HPLC under conditions 1 and 2 of the powdered and lyophilized samples of YTR-830H after storage at 60 °C for six months. The degradation products YTR-830H-II, YTR-830H-IIa, YTR-830H-III and 1,2,3-triazole were found. As described previously,[1] YTR-830H-III could be detected by post-column alkalization. Furthermore, YTR-830H-IV, a new degradation product, was also detected. YTR-830H-IV was not detected following the degradation in various buffers, distilled water,

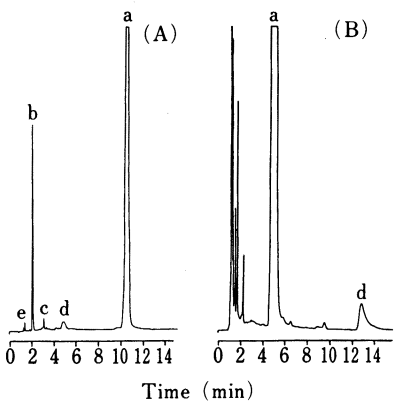

Fig. 1. Chromatograms under HPLC Conditions (A) 1 and (B) 2 of YTR-830H after Storage in the Solid State at 60 °C for 6 Months

a, YTR-830H; b, YTR-830H-II; c, YTR-830H-IIa; d, 1,2,3-triazole.

aqueous alkaline or methanolic alkaline solution. The retention time on a chromatogram of YTR-830H-IV was shorter under HPLC condition 1 and longer under HPLC condition 2 than the times for intact YTR-830H. YTR-830H-IV was isolated and purified by CHP-20P column chromatography.

Chemical Structure of YTR-830H-IV

YTR-830H-IV gave a quasi-molecular ion peak of m/z 168 $(M+1)^+$ in both the FAB- and the EI-MS. The molecular formula $C_7H_{10}N_3O_2$ (Found, 168.0757; Calcd, 168.0772) was obtained from high-resolution EI mass spectrometry. The IR spectrum of YTR-830H-IV showed the characteristic absorptions of a carboxyl group ($\nu_{C=O}$) at 1700 cm^{-1} and an alkene group at 1660 cm^{-1} but not that of β-lactam ($\nu_{C=O}$). In the ^{1}H-NMR spectrum of YTR-830H-IV measured in CD_3OD, the doublet signal of CH_3 at δ 1.74, the doublet signals of =CH at the C-4′ and C-5′ positions of the triazole moiety at δ 7.56 and δ 8.01 (each $J=1$ Hz), the singlet signal of CH_2 at δ 5.69 and the quartet signal of =CH at δ 5.96, showing a long range coupling ($J=1.5$ Hz), were observed. Furthermore, a nuclear Overhauser effect (NOE) of approximately 6% between the protons of CH_3 at δ 1.74 and =CH at δ 5.96 was observed.

YTR-830H-IV could be methylated by the addition of diazomethane-saturated ethyl ether

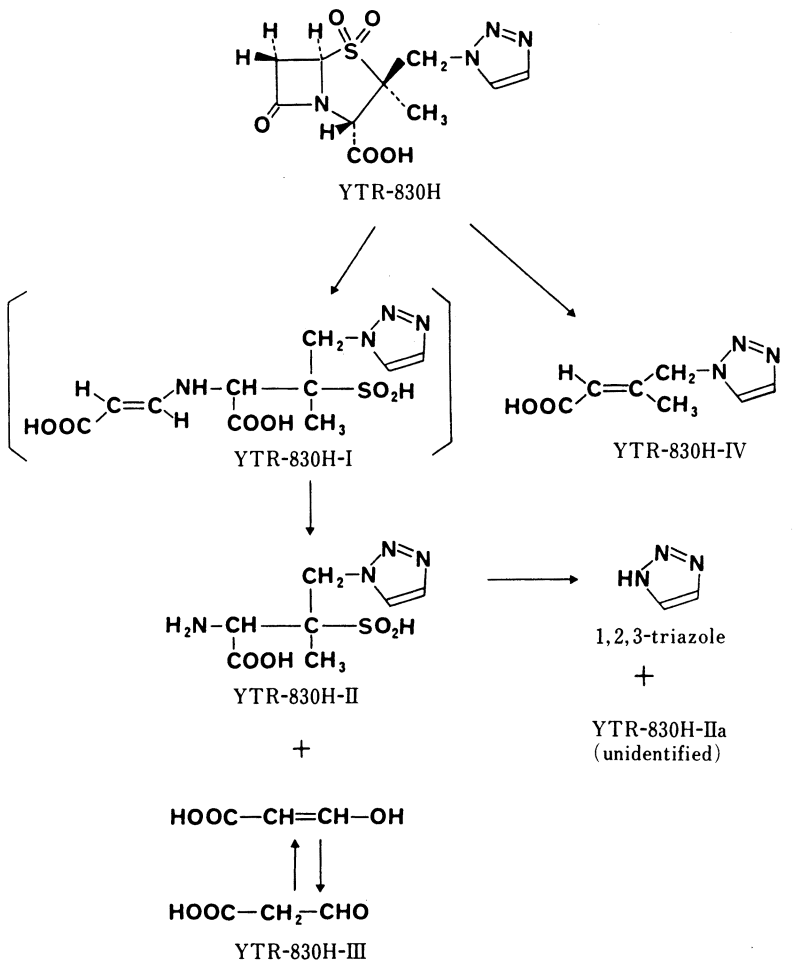

Chart 1. Chemical Structures of the Thermal Degradation Products of YTR-830H in the Solid State

solution: this methyl ester showed a molecular ion peak of m/z 181 $(M)^+$ in its EI-MS and approximately a 9% NOE (between CH_3 and $=CH$) in its ^1H-NMR spectrum.

On the basis of the above results, YTR-830H-IV was identified as (*Z*)-3-methyl-4-(1*H*-1,2,3-triazol-1-yl)-2-butenoic acid.

Presumed Degradation Pathways of YTR-830H

As shown in Chart 1, it can be postulated that there is another degradation pathway followed by YTR-830H undergoing thermal degradation in the solid state, compared with the degradation schemes determined for various buffer solutions, distilled water, aqueous NaOH solution and NaOH-saturated methanol.[1)]

References

1) T. Marunaka, E. Matsushima, Y. Minami, K. Yoshida and R. Azuma, *Chem. Pharm. Bull.*, **36**, 4478 (1988).
2) R. G. Micetich, S. N. Maiti, P. Spevak, T. W. Hall, S. Yamabe, N. Ishida, M. Tanaka, T. Yamazaki, A. Nakai and K. Ogawa, *J. Med. Chem.*, **30**, 1469 (1987).
3) R. G. Micetich, T. W. Hall, S. N. Maiti, P. Spevak, S. Yamabe, N. Ishida, K. Ogawa, M. Tanaka, T. Yamasaki and A. Nakai, Proceedings of the 14th International Congress of Chemotherapy, Kyoto, Japan, 1985, p. 249; N. Ishida, A. Hyodo, C. Hanehara, Y. Miyake, Y. Kawaguchi and S. Yamabe, Proceedings of the 14th International Congress of Chemotherapy, Kyoto, Japan, 1985, p. 1274; M. R. Jacobs, S. C. Aronoff, S. Johenning, D. M. Shlaes and S. Yamabe, *Antimicrob. Agents Chemother.*, **29**, 980 (1986); M. R. Jacobs, S. C. Aronoff, S. Johenning and S. Yamabe, *J. Antimicrob. Chemother.*, **18**, 177 (1986); P. C. Appelbaum, M. R. Jacobs, S. K. Spangler and S. Yamabe, *Antimicrob. Agents Chemother.*, **30**, 789 (1986); F. Moosdeen, J. Keeble and J. D. Williams, *Reviews of Infectious Diseases*, **8**, supp. 5, S562 (1986).