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Reaction Products of Sulbactam with Pyrazole, 1,2,4-Triazole and Imidazole: High-Performance Liquid Chromatographic Behavior and Structural Investigation

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The reactions of sulbactam (I) with pyrazole, 1,2,4-triazole and imidazole in weakly alkaline solutions yielded 1-(5-carboxy-6-methyl-6-sulfino-4-aza-2-heptenoyl)-pyrazole (III), -1,2,4-triazole (IV), and -imidazole (V), respectively. The proton nuclear magnetic resonance spectra and high-performance liquid chromatographic (HPLC) behavior indicate that III, IV and V each exist as a mixture of *E*- and *Z*-isomers in a ratio of about 2 : 1 in aqueous solutions at low temperature, and that rapid interconversion between the *E*- and *Z*-isomers occurs at high temperature. Compounds III, IV and V were converted to methyl 5-carboxy-6-methyl-6-sulfino-4-aza-2-heptenoate (II) in methanol.

Keywords——sulbactam; sulbactam degradation; sulbactam reaction product NMR; sulbactam reaction product HPLC; sulbactam reaction product interconversion

Introduction

Sodium sulbactam, sodium (2*S*,5*R*)-3,3-dimethyl-7-oxo-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylic acid 4,4-dioxide (I), is a potent semi-synthetic β-lactamase inhibitor.[1)]

In a previous paper,[2)] we reported a spectrophotometric assay method for sulbactam, based on the UV (ultraviolet) absorbance at 326 nm of the reaction product with 1,2,4-triazole in solution (pH 10.0) at 60 °C. In the subsequent paper,[3)] we reported a high-performance liquid chromatographic (HPLC) method for the determination of sulbactam in urine and plasma using the reaction with 1,2,4-triazole. The present paper deals with the HPLC behavior and a structural investigation of the reaction products of sulbactam with 1,2,4-triazole, pyrazole and imidazole.

Experimental

Reagents and Materials——Sodium sulbactam was kindly donated by Pfizer Taito Co., Ltd. (Tokyo, Japan). Tetra-*n*-butylammonium bromide (TBAB), buffer salts, and other chemicals of analytical reagent grade were purchased from Nakarai Chemicals Co. (Kyoto, Japan). De-ionized, glass-distilled water and distilled methanol were used for the preparation of the eluents for HPLC.

Isolation of Reaction Product of Sulbactam with Pyrazole——Sodium sulbactam (50 mg) was dissolved in 50 ml of 2 M pyrazole solution (pH 9.0) and reacted at 60 °C for 3 h. The pH of the reaction solution was adjusted to 7.0 by addition of 0.1 M HCl, then the solvent was removed by evaporation, and the residue was dried under reduced pressure. After removal of excess pyrazole by extraction with ethylether, the residue was dissolved in 2 ml of distilled water, and subjected to preparative column chromatography (LiChroprep RP-8, 310 × 25 mm i.d., E. Merck, Darmstadt, West Germany). The column was eluted with water, and the fraction of the eluate between 70 and 90 ml was collected. Removal of the solvent by evaporation gave an yellowish-white solid (product A). Mass spectra of

product A were not obtained.

Measurements——UV Spectra: The UV spectra of HPLC peaks were obtained on a model 228 spectrophotometer (Hitachi Co., Tokyo, Japan) with a flow-through cell by the stopped-flow technique.

HPLC: All separations were performed using a liquid chromatograph (TRIROTAR-V, Japan Spectroscopic Co., Tokyo) equipped with a variable-wavelength detector (UVIDEC-100-V, Japan Spectroscopic Co.) or a model 228 spectrophotometer (Hitachi Co.). The stationary phase used was Develosil ODS-5 (5 μm particle size, Nomura Chemicals, Seto, Japan) packed in a stainless steel column (150 mm × 4.6 mm i.d.). The eluents (flow rate, 0.8 ml/min) were as follows: 5 mM TBAB plus 1 mM Na_2HPO_4 and 1 mM NaH_2PO_4 : methanol (1.6 : 1, v/v) for the reaction product of sulbactam with pyrazole, 5 mM TBAB plus 1 mM Na_2HPO_4 and 1 mM NaH_2PO_4 : methanol (1.8 : 1, v/v) for those with 1,2,4-triazole and imidazole, and 5 mM TBAB plus 1 mM Na_2HPO_4 and 1 mM NaH_2PO_4 : methanol (1.7 : 1, v/v) for degradation of the reaction product of sulbactam with pyrazole in methanol. The detection was performed at 325 or 300 nm. The column was maintained at the desired temperature (5—50 °C) using a thermostated water-bath (RTE-8, Neslab Instruments, Inc., Portsmouth, U.S.A.).

Proton Nuclear Magnetic Resonance (^{1}H-NMR) Spectra: ^{1}H-NMR spectra were measured on a JNM-FX 200 spectrometer (JEOL, Tokyo). The sample was dissolved in D_2O. The chemical shifts are given in parts per million (ppm) from internal sodium 3-(trimethylsilyl)propionate-d_4.

HPLC Separations of Reaction Products of Sulbactam with Pyrazole, 1,2,4-Triazole and Imidazole——A 1-ml aliquot of sodium sulbactam solution (100 μg/ml) was mixed with 1 ml of 2 M pyrazole, 1,2,4-triazole or imidazole solution (pH 9.0), and the solution was kept at 60 °C for 30 min for the reactions with 1,2,4-triazole and imidazole, and for 60 min for that with pyrazole. The reaction solution was cooled to ambient temperature (20 °C), and a 10-μl portion of the solution was injected into the HPLC under the conditions described above (Figs. 1, 3 and 4).

The isolated product A was dissolved in methanol, and the solution was allowed to stand in a thermostated water-bath at 60 °C. Aliquots were withdrawn at predetermined time intervals, and immediately injected into the HPLC column (Fig. 2).

Results and Discussion

The reactions of sulbactam with pyrazole, 1,2,4-triazole and imidazole yield products having absorption maxima (λ_{max}) at 325, 326 and 320 nm, respectively,[2] and the reaction product with 1,2,4-triazole is eluted from a reversed-phase HPLC column as a broad peak with a shoulder at ambient column temperature, and as a sharp single peak at elevated temperature (50 °C).[3] Such a temperature-dependent change in the peak shape suggests that the multiple species could be involved in the reaction products. It was difficult, however, to isolate the reaction products because of their hydrophilicity and instability in methanolic solution. In subsequent trials to isolate the products formed by the reactions with pyrazole and imidazole, it was found that reaction of sulbactam with pyrazole yielded relatively stable products that could be isolated as a yellowish-white solid (product A, see Experimental). The product A thus isolated was subjected to ^{1}H-NMR and HPLC analyses. The observed ^{1}H

TABLE I. ^{1}H Chemical Shifts and Spin–Spin Coupling Constants of Reaction Product of Sulbactam with Pyrazole

Assignment	Chemical shift[a]	
	Z-Isomer	*E*-Isomer
2C-H	5.66 (d)[b]	5.80 (d)
3C-H	7.28 (d)	7.99 (d)
5C-H	4.00 (s)	4.02 (s)
6C-CH_3	1.09 (s), 1.18 (s)	1.06 (s), 1.15 (s)
Pyrazole	6.55—6.58 (m)	6.55—6.58 (m)
	7.81—7.83 (m)	7.81—7.83 (m)
	8.30—8.32 (m)	8.30—8.32 (m)
Coupling constant[c]		
$J_{2C\text{-}H,3C\text{-}H}$	7.8	13.0

a) In ppm. *b*) The abbreviations used are as follows: s, singlet; d, doublet; m, multiplet. *c*) In Hz.

chemical shifts, spin–spin coupling constants and assignments of product A are listed in Table I. In a previous paper,[4] we reported that sodium sulbactam was degraded in alkaline methanolic solution to yield methyl 5-carboxy-6-methyl-6-sulfino-4-aza-2-heptenoate (II), and II existed as a 3 : 1 mixture of *E*- and *Z*-isomers in methanol. These results suggest that product A may be 1-(5-carboxy-6-methyl-6-sulfino-4-aza-2-heptenoyl)pyrazole (III). Further, from the signal intensities and coupling constants in the ^{1}H-NMR spectra, III may also exist as a 2 : 1 mixture of *E*- and *Z*-isomers. These conclusions were confirmed by the HPLC behavior described below.

Figure 1 shows the chromatograms of the reaction product of sulbactam with pyrazole (product A) obtained at various column temperatures (5—50 °C). Two well-separated peaks with an area ratio of 2 : 1 were observed on chromatograms at column temperatures of 5 and 20 °C. When the effluents containing the peak 1 and 2 compounds were fractionated and rechromatographed immediately under the same HPLC conditions, peaks 1 and 2 were again observed in the chromatogram of each fraction. This suggests that relatively rapid interconversion occurs between these compounds. The λ_{max} values of peaks 1 and 2 in Fig. 1 measured by the stopped-flow technique were 318 and 326 nm, respectively. It is known[5] that λ_{max} of the *E*-isomer of a vinylogous urethan (–NC=C–COOR) appears at shorter wavelength by about 10 nm than that of the *Z*-isomer. At a column temperature of 50 °C, these peaks became unresolved, giving a broad peak with a shoulder. These results confirm that peaks 1 and 2 are due to the *E*- and *Z*-isomers of III, respectively. At more elevated column temperature, these substances will not be distinguishable, because of their rapid interconversion. Figure 2 shows chromatograms of the isolated product A degraded in

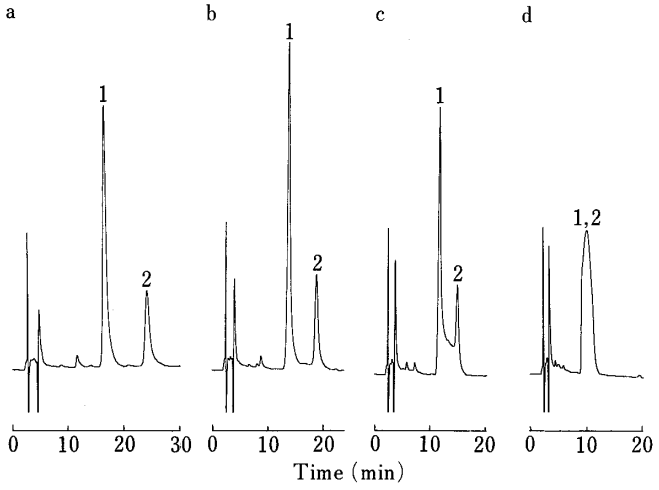

Fig. 1. Chromatograms of Reaction Product of Sulbactam with Pyrazole at Various Column Temperatures

Column temperature: a, 5 °C; b, 20 °C; c, 35 °C; d, 50 °C. Detection: 325 nm. Sensitivity: 0.032 absorbance unit full scale (a.u.f.s.). Injection volume: 10 μl. Assignments: 1, *E*-isomer and 2, *Z*-isomer of 1-(5-carboxy-6-methyl-6-sulfino-4-aza-2-heptenoyl)pyrazole (III). Reaction conditions: see Experimental.

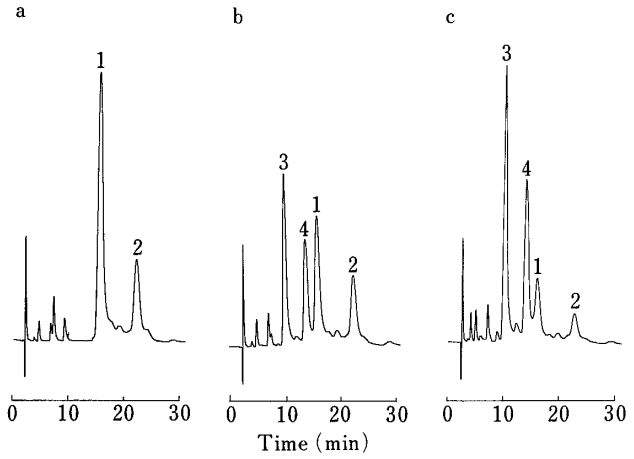

Fig. 2. Chromatograms of Reaction Product of Sulbactam with Pyrazole Degraded in Methanol at 60 °C

Reaction time: a, 0 min; b, 30 min; c, 80 min. Column temperature: 20 °C. Detection: 300 nm. Sensitivity: 0.032 a.u.f.s. Injection volume: 10 μl. Assignments: 1, *E*-isomer and 2, *Z*-isomer of 1-(5-carboxy-6-methyl-6-sulfino-4-aza-2-heptenoyl)pyrazole (III); 3, *E*-isomer and 4, *Z*-isomer of methyl 5-carboxy-6-methyl-6-sulfino-4-aza-2-heptenoate (II).

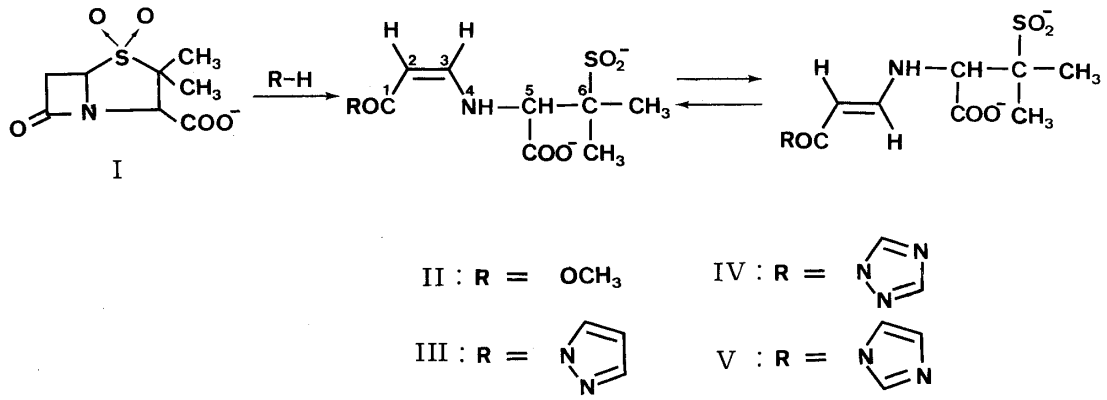

Chart 1. Reaction Pathways of Sodium Sulbactam

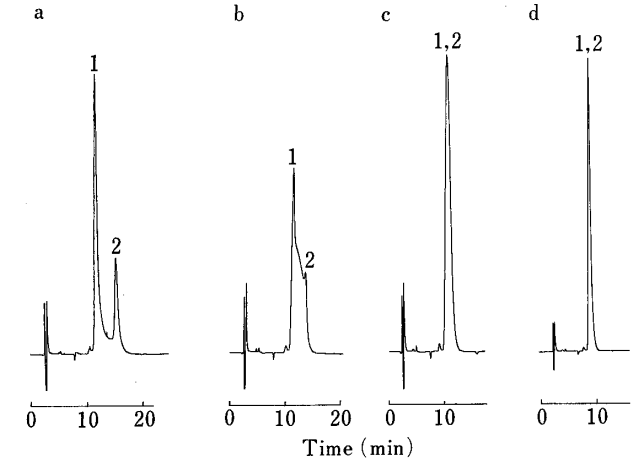

Fig. 3. Chromatograms of Reaction Product of Sulbactam with 1,2,4-Triazole at Various Column Temperatures

Column temperature: a, 5 °C; b, 20 °C; c, 35 °C; d, 50 °C. Detection: 325 nm. Sensitivity: 0.064 a.u.f.s. except for d (0.128 a.u.f.s.). Injection volume: 10 μl. Assignments: 1, *E*-isomer and 2, *Z*-isomer of 1-(5-carboxy-6-methyl-6-sulfino-4-aza-2-heptenoyl)-1,2,4-triazole (IV). Reaction conditions: see Experimental.

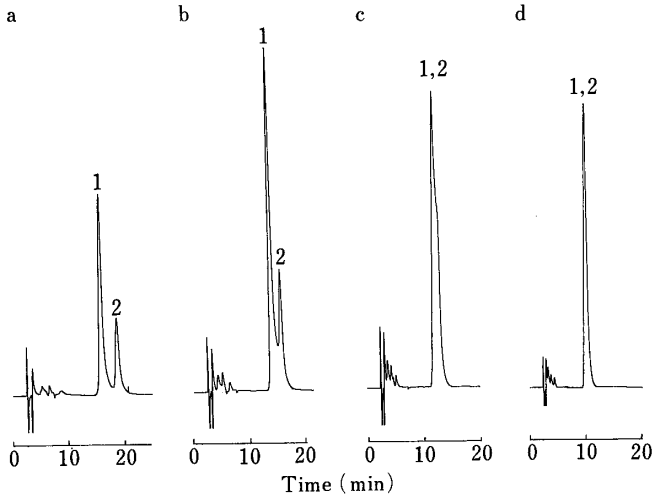

Fig. 4. Chromatograms of Reaction Product of Sulbactam with Imidazole at Various Column Temperatures

HPLC conditions were the same as those in the legend to Fig. 3. Assignments: 1, *E*-isomer and 2, *Z*-isomer of 1-(5-carboxy-6-methyl-6-sulfino-4-aza-2-heptenoyl)imidazole (V). Reaction conditions: see Experimental.

methanol at 60 °C for various time periods. With increasing reaction time, the intensities of peaks 1 and 2 decrease with concomitant appearance and increase of peaks 3 and 4. The retention times of peaks 3 and 4 agreed exactly with those of the *E*- and *Z*-isomers of II. From the ^{1}H-NMR spectra (Table I) and HPLC results described above, it is concluded that the reaction of sodium sulbactam with pyrazole in weakly alkaline solution yields a mixture of *E*- and *Z*-isomers of III (Chart 1).

Figures 3 and 4 show chromatograms of sulbactam reacted with 1,2,4-triazole and imidazole, respectively, obtained at column temperatures between 5 and 50 °C. The λ_{max}

values of peaks 1 and 2 in Fig. 3 measured by the stopped-flow technique were 327 and 331 nm, respectively, and those of the peaks in Fig. 4 were 324 and 330 nm. Based on the same reasoning as above, the structures of these reaction products could be elucidated as 1-(5-carboxy-6-methyl-6-sulfino-4-aza-2-heptenoyl)-1,2,4-triazole (IV) and -imidazole (V) (Chart 1). Compounds IV and V also each existed as a 2 : 1 mixture of *E*- and *Z*-isomers in aqueous solution. At a column temperature of 50 °C, IV and V each gave a sharp single peak, indicating rapid interconversion between the *E*- and *Z*-isomers. It is also clear from Figs. 1, 3 and 4 that the interconversion between *E*- and *Z*-isomers of IV and V occurs more rapidly than that of III. In a previous paper,[3] we observed IV as a broad peak with a shoulder at ambient column temperature, and as a single peak at 50 °C, and it is now clear that this was due to the difference in the rate of interconversion of the *E*- and *Z*-isomers of IV. Compounds IV and V were also converted to II in methanol.

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