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Review

Diastereomeric β-lactam antibiotics Analytical methods, isomerization and stereoselective pharmacokinetics

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Abstract

Stereospecific HPLC methods for the determination of various diastereomeric β-lactam antibiotics are reviewed. Stereoselectivity in the absorption, distribution and excretion of several diastereomeric β-lactams is summarized. The isomerization of β-lactam isomers and its influence on the pharmacokinetics and pharmacodynamics are discussed.

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1. Introduction

Pencillins and cephalosporins are prepared by reaction of the appropriate acylating agent with 6-aminopenicillanic acid or 7-aminocephalosporanic acid. If the acylating agent is a mixture of optical isomers, the penicillin or cephalosporin obtained is a mixture of two diastereomers with a racemic side-chain. It is known that the antimicrobial activities of the diastereomers thus obtained are not identical [1–4]. As the D-epimers of ampicillin, amoxicillin, azidocillin, cephalexin and cephaloglycin are considerably more potent than their corresponding L-epimers, only the more active epimer is prepared as a commercial form. The antimicrobial activities are also different between the epimers of phenethicillin, propicillin, clometocillin. However, as the differences in antimicrobial activities are small, the use of pure epimers may not be necessary for these penicillins. On the other hand, the biological activities of carbenicillin epimers have been reported to be equivalent, probably owing to rapid epimerization during biological assay procedures.

However, the information appears to be limited with regard to the stereospecific analytical methods and the differences in pharmacokinetics for diastereomeric β-lactam antibiotics. In this review, methods for the determination of various diastereomeric β-lactam antibiotics are summarized. The discussions are extended to the isomerization of the diastereomers, its influence on the pharmacokinetics and the differences between diastereomers in the processes of absorption, distribution and disposition.

2. Analytical methods

Proton nuclear magnetic resonance (^{1}H NMR) has been successfully utilized to determine the configuration at C-10 for the penicillins which possess an aromatic group attached to C-10 [4,5]. The differences in chemical shifts between the epimers were attributed to the shielding effect of the aromatic ring in the side-chain, which was caused by the folded conformation. Although the ratios of sulbenicillin epimers in various preparations were quantitatively determined by ^{1}H NMR spectroscopy, high-performance liquid chromatography (HPLC) appears to be most convenient for the determination of diastereomeric β-lactam antibiotics. Therefore, stereospecific HPLC methods reported for various diastereomeric β-lactams (Fig. 1) are summarized in this section.

2.1. Carbenicillin

Carbenicillin epimers (**1**) were determined by reversed-phase HPLC using a μBondapak C_{18} column (300 × 4.0 mm I.D.) [6]. The mobile phase was methanol–0.01 *M* tetrabutylammonium bromide (4:7, v/v) at a flow-rate of 3.0 ml/min. Carbenicillin was detected at 254 nm. Two epimers were incompletely resolved under these conditions. When the mobile phase changed to methanol–0.05% acetic acid, the resolution was even poorer. On the other hand, the D-(−)-epimer content in the commercial preparation was 55% as determined by ^{1}H NMR.

Carbenicillin epimers were also determined by Gupta and Stewart [7]. The analytical column used was μBondapak phenyl (300 × 4 mm I.D.) with 0.01 *M* ammonium acetate as the mobile phase. The flow-rate was 1.6 ml/min and the epimers were detected at 245 nm. The two epimers were resolved close to the baseline, but the absolute configurations of the eluted peaks were not determined.

An HPLC method for the determination of carbenicillin epimers was also reported by Twomey [8]. Carbenicillin epimers were determined with a Spherisorb ODS column (150 × 4.6 mm I.D.) using methanol–0.05 *M* KH_2PO_4 (37:63, v/v) as the mobile phase. Tetrabutylammonium bromide (0.1%, w/v) was added as the ion-pair reagent, and the pH was adjusted with 10% H_3PO_4. The flow-rate was 1.2 ml/min and the epimers were detected at 220 nm. Carbenicillin epimers could be either resolved or eluted as a single peak, depending on the mobile phase pH. The resolution was very poor at pH 3.00 and 3.70, and the epimers eluted as a single peak at

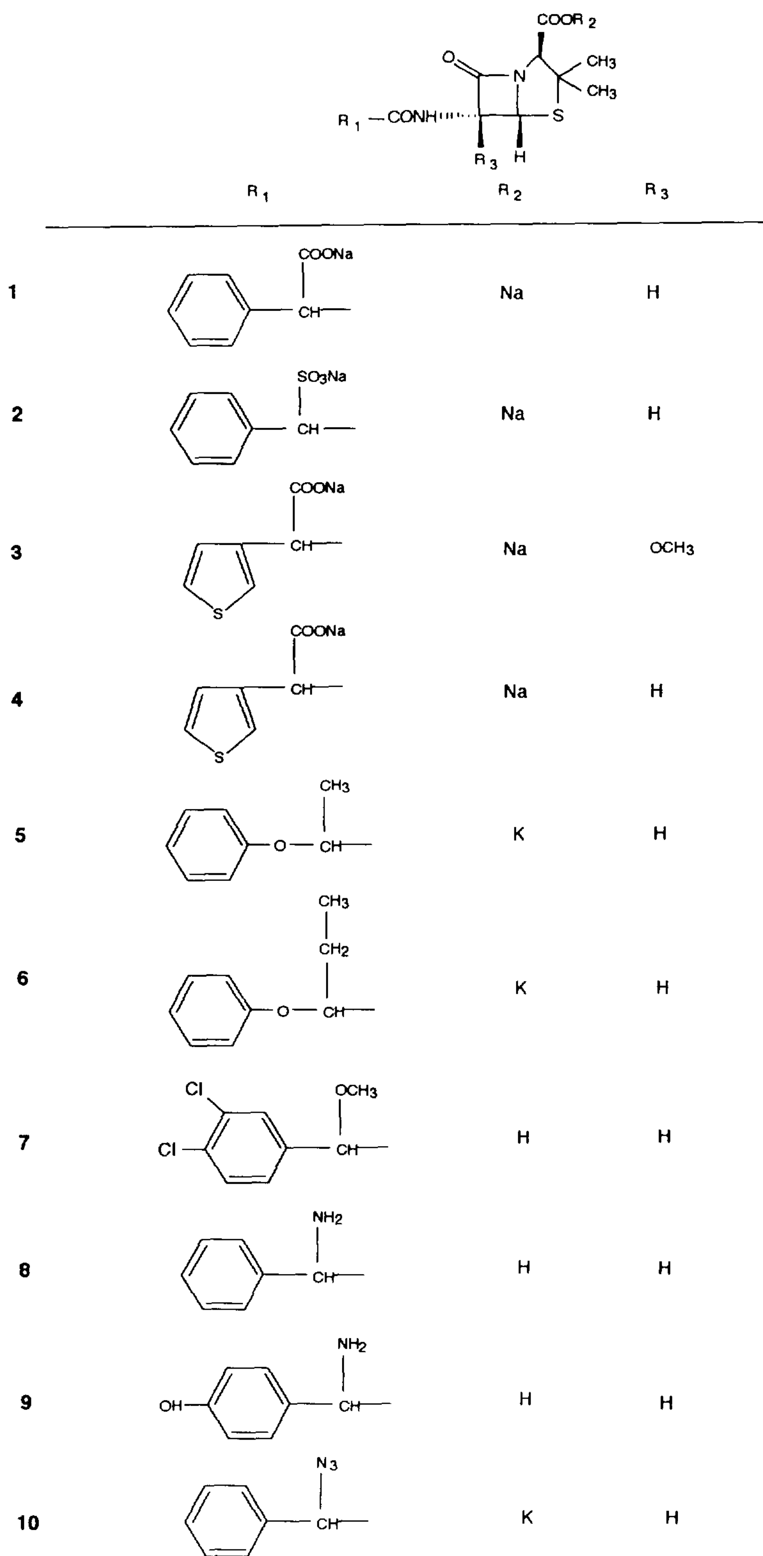

Fig. 1. (Continued on p. 198.)

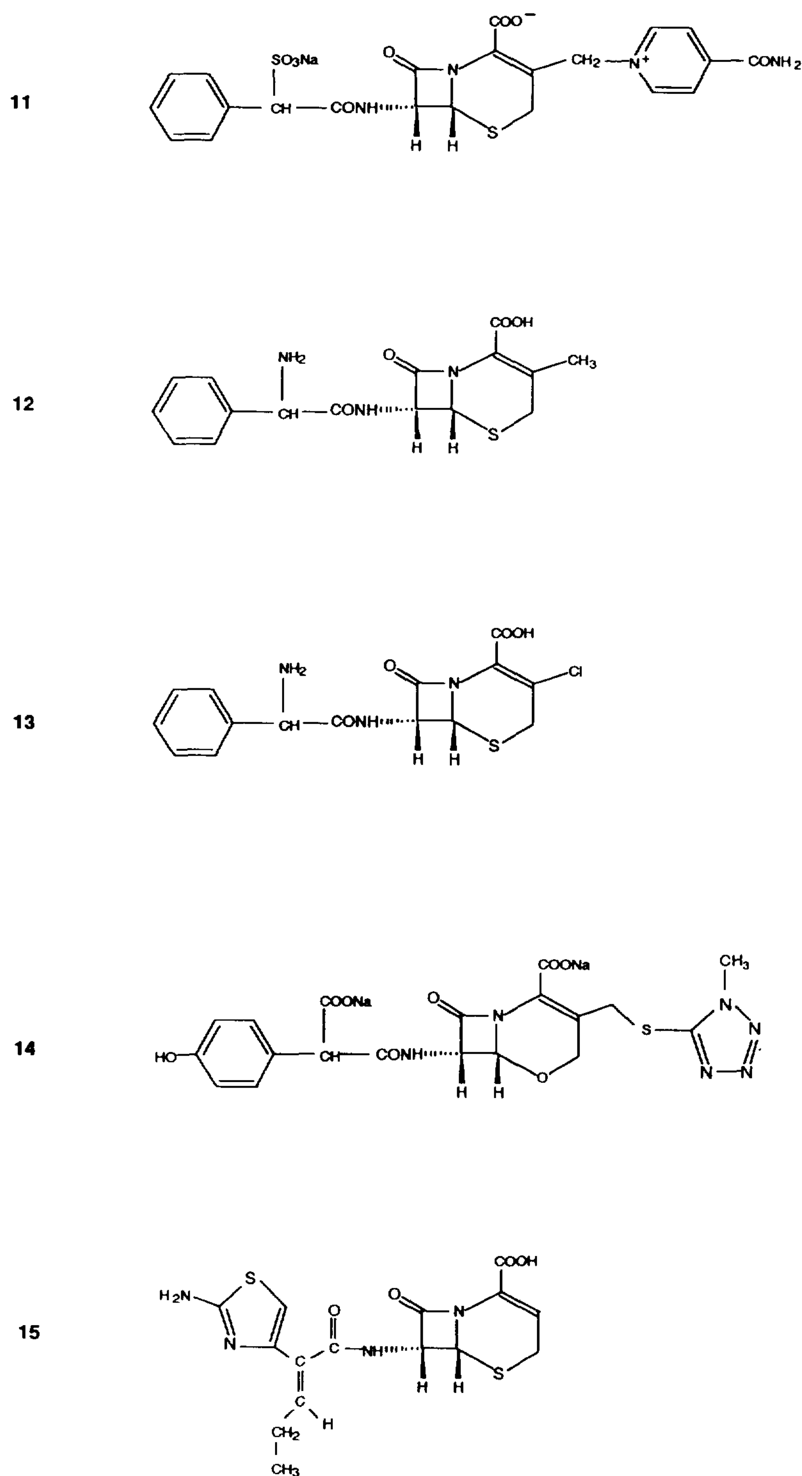

Fig. 1. Structures of diastereomeric β-lactam antibiotics.

pH 3.35. Although carbenicillin epimers were fairly well resolved at pH 4.35, baseline resolution was not achieved. Absolute configurations of the eluting epimers were not determined.

An HPLC method developed by Hoogmartens et al. [4] employed a Zorbax C_8 column (250 × 4.6 mm I.D.) with a mobile consisting of methanol–water–5% 0.2 *M* phosphate buffer (pH 7.0). The flow-rate was 1.0 ml/min and carbenicillin epimers were detected at 254 nm. With 5% methanol in the mobile phase, the two epimers were resolved close to the baseline. As the absolute configurations of the eluting epimers were not determined, it was not known which epimer eluted faster under their HPLC conditions. However, it is most likely that the D- (or *R*-) epimer eluted faster according to the results obtained by Aso et al. [9]. They also found that the content of the first-eluting epimer was ca. 54% in commercial preparations, which suggested that the commercial preparations did not contain equal amounts of each epimer.

Hashimoto et al. [10] also reported an HPLC method for epimerization studies in aqueous solutions. A Nucleosil $5C_{18}$ column (150 × 4.6 mm I.D.) with a mobile phase consisting of 0.05 *M* ammonium acetate–methanol (17:1) was used for the determination of carbenicillin epimers. Also, a glass column (86 × 3 cm I.D.) packed with porous polystyrene was used to isolate each carbenicillin epimer in their study.

For epimerization studies in aqueous solutions, Aso et al. [9] developed an HPLC method for carbenicillin epimers. A TSKgel ODS-80TM column (150 × 4.6 mm I.D.) with a mobile phase consisting of 0.05 *M* phosphate (pH 7.0)–methanol (4:1) was used and carbenicillin epimers were detected at 220 nm. The D- (or *R*-) epimer eluted faster than the L- (or *S*-) epimer with retention times of ca. 5 and 6 min for D- and L-carbenicillin, respectively. The two epimers were baseline separated.

Ishida et al. [11] reported an HPLC method for the determination of carbenicillin epimers in biological fluids. Plasma and urine samples were prepared for HPLC by solid-phase extraction using SAX-Bond Elut. Analytical column used was Cosmosil $5C_{18}$-AR (250 × 4.6 mm I.D.) with a mobile phase consisting of 0.05 *M* ammonium acetate–methanol (9:1, v/v). The flow-rate was 1.2 ml/min and carbenicillin epimers were detected at 254 nm. The method was applicable to plasma and urine samples from humans, rats and rabbits. The epimers were resolved to the baseline with no interfering peaks (Fig. 2). The *R*-epimer eluted faster than the *S*-epimer with retention times of ca. 18 and 23 min for the *R*- and *S*-epimers, respectively. In their studies, carbenicillin epimers were resolved and isolated using a glass column (100 × 3 cm I.D.) packed with porous polystyrene (250–800-μm particle size), and the absolute configurations of the epimers were assigned using ^{1}H NMR. The *R*- to *S*-epimer ratio (*R*/*S* ratio) was ca. 1.1 for carbenicillin disodium salt purchased from Sigma.

2.2. Cefsulodin

An HPLC method for the determination of cefsulodin epimers (**11**) was reported by Fujita and Koshiro [12] using a μBondapak C_{18} column (300 × 3.9 mm I.D.). They used two kinds of mobile phase compositions; mobile A consisted of 0.0168 *M* dibasic ammonium phosphate–acetic acid–methanol (100:1.68:5.98) containing 5 m*M* triethylamine, and mobile phase B consisted of an aqueous solution (containing 0.0388 *M* ammonium acetate, 0.292 m*M* dibasic ammonium phosphate and 9.363 μ*M* triethylamine) – acetonitrile – methanol – dimethylformamide

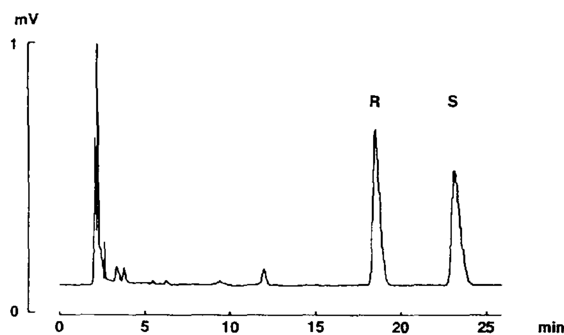

Fig. 2. Chromatogram of human plasma spiked with carbenicillin. R = *R*-epimer and S = *S*-epimer.

–acetic acid (1000:7.6:1.05:1.31:0.30). For both mobile phase compositions, the flow-rate was 1.2 ml/min and cefsulodin epimers were detected at 260 nm. Phthalic acid and tegafur were used as internal standards for mobile phase A and B, respectively. For both mobile phase compositions L-(+)-cefsulodin eluted faster than D-(−)-cefsulodin, and the two epimers were separated to the baseline. Commercial preparations contain only the D-(−)-epimer, which is the more active isomer.

2.3. Ceftibuten

Ceftibuten (**15**) is an oral cephem antibiotic which possesses a double bond in the side-chain. Although commercially available formulations contain only the *cis* isomer, HPLC methods for the determination of both *cis* and *trans* isomers were developed because the isomerization from the *cis* to *trans* isomer was observed in vivo. Matsuura et al. [13] developed an automated HPLC method for the determination of ceftibuten isomers in plasma and urine using a column-switching technique. Nucleosil $5C_{18}$ (150 × 4.6 mm I.D.) was used as an analytical column. The mobile phase for plasma assay was [10 m*M* tetra-*n*-butylammonium bromide–2 m*M* monobasic ammonium phosphate (pH 5.0)]–acetonitrile–methanol (25:6:3, v/v/v), and that for urine assay was an aqueous mixture of three salts (5 m*M* tetra-*n*-butylammonium bromide, 5 m*M* tetra-*n*-amylammonium bromide and 8 m*M* monobasic ammonium phosphate)–acetonitrile–methanol (65:25:10, v/v/v). The flow-rate was 1.5 ml/min and the isomers were detected at 256 nm in both plasma and urine assays. In the plasma assay, the *trans* isomer eluted faster than the *cis* isomer with retention times of 20.8 and 22.9 min, respectively. The *trans* isomer also eluted faster than the *cis* isomer in the urine assay with the retention times of 18.6 and 20.0 min, respectively.

An HPLC method for ceftibuten isomers was also reported by Shimada et al. [14]. A Nucleosil $10C_{18}$ column (150 × 4 mm I.D.) was used with a mobile phase consisting of tetrabutylammonium phosphate–acetonitrile–methanol (50:6:3, v/v/v). The flow-rate was 1.2 ml/min and the isomers were detected at 256 nm. The retention times of the *cis* and *trans* isomers were 10.7 and 12.3 min, respectively. Although the HPLC conditions were similar to those reported by Matsuura et al., the elution order was different.

2.4. Cephalexin

Cephalexin epimers (**12**) were determined by Salto [3] with a μBondapak C_{18} column (300 × 4 mm I.D.) The mobile phase was 0.1 *M* phosphate buffer (pH 3.5) containing 5% methanol at a flow-rate of 1.5 ml/min and the epimers were detected at 254 nm. The two epimers were separated to the baseline with retention times of ca. 10 and 20 min for the L- and D-epimers, respectively.

Cephalexin epimers in rat serum and urine were separated by Tamai et al. [15] using TSKgel ODS-80TM (150 × 4.6 mm I.D.) as an analytical column. The mobile phase compositions were methanol–10 m*M* ammonium acetate (20:80) for the determination of the D-epimer and methanol–10 m*M* phosphate buffer (pH 3.0) containing 10 m*M* ammonium acetate and 10 m*M* pentanesulphonic acid (10:90) for the L-epimer. The flow-rate was 1.0 ml/min and the epimers were detected at 260 nm. No information on the resolution was given.

HPLC methods for cephalexin have also been reported by Hendrix et al. [16] using various ODS columns under the conditions prescribed in the European Pharmacopoeia and the US Pharmacopeia. The mobile phase composition was methanol–acetonitrile–1.36% (w/v) aqueous KH_2PO_4–water (2:5:10:85, v/v) or water–acetonitrile–methanol–triethylamine–sodium 1-pentanesulphonate (850:100:50:15:1, v/v/v/v/w). The flow-rate was 1.5 ml/min and cephalexin epimers were detected at 254 nm. Resolution of the epimers appeared to be good as judged by the differences in capacity factors.

Cephalexin epimers were also separated using poly(styrene–divinylbenzene) as the stationary phase [16,17]. The mobile phase was acetonitrile–0.02 *M* sodium 1-octanesulphonate–0.2 *M* phosphoric acid–water (15.5:10:5:69.5,

v/v) at a flow-rate of 1.0 ml/min. Cephalexin epimers were detected at 254 nm. Baseline separation of the two epimers was observed under the HPLC conditions.

2.5. Moxalactam

There have been several reports on HPLC method for the determination of moxalactam epimers (**14**). Konaka et al. [18] used Nucleosil $10C_{18}$ (300 × 4 mm I.D.) as an analytical column. The mobile phases used were as follows: methanol–0.05 *M* monobasic potassium phosphate (5:95) adjusted to pH 6.5 at a flow-rate of 2.0 ml/min for raw material analysis, and methanol–0.005 *M* tetra-*n*-butylammonium phosphate (25:75) adjusted to pH 6.0 at a flow-rate of 1.0 ml/min for the urine analysis. Moxalactam epimers were detected at 254 nm. Urine samples were prepared for HPLC by solid-phase extraction using a Sep-Pak cartridge. The *R*-epimer eluted faster than the *S*-epimer under both HPLC conditions. The *R*/*S* ratios in the raw materials were 1.05–1.11.

Determination of moxalactam epimers in plasma and urine was also reported by Miner et al. [19]. Plasma samples were deproteinized with ice-cold methanol and the epimers were separated with a Chromegabond C_{18} column (30 × 4.6 mm I.D.) using 0.1 *M* ammonium acetate–acetonitrile (95:5) adjusted to pH 6.5 as the mobile phase. The flow-rate was 1.5 ml/min, and moxalactam epimers were detected at 270 nm. Urine samples were analysed with a Zorbax TMS column (250 × 4.6 mm I.D.) using 5 m*M* *n*-heptylamine in 11% methanol–distilled water (pH adjusted to 6.0). The flow rate was 1.4 ml/min, and the epimers were detected at 280 nm. The *R*-epimer eluted faster than the *S*-epimer under their HPLC conditions. After oral administration of moxalactam, the plasma concentrations were determined with both HPLC and biological assays, with a good correlation between the two methods.

The HPLC method developed by Aravind et al. [20] employed a Perkin-Elmer C_{18}-ODS-HC-SIL-X column (250 × 4.6 mm I.D.). The mobile phase was methanol–10 m*M* ammonium acetate (pH 6.5) (4:96). The flow-rate was 1.0 ml/min and moxalactam epimers were detected at 230 nm. The retention times of moxalactam epimers were 2.16 and 2.63 min, but the absolute configurations of the eluting epimers were not determined. In the commercial preparation, the ratio of the first to second peak was 54:46, suggesting that the content of one epimer was greater than that of the other. Serum, cerebrospinal fluid and urine samples were also analysed with the same HPLC method after deproteinization with ice-cold methanol. 8-Chlorotheophylline was used as an internal standard.

Ziemniak et al. [21] reported an HPLC method for moxalactam using a μBondapak C_{18} column. The mobile phase was acetonitrile–0.05 *M* ammonium acetate (3:97) with a flow-rate of 1.5 ml/min. Moxalactam epimers were detected at 275 nm. Plasma and urine samples were acidified with hydrochloric acid and were extracted with ethyl acetate. The organic layer was then back-extracted into tromethamine buffer (pH 8.0), which was subjected to HPLC analysis. Allopurinol was used as an internal standard. The D-epimer eluted faster than the L-epimer, with retentions times of 7.8 and 10.5 min, respectively. The two epimers were separated to the baseline with no interfering peaks on the chromatogram.

Moxalactam epimers in serum and myometrial tissue were separated by Bawdon et al. [22] using a μBondapak C_{18} column. The mobile phase was 0.1 *M* sodium phosphate–methanol (84:16) at pH 3.2. The flow-rate was 2.0 ml/min and the epimers were detected 254 nm. The *R*-epimer eluted faster than the *S*-epimer under their HPLC conditions. They observed a good correlation between the HPLC and microbiological assay methods.

2.6. Sulbenicillin

The contents of D-(−)- and L-(+)-epimers of sulbenicillin (**2**) were determined by Nomura et al. [2] using ^{1}H NMR spectroscopy. Owing to folded conformations, the shielding effects of the side-chain aromatic ring were different between

the two epimers, resulting in differences in the chemical shifts assigned to the 3-CH_3 groups. Good correlation was observed between the bioactivity and the D-(−)-epimer content, which was consistent with the fact that the D-(−)-epimer is far more potent than the L-(+)-epimer. The antimicrobial activity of the L- (or *S*-) epimer was only 2.7% of that of the D- (or *R*-) epimer.

For the determination of sulbenicillin epimers, Yamaoka et al. [6] applied the same HPLC method as described above for carbenicillin, which resulted in no resolution of the sulbencillin epimers. However, the D-(−)-epimer content in the commercial preparation was ca. 77% as determined by ^{1}H NMR.

We also separated sulbenicillin epimers by HPLC with methods similar to those reported by Ishida et al. [11] for carbenicillin, and obtained a baseline separation of sulbenicillin epimers (unpublished results). The *R/S* (or D/L) ratio was approximately 3 in the commercial preparation as determined by HPLC. Therefore, the more potent isomer appears to be the dominant isomer in the commercial preparation.

2.7. Temocillin

Temocillin epimers (**3**) were determined by Bird et al. [23] using two kinds of reversed-phase HPLC conditions. For the first HPLC system, a μBondapak C_{18} column (300 × 3.9 mm I.D.) was used with a mobile phase consisting of methanol–0.1 *M* phosphate buffer (pH 7.0) (1:9, v/v) at a flow-rate of 2.0 ml/min. Temocillin epimers were detected at 230 nm. The retention times were 6.0 and 7.5 min for the *R*- and *S*-epimer, respectively, with baseline separation. For the second system, Zorbax C_8 (250 × 4.6 mm I.D.) was used as an analytical column with a mobile phase consisting of methanol–0.1 *M* phosphate buffer (pH 7.0) (16:84, v/v). The flow-rate was 1.5 ml/min and temocillin epimers were detected at 230 nm. The retention times were 8.0 and 9.5 min for the *R*- and *S*-epimer, respectively. The second system gave a better resolution of the epimers. In their studies, the *R*-epimer was separated by crystallization of the dibenzylethylenediamine salt and the *S*-epimer by preparative HPLC. Absolute configurations of both epimers were assigned from the ^{1}H NMR spectra in comparison with those of ticarcillin.

2.8. Ticarcillin

Gupta and Stewart [7] reported the determination of ticacillin epimers (**4**) in an aqueous solution with the same HPLC method as described above for carbenicillin [7]. The two epimers were resolved, but not to the baseline.

Hoogmartens et al. [4] used the same HPLC method as described above for carbenicillin for the determination of ticarcillin epimers. Two epimers were resolved, but the absolute configurations of the eluting epimers were not determined. They found that the amount of the first-eluting epimer was approximately 56% in the commercial preparations, suggesting that the commercial preparations were not 1:1 mixtures of the epimers.

An HPLC method was also reported by Watson [24] for the determination of ticarcillin epimers in serum and urine samples from patients. Serum and diluted urine samples were acidified with hydrochloric acid, and ticarcillin epimers were extracted with dimethyl ether. A Hypersil ODS column (100 × 5 mm I.D.) was used with a mobile phase consisting of methanol–0.05 *M* phosphoric acid (25:75 for serum and 30:70 for urine). The flow-rate was 2.0 ml/min and ticarcillin epimers were detected at 214 nm. Thienylbutyric acid was used as an internal standard. For the serum sample the two epimers appeared to be separated to the baseline, although an interfering peak partially overlapped one of the epimers. For the urine sample no interfering peaks were observed with a baseline separation of the epimers. Ticarcillin sodium salt in the commercial preparation was a 45:55 mixture of the two epimers (according to the peak ratio on the chromatogram). As absolute configurations were not assigned to the eluting peaks, it was not known which epimer content was greater.

2.9. Other β-lactams

Hoogmartens et al. [4] developed HPLC methods for the determination of the epimers of

phenethicillin (**5**), propicillin (**6**) and clometocillin (**7**). Zorbax C_8 (250 × 4.6 mm I.D.) was used as an analytical column with a mobile phase composed of methanol–water–5% 0.2 *M* phosphate buffer (pH 7.0). The flow-rate was 1.0 ml/min and the epimers were detected at 254 nm. The methanol contents in the mobile phase were 37.5, 45 and 50% for phenethicillin, propicillin and clometocillin, respectively. The epimers were resolved close to the baseline, and the D-epimer eluted faster than the L-epimer for all three penicillins. It was found that the commercial preparations were not 1:1 mixtures of the epimers and that the epimer ratio in the preparation varied from one manufacturer to another. The D-epimer contents of phenethicillin, propicillin and clometocillin varied from 33 to 46%, 24 to 49% and 56 to 59%, respectively.

They used the same HPLC method for the determination of ampicillin, amoxicillin and azidocillin (**10**), except that the methanol content was varied between 10 and 40%. The L-epimer eluted faster for ampicillin, whereas the D-epimer eluted faster for amoxicillin and azidocillin. The less active epimers were not detected in the commercial preparations of these penicillins.

For the determination of carfecillin, they applied the same HPLC method with various methanol contents and pH. All the HPLC conditions resulted in only one peak, although the presence of two diastereomers was confirmed by ^{1}H NMR.

HPLC methods were reported by Salto [3] for the determination of ampicillin and α-phenoxyethylpenicillin using a μBondapak C_{18} column. The mobile phases were mixtures of methanol and 0.1 *M* phosphate buffer in various proportions. The mobile phase pH was also varied. Under the HPLC conditions studied, the L-epimer eluted faster than the D-epimer for ampicillin, whereas the D-epimer eluted faster for α-phenoxyethylpenicillin. The epimers seemed to be well resolved for both penicillins.

Epimers of 7-ureidoacetamido cephalosporins were determined by Young [25] using a μBondapak C_{18} column. The mobile phase was 0.01 *M* diammonium hydrogenphosphate containing 5–20% methanol. For the series of cephalosporins studied, the L-epimers eluted faster than D-epimers. The epimers were well resolved under the HPLC conditions.

Ampicillin prodrugs, such as bacampicillin and talampicillin, are commercially available as mixtures of two epimers due to the chirality of the prodrug moiety. However, no information is available on the stereoselective analytical method or the stereoselectivity in the pharmacokinetics of the ampicillin prodrugs, as the prodrugs are rapidly hydrolysed to release ampicillin once they are absorbed in the body.

3. Isomerization

Hoogmartens et al. [4] studied the epimerization of carbenicillin in aqueous solution at pH 7.0. The epimerization rate from the second-eluting epimer in their HPLC method (probably the *S*-epimer as mentioned above) to the first-eluting epimer was faster than that from the first- to the second-eluting epimer. The results suggest that owing to epimerization it may be impossible to determine the activity of isolated epimers by the usual microbiological methods [1]. The same held true for ticarcillin, i.e. the epimerization rate from the second-eluting epimer to the first-eluting epimer was faster than that from the first- to the second-eluting epimer. When the results obtained in our laboratory are compared with those obtained by Hoogmartens et al. [4] and Aso et al. [9], the *R*-epimer is likely to elute faster than the *S*-epimer under their HPLC conditions. It is also probably true that owing to the epimerization the activity of isolated ticarcillin epimers may not be determined by the conventional microbiological methods, as stated by Hoogmartens et al.

Epimerization of carbenicillin in aqueous solutions was studied by Hashimoto et al. [10]. It was found that the epimerization rate was greater at pH 11 than at pH 9, which was catalysed by hydroxide ion. Epimerization of carbenicillin was also studied by Aso et al. [9] at pH 7.4 in the absence and presence of human serum albumin (HSA). The epimerization rate constant increased and reached a plateau value as the HSA concentration increased. It was revealed that the

epimerization was catalysed by HSA and that the Michaelis–Menten-type complex was formed between carbenicillin and HSA.

Epimerization of cefsulodin in aqueous solutions was studied by Fujita and Koshiro [12]. D-(−)-Cefsulodin was stable to epimerization below neutral pH but was sensitive to epimerization in alkaline solution, suggesting that the epimerization was catalysed by hydroxide ion. The epimerization constant increased as the temperature increased, with an activation energy of ca. 27 kcal/mol. Moreover, the concentration of the D-(−)-epimer was slightly greater than that of the L-(+)-epimer at equilibrium, which suggested that the conformation of the D-(−)-epimer was more stable than the L-(+)-epimer.

Isomerization of *cis*-ceftibuten to the *trans* isomer in serum was measured by Shimada et al. [14]. The isomerization rate constants of both isomers correlated well with their binding percentages in serum, suggesting that the binding to serum protein exerted a catalytic effect on the isomerization. When the isomerization rate was compared between serum and albumin-deficient serum, it was found that the isomerization was accelerated in the presence of HSA and that the catalytic effect was inhibited by warfarin. However, since the inhibitory effect of warfarin was observed only at higher warfarin to HSA molar ratios, the isomerization may be catalysed not only by the warfarin site but also by other binding sites. On the other hand, the presence of α_1-acid glycoprotein did not affect the isomerization.

Epimerization of temocillin in aqueous solutions was studied by Bird et al. [23], who observed significant effects of pH and temperature on the epimerization rate. The equilibration half-life ($t_{1/2}$) increased as the pH increased, the $t_{1/2}$ values at 25°C being 0.22 and 5.0 h at pH 5.0 and 7.0, respectively. On the other hand, $t_{1/2}$ decreased as the temperature increased; the $t_{1/2}$ values at pH 7.0 were 5.0 and 2.0 h at 25 and 37°C, respectively. The equilibration rate was also affected by the phosphate concentration with the half-life at pH 7.0 decreasing at higher phosphate concentrations, suggesting a catalytic effect of phosphate on epimerization. Moreover, the percentage of the *R*-epimer at equilibrium was dependent on the temperature; the percentage of the *R*-epimer at equilibrium was 70.5, 65.0 and 62.5% at 0, 25 and 37°C, respectively. However, the pH did not affect the *R*-epimer percentage at equilibrium.

Hashimoto and co-workers [10,26] studied the epimerization of moxalactam epimers in aqueous solutions at various pH. The epimerization rate constants for both epimers were smallest around pH 7.0 and were greater in both acidic and basic solutions. It was found that the epimerization was water catalysed in acidic solutions and that the epimerization in basic solutions was catalysed by hydroxide ion.

Epimerization of moxalactam was also studied in frozen aqueous solutions [27]. Although the epimerization rate constant decreased as the temperature decreased, the epimerization did not stop until the temperature fell below the collapse temperature of the moxalactam aqueous solution (−19°C). Also, the *R*/*S* ratio at equilibrium increased as the temperature decreased, which could be ascribed to the difference in the activation energy of epimerization between the epimers.

Epimerization of moxalactam was further studied by Hashimoto et al. [28] in frozen urine and plasma samples during long-term storage. The *R*/*S* ratio at equilibrium in plasma was found to differ from that in aqueous solutions, owing to the difference in plasma protein binding between the epimers. Urine components also affected the *R*/*S* ratio at equilibrium. These observations suggested that the *R*/*S* ratio may change during storage depending on the components in samples. It was recommended that biological samples should be stored at or below −70°C to prevent epimerization.

4. Stereoselectivity in absorption

The stereospecific absorption and degradation of cephalexin have been reported by Tamai et al. [15]. After oral administration to rats the D-epimer was detected in serum and urine, whereas the L-epimer was not detected in either serum or urine. However, the L-epimer competitively inhibited the uptake of the D-epimer by the

everted intestine in vitro, suggesting that both epimers can be absorbed from the intestine. Moreover, the affinity to the transporter, probably the dipeptide transporter, was found to be greater for the L-epimer than the D-epimer. On the other hand, the L-epimer rapidly degraded in the intestinal tissue homogenate, serum and urine, whereas no appreciable degradation was observed for the D-epimer. It was concluded that both cephalexin epimers may be absorbed from the intestine but that only the D-epimer can be detected in the body owing to immediate hydrolysis of the L-epimer after absorption.

Kramer et al. [29] measured the uptake of cephalexin epimers into rabbit small intestinal brush border membrane vesicles. The D-epimer was taken up by the vesicles, whereas the L-epimer was not taken up to a significant extent. After reconstitution of brush border membrane proteins into proteoliposomes, only liposomes containing the M_r 127 000 binding protein showed a significant uptake of the D-epimer, whereas the L-epimer was not transported by the proteoliposomes. However, the L-epimer was slightly more effective than the D-epimer in preventing the photolabelling of the M_r 127 000 protein, suggesting that the affinity to the intestinal dipeptide transport system may be greater for the L-epimer than for the D-epimer. This observation appeared to be consistent with that reported by Tamai et al. as described above.

Uptake of cefaclor (**13**) was studied by Dantzig et al. [30] using human CaCo-2 cell line. It was found that cefaclor was transported by a dipeptide carrier and that the uptake was inhibited to a greater extent by the presence of the L-isomer than the D-isomer of the dipeptide, Gly–Phe. The concentration required for 50% inhibition was ten times greater for Gly–D-Phe than for Gly–L-Phe, suggesting that the carrier may have a preference for the L-isomer.

The transport of ceftibuten and its *trans* isomer into rat intestinal brush border membrane vesicles was studied by Yoshikawa et al. [31] and Muranushi et al. [32]. *cis*-Ceftibuten was transported via the oligopeptide transport system, and its uptake into the vesicles was stimulated in the presence of an H^+ gradient. In contrast, the uptake of the *trans* isomer was very small, and was not stimulated by the H^+ gradient. As the lipophilicity was similar between the *cis* and *trans* isomers, it was concluded that the uptake of ceftibuten and its *trans* isomer was stereoselective.

5. Stereoselectivity in distribution and disposition

Plasma protein binding of carbenicillin epimers was measured in vitro in humans [11,33]. The binding was stereoselective with the unbound fraction of the *R*-epimer being greater than that of the *S*-epimer (Fig. 3). It was revealed that the stereoselectivity in human plasma resulted from that in the binding to albumin (HSA) [34]. There were at least three binding sites on HSA for both epimers, and only one of the binding sites showed stereoselectivity; the affinity of the *S*-epimer to the stereoselective site was about four times greater than that of the *R*-epimer. The affinities to other binding sites were similar between the epimers. Moreover, the epimers seemed to compete at all the binding sites.

Carbenicillin was intravenously administered to human volunteers and the plasma concentrations and urinary excretion were measured [33]. Renal clearance was greater for the *R*-epimer than for the *S*-epimer, indicating the stereoselectivity in renal excretion. Renal clearance of unbound drug was greater than the

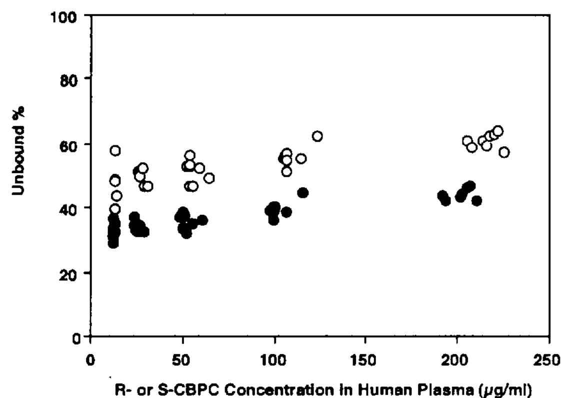

Fig. 3. Unbound fraction of (○) *R*- and (●) *S*-epimers of carbenicillin (CBPC) in human plasma.

glomerular filtration rate (GFR) for both epimers, suggesting that both epimers are actively secreted. Moreover, when probenecid was coadministered, the renal clearances decreased, and the renal clearances for unbound drug were almost equal to the GFR for both epimers. These results indicate that both epimers are secreted by the organic anion transport system in the renal proximal tubules. The secretion rate calculated was greater for the *S*-epimer than for the *R*-epimer, suggesting that the organic anion transport system may distinguish carbenicillin epimers. However, in the glomerular filtration process the *R*-epimer is cleared faster than the *S*-epimer owing to the greater unbound fraction of the *R*-epimer in plasma. Therefore, in the overall renal excretion process, the faster glomerular filtration of the *R*-epimer overrides the faster renal tubular secretion of the *S*-epimer, which results in greater renal clearance of the *R*-epimer.

In rabbits, the stereoselectivity in plasma protein binding was opposite to that in humans, i.e., the unbound fraction was greater for the *S*-epimer than for the *R*-epimer (Fig. 4) [11]. This suggests that the stereoselectivity in binding may differ from one species to another, which will make it difficult to predict the stereoselectivity in humans from animal data.

Binding of *cis*-ceftibuten and its *trans* isomer in human serum was also stereoselective; the binding was more extensive for the *cis* than for the *trans* isomer [14]. The major binding protein appeared to be HSA, and both isomers were mainly bound to the warfarin binding site. Therefore, it was most likely that the warfarin site exhibited the stereoselectivity in the binding of ceftibuten and its *trans* isomer.

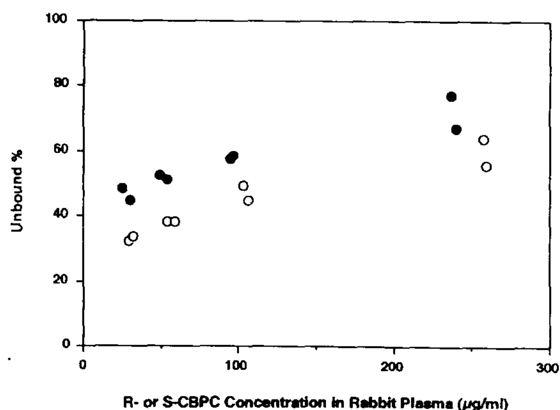

Fig. 4. Unbound fraction of (○) *R*- and (●) *S*-epimers of carbenicillin (CBPC) in rabbit plasma.

The effect of isomerization on the pharmacokinetics of ceftibuten was studied in humans [14]. Plasma concentrations and urinary excretion of both *cis* and *trans* isomers following oral administration of *cis*-ceftibuten were simulated using a physiological model. The model incorporated the isomerization of ceftibuten in plasma and interstitial fluid. The urinary excretion of the *trans* isomer was fairly well predicted, which confirmed that the isomerization of ceftibuten to its *trans* isomer actually occurs in the body.

The isomerization of ceftibuten to its *trans* isomer in the body was also suggested by Matsuura et al. [13]. In their studies, after oral adminstration of ceftibuten to humans, approximately 9% of the dose was recovered in urine as the *trans* isomer, whereas the urinary recovery of ceftibuten was ca. 70%.

Binding of moxalactam in human plasma was measured in vitro using an ultrafiltration method [35]. The binding was stereoselective with the unbound fraction of the *R*-epimer being greater than the *S*-epimer (47% and 33%, respectively). After intravenous administration of moxalactam to humans, more than 90% of the dose was recovered unchanged in urine, indicating that the urinary excretion is the major elimination route for both epimers. Although renal clearance was greater for the *R*-epimer than for the *S*-epimer, the renal clearances for the unbound drug were similar between the epimers. Moreover, the renal clearance values for the unbound epimers were very close to the GFR. These observations suggested that the contribution of renal secretion was insignificant in the renal excretion of moxalactam epimers and that the difference in the renal clearances between the epimers reflected that in the plasma protein binding.

Binding of moxalactam epimers in dog plasma was also stereoselective, with the unbound fraction of the *R*-epimer being greater than that of the *S*-epimer (76% and 55%, respectively) [36].

Urinary excretion was the major elimination route, and the glomerular filtration was dominant with almost no contribution from tubular secretion. The difference in the renal clearances between the epimers could be accounted for by the difference in plasma protein binding. Therefore, the pharmacokinetics of moxalactam epimers in dogs appeared to be similar to those in humans.

Pharmacokinetics of moxalactam epimers were also studied in rats [37]. The plasma protein binding was not stereoselective (unbound fraction of ca. 50% for both epimers), nor was the renal clearance. The results indicated that the stereoselectivity may differ among animal species and that it may be difficult to predict the stereoselectivity unless a suitable model animal is chosen.

6. Conclusions

β-Lactam antibiotics have been used clinically for a number of years. Most of the β-lactams, e.g., ampicillin, are used as a single isomer owing to the significant difference in activity between isomers. However, some β-lactams are used as mixtures of diastereomers in spite of the fact that the activities of the isomers are not equal. For sulbenicillin commercial preparations, the content of the more potent epimer is greater than that of the less active epimer, and the epimer/epimer ratio appears to be somewhat controlled. For other β-lactams, the commercial preparations are ca. 1:1 mixtures of isomers, but the content of the more active isomers seem to vary from one manufacturer to another, the clinical importance of which is yet to be clarified.

There have been numerous studies to determine antimicrobial activity, pharmacokinetics and pharmacodynamics of the diastereomeric β-lactam antibiotics. However, most studies employed conventional microbiological methods which failed to distinguish diastereomers. Also, many HPLC methods failed to resolve diastereomers, which again failed to give information on the differences between isomers with regard to activity, stability, pharmacokinetics and pharmacodynamics.

Stereoselectivity appears to exist for diastereomeric β-lactams in various processes in the body, such as absorption, distribution and excretion. If the drug is transported by the carrier system, diastereomers may be distinguished by the system, e.g., cephalexin, cefaclor and ceftibuten in the absorption process and carbenicillin in the secretion process. As it is well known that many β-lactams are absorbed from the intestine by the dipeptide carrier system and are secreted in the renal proximal tubule by the organic anion transport system, it is very likely that these processes are stereoselective for many β-lactam isomers.

Stereoselectivity may also exist in distribution. Plasma protein binding has been shown to be stereoselective for carbenicillin and moxalactam. The binding of the geometric isomer ceftibuten is also stereoselective. Moreover, epimer–epimer interaction has been observed in the binding of carbenicillin epimers to HSA. It has to be clarified if the differences in binding and the existence of isomer–isomer interactions are clinically important. The isomer–isomer interaction may also occur in the processes of absorption and secretion, the possibility of which has not been studied.

As the L-epimer of cephalexin is so susceptible to degradation, the significant difference in activity between cephalexin isomers may be due to the difference in stability, not to the difference in intrinsic activity. In contrast, isomerization during microbiological assay procedures may result in a lack of difference in the activity between the isomers of some β-lactam antibiotics, as has been suggested by Butler et al. [1] and also by Hoogmartens et al. [4]. The difference in stability between isomers and also the isomerization have to be taken into account when the activities between isomers are compared.

Isomerization may occur not only in solutions but also in the body, as has been shown for ceftibuten. Further, isomerization in the body may occur for many diastereomeric β-lactams. Indeed, epimerization in serum has been observed for carbenicillin, moxalactam and sul-

benicillin. Isomerization is one of the critical factors in evaluating chiral drugs as to whether the drug should be used as a single isomer or can be used as a mixture of isomers. Even if the single isomer is isolated and administered, the efficacy of the single isomer may be equal to that of the isomeric mixture owing to isomerization. Therefore, isomerization of diastereomeric β-lactams and the differences in activity and stability should be thoroughly studied in order to justify the clinical use of diastereomeric mixtures. Moreover, as the isomerization may occur both in frozen biological samples and in solutions, the storage conditions for diastereomeric β-lactams should be studied beforehand.

Despite the long history of clinical use of diastereomeric β-lactam antibiotics, the differences between isomers are not very well understood with respect to activity, stability, pharmacokinetics and pharmacodynamics. A better understanding of the differences between β-lactam isomes will contribute to the better clinical use of conventional diastereomeric β-lactam antibiotics and the development of novel chiral drugs.

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