PHARMACOLOGICAL STUDIES ON CARBAPENEM ANTIBIOTICS

III. CHEMICAL STRUCTURE OF PS-5D III, THE PRIMARY RENAL METABOLITE OF PS-5

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The chemical structure of PS-5D III, the primary breakdown product of PS-5 by particle-bound renal dipeptidase, is elucidated to be 3-(2-acetoamidoethyl)thio-5-(1-carboxypropyl)-1-pyrroline-2-carboxylic acid.

Unlike the penicillins and cephalosporins, PS-5 and related carbapenem compounds undergo a severe metabolism in kidneys to give the poor blood levels and urinary recoveries.[1] For correction of this defect of carbapenem antibiotics by chemical and biological methods without reducing the unexpectedly excellent and broad antimicrobial spectrum and the β-lactamase-inhibitory activity, it is vitally important to understand the detailed mechanism of inactivation of carbapenem compounds in kidneys, particularly by renal dipeptidase.[2,3]

This report elucidates the chemical structure of PS-5D III that is the primary metabolite of PS-5 by renal dipeptidase. It corresponds with the theoretical breakdown product of PS-5 by β-lactamase.[4]

Materials and Methods

PS-5D III

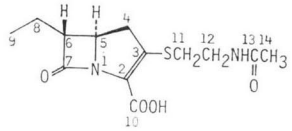
PS-5D III was prepared from PS-5 by partially purified particle-bound dipeptidase of rat kidney as described in the preceding paper.[3]

N-Acetyl PS-5D III Dimethyl Ester

A suspension of PS-5D III disodium salt (30 mg) in 2 ml of dimethylformamide (DMF) was mixed with 1 ml of acetic anhydride and was kept stirred for 60 minutes at 0°C. After acetylation, the reaction mixture was subjected to repeated extractions with 50 ml portions of *n*-hexane followed by rinsing with small volumes of ethyl ether and *n*-hexane to give a solid residue. The residue was dissolved in 4 ml of ice-cold DMF and was mixed with 0.2 ml of triethylamine and 1 ml of methyl iodide. After agitation for 2 hours at room temperature, the reaction mixture was poured into 40 ml of 1/5 M phosphate buffer, pH 7.4, to give a suspension. The product was extracted from the suspension with 40 ml of ethyl acetate. The ethyl acetate extract was collected, dried over anhydrous sodium sulfate and then concentrated to about 2 ml under reduced pressure. The concentrated extract was charged on a column (11 × 900 mm) of Bio-Beads S × 3 and developed with benzene as eluent. Under monitoring by silica gel thin-layer chromatography (pre-coated silica gel thin layer plates, silica gel 60 F_{254}; E. Merck, Darmstadt) using a solvent system of chloroform - methanol (10: 1), fractions containing a UV-absorptive compound at Rf 0.60 (corresponding with *N*-acetyl PS-5D III dimethyl ester) were collected and combined. Concentration to dryness under reduced pressure gave a residue. The residue was dissolved in 1 ml of benzene and was subjected to preparative thin-layer chromatography using the said solvent system on silica gel thin-layer plates which had been thoroughly washed with methylene chloride - methanol (4: 1). The areas of silica gel containing the UV-absorptive compound at Rf 0.60 were marked under a UV lamp and scra-

ped off from glass plates. *N*-Acetyl PS-5D III dimethyl ester was eluted from the silica gel with chloroform - methanol (10: 1) and the eluate was concentrated to dryness under reduced pressure. The evaporation residue was dissolved in a small volume of acetone and applied on a column (11 × 900 mm) of Sephadex LH-20. The column was developed with acetone. Fractions containing *N*-acetyl PS-5D III dimethyl ester were collected on a fraction collector. After removal of acetone by evaporation *in vacuo*, 11.0 mg of *N*-acetyl PS-5D III dimethyl ester was obtained.

Fig. 1. Numbering of PS-5.



General Methodology

UV, IR, mass, ^{1}H NMR and ^{13}C NMR spectra were recorded with a Hitachi 200–20 UV/visible spectrometer, a Hitachi 260–30 IR spectrometer, a Hitachi RMU-7 mass spectrometer, a JEOL PS-100 spectrometer and a JEOL FX-100 spectrometer, respectively.

In this paper we employ the numbering system as shown in Fig. 1.

Results

Spectroscopic Properties of PS-5D III

PS-5D III, the primary metabolite of PS-5 by renal dipeptidase, exhibited no characteristic UV absorption, whereas PS-5 and related carbapenems having the saturated C-3 side chain produce a distinct peak around 300 nm. Thus the carbapenem skeleton seemed to be broken.

Fig. 2 represents the IR spectrum of PS-5D III disodium salt in KBr. The absorption band at 1760 cm^{-1} assigned to the carbonyl group of the β-lactam ring of PS-5[5] is not seen in Fig. 2, suggesting that the metabolite has no β-lactam structure.

The 100 MHz ^{1}H NMR spectrum of PS-5D III disodium salt in D_2O is shown in Fig. 3. Compared with PS-5, it is noteworthy that the metabolite gave some proton peaks as mixtures of closely resembling signals. For example, two triplets of methyl at δ 0.86 and δ 0.88 and two triplets of methylene at δ 2.71 and δ 2.74 were obviously identified. Thus PS-5D III was considered to be a mixture of diastereomers in solution. In addition, a new proton signal is overlapping to the C-5 H at δ 4.0 ~ 4.5.

Table 1 summarizes the comparative spectroscopic data of PS-5D III and PS-5.

On the basis of these findings, PS-5D III was assumed to have a chemical structure similar to that of the non-β-lactam compound II of MC696-SY-2-A.[6]

Fig. 2. IR Spectrum of PS-5D III 2Na (KBr).

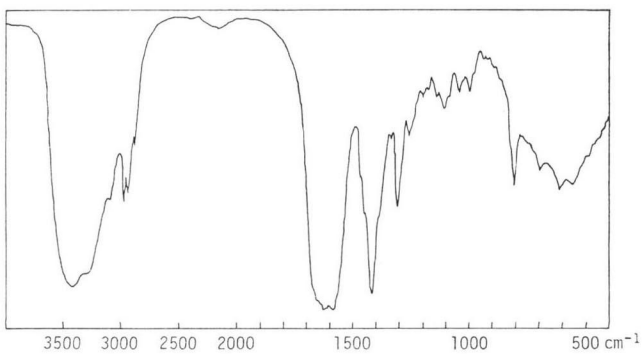


Fig. 3. 100 MHz ^{1}H NMR spectrum of PS-5D III 2Na in D_2O.

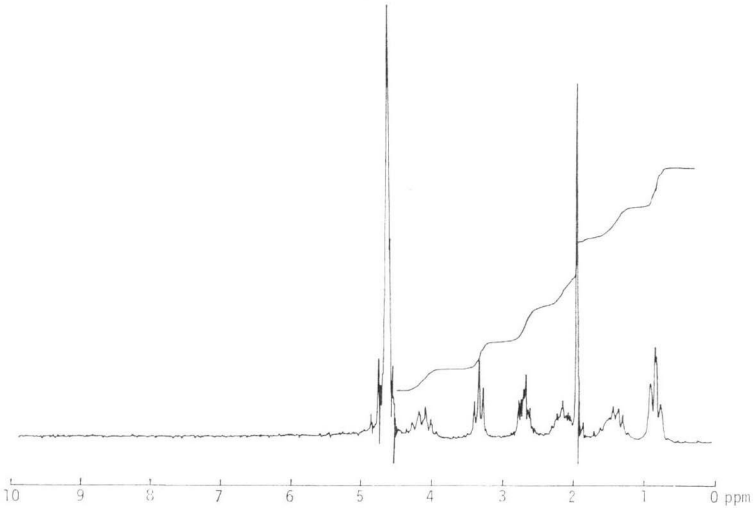


Table 1. Comparative spectroscopic data of PS-5D III and PS-5.

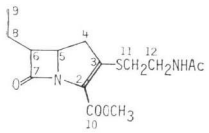
	PS-5D III 2Na	PS-5 Na
^{1}H NMR (D_2O)	(DSS)	(TSP)
9-CH_3	0.86, 0.88 (3H, t×2, J=7 Hz)	1.06 (3H, t, J=7 Hz)
8-CH_2	1.45~1.75 (2H, m)	1.72~2.0 (2H, m)
6-CH	1.95~2.65 (1H, m)	2.88~3.58 (1H, m)
5-CH	3.95~4.45 (1H, m)	4.04 (1H, dt, J=3.0, 9.2 Hz)
4-CH_2	1.95~2.65 (2H, m)	2.88~3.58 (2H, m)
11-CH_2	2.71, 2.74 (2H, t×2, J=6.5 Hz)	2.88~3.58 (2H, m)
12-CH_2	3.40 (2H, t, J=6.5 Hz)	2.88~3.58 (2H, m)
14-CH_3	1.97 (3H, s)	2.05 (3H, s)
3-CH	3.95~4.45 (1H, m)	—
$UV_{max}^{H_2O}$ nm	End absorption	301
IR (KBr; cm^{-1})	1680, 1630, 1580, 1420	1760, 1650, 1600, 1555, 1400
FD-MS (m/z)	383 $(M+Na)^+$	—

Chemical Structure of *N*-Acetyl PS-5D III Dimethyl Ester

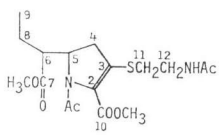
For structural elucidation, PS-5D III was acetylated and methylated to give *N*-acetyl PS-5D III dimethyl ester which yielded useful information in comparison with PS-5 methyl ester.[7] The spectroscopic data of *N*-acetyl PS-5D III dimethyl ester and PS-5 methyl ester are comparatively shown in Table 2.

N-Acetylation resulted in the restoration of the UV absorption peak at 301 nm characteristic of carbapenem compounds having the saturated C-3 side chain, indicating that the double bond was reestablished between C-2 and C-3. The cleavage of the β-lactam ring was again evidenced by the absence of the characteristic IR absorption band at 1760^{-1} cm and by the low-field chemical shift of C-5 H combined with the high-field chemical shift of C-6 H in the ^{1}H NMR spectrum. The similar change of chemical shifts is reported for 1,7-seco-*N*,*N*-diacetylthienamycin dimethyl ester.[8] The molecular ion peak of

Table 2. Spectral data of *N*-acetyl PS-5D III dimethyl ester and PS-5 methyl ester.



PS-5 methyl ester



N-Acetyl PS-5D III dimethyl ester

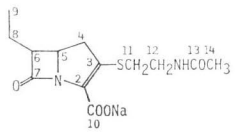
	IR (cm^{-1})	UV (nm)
N-Acetyl PS-5D III dimethyl ester	1725, 1660	301
PS-5 methyl ester	1780, 1710, 1680, 1560, 1505	316

^1H NMR

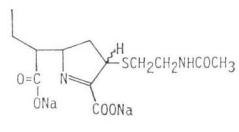
	9-CH_3	8-CH_2	6-CH	5-CH	4-CH_2	11-CH_2	12-CH_2	Ac	CH_3O-
N-Acetyl PS-5D III dimethyl ester	0.96 ppm (t)	1.62 (q)	2.5~2.9 (m)	4.64 (m)	2.61~3.21 (m)	2.5~3.0 (m)	3.39 (t)	2.00 (s) 2.08 (s)	3.68 (s) 3.82 (s)
PS-5 methyl ester	1.04 (t)	1.87 (m)	3.04 (m)	3.94 (d.t.) (J=3.0, 9.2 Hz)	2.80~3.56 (m)	2.80~3.56 (m)	2.80~3.56 (m)	1.95 (s)	3.81 (s)

^1H NMR spectra were recorded at 100 MHz in $CDCl_3$. Chemical shifts are given in parts per million relative to TMS as internal standard.

Table 3. Comparative ^{13}C NMR data of PS-5D III 2Na and PS-5 Na.



PS-5 Na



PS-5D III 2Na

Carbon No.	10	13	7	2	3	5	6
PS-5D III 2Na	183.6 (s)	177.5* (s)	174.2* 173.5* (s)	171.8* 171.1* (s)	57.7# 56.9# (d)	74.8 74.4 (d)	50.6# 49.8# (d)
PS-5 Na	184.0 (s)	175.0 (s)	169.3 (s)	141.1* (s)	130.4* (s)	60.2 (d)	55.6 (d)
Carbon No.	12	11	4	8	14	9	
PS-5D III 2Na	39.7 (t)	35.8 35.1 (t)	31.3 30.8 (t)	23.3 22.6 (t)	22.8 (q)	12.5 (q)	
PS-5 Na	40.0# (t)	39.9# (t)	31.5 (t)	22.5 (t)	22.6 (q)	11.4 (q)	

Unit in ppm

* and #: Assignments may be interchanged among indicated columns.

^{13}C NMR spectra were recorded in D_2O with dioxane as internal standard.

Fig. 4. Chemical structure of *N*-acetyl PS-5D III dimethyl ester.

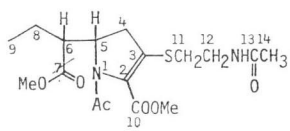
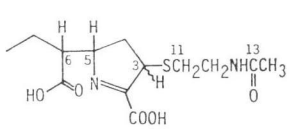


Fig. 5. Chemical structure of PS-5D III.



N-acetyl PS-5D III dimethyl ester was observed at *m/z* 386 in the mass spectrum.

The above-described spectral data allows a conclusion that *N*-acetyl PS-5D III dimethyl ester has a chemical structure as shown in Fig. 4

Chemical Structure of PS-5D III

The chemical structure of *N*-acetyl PS-5D III dimethyl ester (Fig. 4) and the spectroscopic data of PS-5D III disodium salt (Figs. 2 and 3 and Table 1) indicate that the chemical structure of PS-5D III is as presented in Fig. 5.

This structure of PS-5D III was supported by the observation that the cluster ion peak was located at *m/z* 383 $(M+Na)^+$ in the field desorption mass spectrum.

^{13}C NMR spectrometry also supported the chemical structure of PS-5D III shown in Fig. 5. Table 3 summarizes the comparative ^{13}C NMR spectroscopic data of PS-5D III and PS-5.

The diastereomeric property of PS-5D III is explicitly seen in the fact that most carbons give two closely neighboring signals.

Discussion

The chemical structure of PS-5D III, the primary metabolite of PS-5 (Fig. 5), indicates that renal dipeptidase cleaves the β-lactam ring of PS-5 to give a breakdown product which is assumed to be identical with the reaction product of β-lactamase. Although *N*-mono or di-methyl dipeptides such as *N*, *N*-dimethylglycyldehydrophenylalanine serve as exceptional substrates with very weak susceptibility,[9] the substrate profile reported by CAMPBELL *et al.*[10] shows that renal dipeptidase requires the presence of free α-amino and carboxyl groups in the dipeptide substrates. In spite of the absence of the free amino group in carbapenem skeleton, the results described in this and preceding papers unexpectedly prove that PS-5 and other carbapenem compounds serve as substrates for renal dipeptidase.

The unusual enzymatic susceptibility of carbapenem antibiotics is also reported in deacetylation of PS-5 by L-amino acid acylase and D-amino acid acylase,[11] although PS-5 is neither *N*-acyl-L-amino acid nor *N*-acyl-D-amino acid.

The cleavage of the β-lactam ring of the penicillins and cephalosporins is known to be catalyzed not only by β-lactamases which are responsible in the drug resistance mechanism of pathogens,[4] but also by DD-carboxypeptidases which are target enzymes of the drugs in pathogens.[12] Carbapenem antibiotics, on the other hand, have originally been isolated as β-lactamase inhibitors and have been found to be highly resistant to most β-lactamases. In these circumstances, carbapenem compounds may be a useful tool to find enzymological relations among β-lactamase, DD-carboxypeptidase, dipeptidase and amino acid acylase.

Some β-lactamases such as *Bacillus cereus* 569 type II β-lactamase (cephalosporinase) are reported to hydrolyze carbapenem antibiotics.[13] As this cephalosporinase requires zinc as the cofactor while dipeptidase is a zinc-containing enzyme, zinc may play an important role in the hydrolysis of the β-lactam ring. (Amino acid acylase requires cobalt for deacylation.)

Acknowledgement

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