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Epimerization of Amoxicillin Piperazine-2,5-dione in Acidic Solutions

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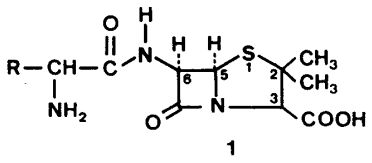
Products formed from amoxicillin by reaction with glucose in solution (pH 9.2) were isolated and examined by high-performance liquid chromatography, nuclear magnetic resonance, ultraviolet and mass spectroscopy, and optical rotation measurement. The products were elucidated to be the corresponding (2*R*)-piperazine-2′,5′-dione and the (2*S*)-epimer. The (2*R*)-piperazine-2′,5′-dione readily epimerized to the (2*S*)-epimer in acidic solutions, and at equilibrium, the (2*R*)- and (2*S*)-epimers existed in a ratio of 2 : 1. The epimerization also took place in alkaline solutions to a small extent. The 2-position in these compounds corresponds to the 5-position in the starting amoxicillin.

Keywords——amoxicillin; amoxicillin piperazine-2,5-dione; amoxicillin piperazine-2,5-dione epimerization; amoxicillin piperazine-2,5-dione NMR

Introduction

Recently, ampicillin piperazine-2,5-dione (**2a**) has been reported to be a metabolite of ampicillin (**1a**) in rat[1] and human[2] urine. Our unpublished results also suggest that amoxicillin piperazine-2,5-dione (**2b**) is a metabolite of amoxicillin (**1b**) in human urine. The *in vitro* formation and preparation of ampicillin piperazine-2,5-dione were first described by Bundgaard and Larsen.[3] Roets *et al.*[4] prepared amoxicillin piperazine-2,5-dione in a similar way. A number of papers[5–12] have dealt with the epimerization of penicilloic acids, and indicated that the epimerization mainly occurs at the 5-position over a wide pH range between 2.5 and 13. The present paper deals with the epimerization of amoxicillin piperazine-2,5-dione at the 2-position (which corresponds to the 5-position in the starting amoxicillin) in acidic media.



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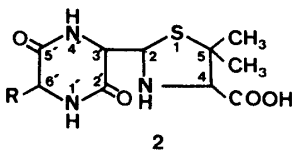

2

1a : R =

1b : R = HO

2a : R =

2b : R = HO

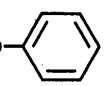

Chart 1

Experimental

Reagents and Materials——Amoxicillin trihydrate was supplied by Beecham Yakuhin Co., Ltd. (Tokyo, Japan). Buffer salts and other chemicals of reagent grade were purchased from Nakarai Chemicals Co. (Kyoto, Japan).

Isolation and Purification of Piperazine-2,5-dione of Amoxicillin——Amoxicillin (100 mg) was dissolved in 10 ml of a 10% (w/v) glucose solution. The reaction solution, whose pH was maintained at 9.2 by occasional addition of 2 M sodium hydroxide solution, was kept at room temperature for 20 h (solution A).

(i) Solution A was neutralized with 5 M hydrochloric acid. The neutral solution was concentrated to a small volume, and subjected to preparative column chromatography (LiChroprep RP-8, 310 × 25 mm i.d., Merck, Darmstadt, West Germany). The column was eluted with H_2O–methanol (2 : 1, v/v) containing 0.5% (v/v) acetic acid. The fraction between 300 and 360 ml was collected. Removal of the solvent by evaporation gave a yellowish-white solid (product A).

(ii) Solution A was acidified to pH 2 by addition of 5 M hydrochloric acid and cooled to about 4 °C until precipitation occurred. The yellowish-white precipitate formed was filtered off, and washed with water. The precipitate was dissolved in a small volume of 20 mM phosphate buffer solution (pH 7.0), and subjected to preparative column chromatography under the same conditions as described above. The fraction between 420 and 510 ml was collected. Removal of the solvent by evaporation gave a yellowish-white solid (product B).

Equipment——The proton nuclear magnetic resonance (^{1}H-NMR) spectra were obtained at 200 MHz on a JNM-FX-200 spectrometer (JEOL, Tokyo). The samples were dissolved in $(CD_3)_2SO$, and the chemical shifts are given in ppm from internal tetramethylsilane. Reversed-phase high-performance liquid chromatography (HPLC) was carried out using an LC-5A pump (Shimadzu, Kyoto), a 15 cm × 4.6 mm i.d. stainless steel column packed with Develosil ODS-5 (5 μm particle size, Nomura Chemicals, Seto, Japan) and a variable wavelength detector (SPD-2AV, Shimadzu). The eluent used was 7 mM sodium dihydrogen phosphate plus 3 mM phosphoric acid–methanol (3 : 1, v/v) (pH 3.2), and the flow rate was maintained at 0.8 ml/min. The detection was performed at 230 nm. Ultraviolet (UV) spectra were measured on a model 228 spectrophotometer (Hitachi, Tokyo). Secondary ion mass spectra (SIMS) were obtained with an M-80A mass spectrometer (Hitachi). Optical rotations were measured with a DIP-4 digital polarimeter (JASCO, Tokyo). The pH values were measured on a model F-8 pH meter (Horiba, Kyoto).

Results and Discussion

Roets *et al.*[4] prepared amoxicillin piperazine-2,5-dione in the same manner as reported by Bundgaard and Larsen;[3] the reaction of amoxicillin with glucose in solution (pH 9.2) for 20 h at room temperature followed by acidification of the reaction solution gave the corresponding piperazine-2,5-dione as a precipitate. However, since the acidification of the reaction solution gave no precipitate, we could not obtain the piperazine-2,5-dione. HPLC of the reaction solution showed two peaks at retention times of about 12 and 16 min. At the initial stage of the reaction, the peak at 12 min appeared; the peak at 16 min was observed as the reaction proceeded. These substances were isolated by reversed-phase preparative column

TABLE I. ^{1}H Chemical Shifts of the Isolated Products A and B

	Chemical shift, δ (J/Hz)	
	Product A	Product B
C-5 methyls	1.21 s, 1.55 s	1.20 s, 1.57 s
4-H	3.58 s	3.55 s
3′-H	3.81 br[a]	4.34 br[b]
6′-H	4.82 br[c]	4.84 br[d]
2-H	5.07 d (J=3.4)	5.09 d (J=2.2)
Aromatics	6.73, 7.09 dd	6.73, 7.10 dd
4′-H	7.66 br[e]	8.61 br[e]
1′-H	8.49 d (J=1.5)[e]	8.48 d (J=1.5)[e]

a) On irradiation at δ 7.66 ppm this signal became a doublet (J=3.4 Hz). *b*) On irradiation at δ 8.61 ppm this signal became a doublet (J=2.2 Hz). *c*) On irradiation at δ 8.49 ppm this signal became a singlet. *d*) On irradiation at δ 8.48 ppm this signal became a singlet. *e*) On addition of D_2O, the signal disappeared.

chromatography (products A and B, see Experimental). The products A and B thus isolated had retention times of 12 and 16 min, respectively. However, the isolated product A contained about 5% product B, and *vice versa*. Bundgaard and Larsen[3] stated that the reaction solution of ampicillin piperazine-2,5-dione with mercury(II) chloride in phosphate buffer solution (pH 7.0) shows a UV absorption maximum at 305 nm. The products A and B in phosphate buffer solution (pH 7.0) in the presence of mercury (II) chloride each showed an absorption maximum at 305 nm. The SIMS of products A and B showed a peak at *m/z* 366, which is assignable to $(M+H)^+$. The optical rotations of products A and B were $+42°$ and $-41°$ ($c=0.01$ in dimethylsulfoxide), respectively. These results suggest that products A and B could be epimers of amoxicillin piperazine-2,5-dione. The ^1H chemical shifts of the isolated products A and B are shown in Table I. The ^1H-NMR spectrum of product A was almost consistent with that of amoxicillin piperazine-2,5-dione reported by Roets *et al.*[4] Product A should be (2*R*)-piperazine-2′,5′-dione, which has the same (*R*) configuration at the 2-position as the starting amoxicillin (the 2-position of the former corresponds to the 5-position in the latter). There are two marked differences in the ^1H chemical shifts and coupling constants (Table I) between products A and B; one is the downfield shifts of the 3′C and 4′C protons in the case of product B, and the other is the smaller coupling constant between the 2C and 3′C protons for product B than A. Busson *et al.*[5] reported downfield shifts of the 6C and amide protons on conversion from the (5*R*)- to (5*S*)-isomer for dimethylbenzylpenicilloate, and a smaller coupling constant between the 5C and 6C protons for the (5*S*)- than the (5*R*)-isomer. The 3′C and 4′C protons of the piperazine-2,5-dione correspond to the 6C and amide protons of the dimethylbenzylpenicilloate. Taking into account the ^1H-NMR data for dimethylbenzylpenicilloate and other spectral data described above, products A and B are concluded to be the (2*R*)-piperazine-2′,5′-dione and the (2*S*)-epimer, respectively. The epimerization of amoxicillin piperazine-2,5-dione, as well as a penicilloic acid, took place at the 5-position of the starting penicillin.

The epimerization of the (2*R*)-epimer to (2*S*)-epimer was preliminarily examined in phosphate buffer solutions at pH 3 and 8 and at 37 °C for 12 h by HPLC. At pH 3, the (2*R*)-configuration was favored at equilibrium; the (2*R*)- to (2*S*)-epimers existed in the ratio of 2 : 1. However, at pH 8, the epimerization took place to a small extent. It is interesting to compare these results with those for the epimerization of penicilloic acids. The epimerization of penicilloic acids at the 5-position took place easily between pH 2.5 and 13; on the other hand, in the case of amoxicillin piperazine-2,5-dione it tended to occur only in acidic media.

A detailed consideration of the epimerization kinetics of amoxicillin and other aminopenicillin piperazine-2,5-diones will be presented elsewhere.

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