[Chem. Pharm. Bull. 28(12)3527—3536(1980)]

Structural Investigation of New Metabolites of Amino-penicillins excreted in Human Urine

Mikio Masada, Yoshihiro Kuroda, Terumichi Nakagawa, and Toyozo Uno

Faculty of Pharmaceutical Sciences, Kyoto University[1]

(Received June 18, 1980)

New metabolites have been found in human urine after the oral administration of some amino-penicillins. HPLC analysis showed that the amino-penilicillin metabolite had the same retention time as an *in vitro* degradation product of the corresponding penicilloic acid in dilute $HgCl_2$ solution or in NaH_2PO_4 solution. The product was not formed by the same treatment of the intact penicillin. NMR, Raman, and FD mass spectra of the product and the visible absorption spectrum of its colored derivative indicated cleavage of the C–S bond in the thiazolidine ring of the penicilloic acid to leave an SH group. These findings indicate that the metabolite is the penamaldic acid of the parent penicillin. Direct evidence for the presence of the penamaldic acid was obtained from the 200 MHz ^1H NMR spectrum of a urine specimen taken after drug administration. Ampicillin and amoxycillin gave metabolites of this kind, but cyclacillin gave no detectable amount of the corresponding metabolite.

Keywords——amino-penicillins; penicilloic acids; penamaldic acids; 6-amino-penicillanic acid; new metabolites; degradation product; NMR spectra; FD mass spectra; hgh performance liquid chromatography; Raman spectra

Introduction

Amino-penicillins such as ampicillin, amoxycillin, and cyclacillin are widely used in clinical chemotherapy because of their acid-stability, low toxicity efficient absorption and low minimum inhibitory concentrations against various gram-negative and gram-positive microorganisms.

Although various investigations have been made on the *in vitro* properties of penicillins, the entire metabolic profile in man is still unclear. It is known that penicillins have two types of specific sites susceptible to metabolic alteration. One is the β-lactam ring, and the other consists of substituent groups on the side chain. The *in vivo* hydrolysis of the former to yield a penicilloic acid is common to most penicillins, while side-chain biotransformation depends on the structure and properties of the substituents. The hydrolysis of a side-chain amide linkage to form 6-aminopenicillanic acid, however, seems suspicious.[2,3] Harken *et al.*,[4] Thijssen *et al.*,[5,6] and Murai *et al.*[7] studied the metabolism of isoxazolyl penicillins, and found that active metabolites, which are sometimes essential for therapeutic effect, because they may be present at higher serum levels than unchanged penicillins, arise by hydroxylation of the 5-methyl group on the isoxazolyl moiety. Phenoxymethylpenicillin is also known to undergo hydroxylation at the para position of the phenyl group.[8] In the case of amino-penicillins,

1) Location: *Yoshida Shimoadachi-cho, Sakyo-ku, Kyoto 606, Japan.*
2) A.R. English, H.T. Huang, and B.A. Sobin, *Proc. Soc. Exp. Biol. Med.* (N.Y.), **104**, 405 (1960).
3) M. Cole, M.D. Kenig, and V.A. Hewitt, *Antimicrob. Agents Chemother.*, 3, 463 (1973).
4) V. Harken, C.W. Dixon, and J.M. Essery, *Pharmacologist*, **12**, 220 (1970).
5) H.H.W. Thijssen and H. Mattie, *Antimicrob. Agents Chemother.*, **10**, 441 (1976).
6) H.H.W. Thijssen, *J. Antibiotics*, **32**, 1033 (1979).
7) Y. Murai, T. Nakagawa, and T. Uno, *Chem. Pharm. Bull.*, **28**, 362 (1980).
8) H. Vanderhaeghe, G. Parmentier, and E. Everard, *Nature* (London), **200**, 891 (1963).

besides the formation of the penicilloic acid by β-lactam ring opening,[3,9–15] a conjugate product of the benzylamino group with formaldehyde was reported as a minor active metabolite of ampicillin in man.[16,17] However, no active metabolite was found in a subsequent bioautographic analysis of human urine after the administration of ampicillin and amoxycillin.[18] The literature survey mentioned above shows that only the penicilloic acid is definitely known as a metabolite of amino-penicillins in man.

In the course of studies on high performance liquid chromatographic determination and pharmacokinetic analysis of amino-penicillins, we found an unknown peak with an appreciable UV intensity due to a new metabolite on a chromatogram of human urine obtained after the oral administration of ampicillin. The peak was completely separated from those of unchanged penicillin, penicilloic acid and endogenous urinary components. From preliminary investigations on the chemical properties and spectral characteristics, the metabolite was considered to be the penamaldic acid of ampicillin.[19] Since this type of metabolism has not previously appeared in the literature and seems essential to penicillin skeleton, we attempted to confirm the structure of the metabolite and also examined the analogous metabolism of other amino-penicillins. The present paper describes our structural investigation of the new type metabolites of some amino-penicillins by using high performance liquid chromatography (HPLC), as well as nuclear magnetic resonance (NMR), Raman, and visible spectroscopy, and field desorption mass spectrometry (FDMS).

Experimental

1. Materials——The chemical structures and abbreviations for the compounds used in this work are presented in Fig. 1. The anhydrate of AB–PC* (potency: 983 μg/mg), the trihydrate of AM–PC** (potency: 845 μg/mg), the anhydrate of AC–PC* (potency: 1000 μg/mg), and 6-APA* were used as standard materials, and AB–PC capsules* (Solcillin® 250 mg as potency), AM–PC capsules** (Sawacillin® 250 mg as potency), and AC–PC capsules* (Vastcillin® 250 mg as potency) were administered to volunteers.

The penicilloic acids were synthesized by hydrolysis of penicillins in alkaline solution. (1) AB–PA: Standard AB–PC (10 mg) was dissolved in 10 ml of 0.02 N NaOH and allowed to stand at 37° for 45 min. The solution was neutralized with 0.02 N HCl. (2) AM–PA: Standard AM–PC (10 mg) was dissolved in 10 ml of 0.04 N NaOH and kept standing at 20° for 40 min. The solution was neutralized with 0.04 N HCl. (3) AC–PA: Standard AC–PC (10 mg) was dissolved in 10 ml of 0.025 N NaOH and kept standing at 20° for 30 min. The solution was neutralized with 0.025 N HCl. (4) 6-APA–PA: Standard 6-APA (10 mg) was dissolved in 10 ml of 0.5 N NaOH and kept standing at room temperature for 5 min. The solution was neutralized with 0.5 N HCl. HPLC analysis of these hydrolyzed products gave a single peak in each case, without unreacted penicillin or other degraded products, and the retention time was consistent with that of the corresponding hydrolyzed product obtained by penicillinase treatment (Tokyo Chemical Ind. Co., Ltd., Tokyo, Japan). Infrared spectra of the freeze-dried residues of these products showed no band at 1770 cm^{-1} ($\nu_{C=O}$ of β-lactam ring).

The degradation of penicilloic acids was carried out by the following two procedures: (1) NaH_2PO_4 method; a 1 ml portion of aqueous solution of a penicilloic acid obtained above was added to 1 ml of aqueous 1 M NaH_2PO_4 solution, and the mixture was kept in a thermostated water-bath for 6 hr at 30°. The degradation rate profile was observed at reaction temperatures from 20° to 40°, concentrations of NaH_2PO_4 from

9) T.L. Lee, L. D'Arconte, and M.A. Brooks, *J. Pharm. Sci.*, **68**, 454 (1979).
10) K. Miyazaki, O. Ogino, H. Sato, M. Nakano, and T. Arita, *Chem. Pharm. Bull.*, **25**, 253 (1977).
11) K. Miyazaki, O. Ogino, and T. Arita, *Chem. Pharm. Bull.*, **22**, 1910 (1974).
12) T. Murakawa, Y. Yokota, Y. Kono, M. Nishida, S. Goto, and S. Kuwahara, *Chemotherapy* (Tokyo), **21**, 1399 (1973).
13) K. Hellstom, A. Rosen, and A. Swahn, *J. Clin. Pharmacol. Ther.*, **16**, 826 (1974).
14) K. Hellstom, A. Rosen, and A. Swahn, *Europ. J. Clin. Pharm.*, **7**, 125 (1974).
15) H. Graber, T. Perenyi, M. Arr, and E. Luding, *Int. J. Clin. Pharmacol.*, **14**, 284 (1976).
16) M. Nishida, T. Murakawa, Y. Mine, S. Fukada, Y. Kono, and Y. Sueda, *J. Antibiotics*, **14**, 641 (1971).
17) T. Murakawa, Y. Kono, and M. Mishida, *J. Antibiotics*, **15**, 421 (1972).
18) M. Cole and B. Ridley, *J. Antimicrob. Chemother.*, **4**, 580 (1978).
19) M. Masada, T. Nakagawa, and T. Uno, *Chem. Pharm. Bull.*, **27**, 2877 (1979).
* Takeda Pharmaceutical Ind. Co., Ltd., Osaka, Japan.
** Fujisawa Pharmaceutical Ind. Co., Ltd., Osaka, Japan.

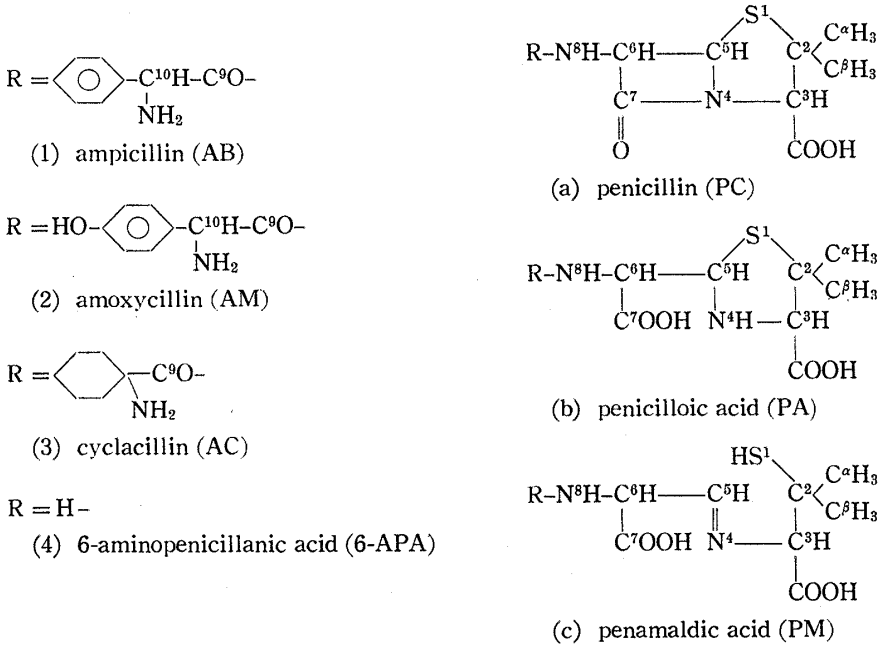


Fig. 1. Structures and Abbreviations

0.25 M to 1.5 M, and reaction times up to 8 hr. (2) $HgCl_2$ method; a 1 ml portion of penicilloic acid solution was added to 1 ml of aqueous 0.00125% $HgCl_2$ solution and the mixture was kept standing at room temperature for 3 min. The degradation rate profile was monitored with various $HgCl_2$ concentrations from 0.0001% to 0.1% and reaction times up to 60 min. Samples of these degradation products were prepared by lyophilization of the reaction solution for NMR and visible spectra measurements, and by isolation using a reversed phase column chromatography (LiChroprep RP-8, 25 mm × 310 mm, E. Merck, West Germany) for measurements of Raman spectra and FD mass spectra.

Sodium *n*-heptylsulfonate used as an ion-pairing agent for HPLC was synthesized by means of the Strecker reaction.[20]

Cyclosilane-d_{18}, sodium 3-trimethylsilyltetradeuteriopropionate (TSP), sodium 2,2-dimethyl-2-silapentane-5-sulfonate (DSS) and deuterium oxide (D_2O) used for NMR measurements were obtained from E. Merck, West Germany. Other chemicals used were of analytical reagent grade.

2. Measurements——1) HPLC: A high performance liquid chromatograph (TRI-ROTAR, JASCO, Tokyo, Japan) equipped with a variable wavelength UV detector (UVIDEC-100, JASCO) was used in a reverse phase mode with a stationary phase of Nucleosil $10C_{18}$ (M. Nagel, West Germany) packed in a 25 cm × 4.6 mm i.d. stainless steel tubing and operated at ambient temperature. A short pre-column (5 cm × 1.5 mm i.d.) filled with LiChrosorb RP-2 (E. Merck) was used to guard the main column. The mobile phase conditions were, (1) AB–PC; a mixture of methanol/water (5/8, v/v) containing 0.011 M sodium *n*-heptylsulfonate, 0.005 M NaH_2PO_4 and 1.3%(v/v) of 0.5 N HCl (pH 2.7) was used at a flow rate of 0.8 ml/min and the effluent was monitored at 218 nm, (2) AM–PC; a mixture of methanol/water (2/5, v/v) containing 0.0085 M sodium *n*-heptylsulfonate, 0.001 M NaH_2PO_4 and 1.3%(v/v) of 0.5 N HCl (pH 2.8) was used at a flow rate of 0.8 ml/min and the effluent was monitored at 228 nm, (3) AC–PC; a mixture of methanol/water (295/500, v/v) containing 0.01 M sodium *n*-heptylsulfonate, 0.001 M NaH_2PO_4 and 1.3%(v/v) 0.5 N HCl (pH 2.8) was used at a flow rate of 0.8 ml/min and the effluent was monitored at 210 nm, and (4) 6-APA; a mixture of methanol/water (1/15, v/v) containing 0.0125 M sodium *n*-heptylsulfonate, 0.0035 M NaH_2PO_4 and 1.3%(v/v) 0.5 N HCl (pH 2.7) was used at a flow rate of 1.5 ml/min and the effluent was monitored at 214 nm. The mobile phase was prepared by microporefiltration (0.45 μm) (Fuji Photo Film Co., Tokyo, Japan) and degassing of a mixture of glass-distilled water and methanol.

2) Visible Spectra: The visible spectra were measured on a UVIDEC-505 (JASCO) UV/VIS spectrophotometer.

3) Raman Spectra: The Raman spectra were measured on a JEOL S-1 (JEOL, Tokyo, Japan) laser Raman spectrophotometer as a methanol solution sealed in a 1 mm i.d. glass capillary tube. The slit width was 14 cm^{-1}. The 488.0 nm line of a Coherent CR-2 Ar^+ laser was used as the excitation source. The output power was about 100 mw at the sample position.

4) FD Mass Spectra: A JMS-01SG-2 mass spectrometer (JEOL) was used; emitter current 17 mA, acceleration voltage 10 kV, peak resolution>1000.

20) R.M. Reed and H.V. Tarter, *J. Am. Chem. Soc.*, **57**, 570 (1953).

5) NMR Spectra: 100 MHz ^1H NMR spectra were measured on a Varian HA-100 D NMR spectrometer. Cyclosilane-d_{18} sealed in a co-axial tube was used to provide an internal lock signal and as an external reference. Chemical shifts are reported downfield with respect to the internal TSP: δ (TSP) = δ (cyclosilane-d_{18}) − 0.52. The conversion constant was determined by using a separate sample containing a solution of TSP in D_2O. The 200 MHz ^1H NMR spectra were measured on a Varian XL-200 NMR spectrometer. Chemical shifts were referenced to internal DSS.

3. **Administration and Pretreatment**——Three healthy male volunteers, 23—24 years old, weighing 58—60 kg, each received two 250 mg capsules (AB–PC, AM–PC, or AC–PC) at once together with 100 ml of water after fasting for 12 hr. They were not permitted to eat until 3 hr after dosing. The subjects received different drugs at least two weeks apart. Urine specimens were collected according to a planned schedule. A 1 ml portion of a urine specimen was diluted with 1 ml of water, and passed through a 0.45 μm pore-size membrane filter (Fuji Photo Film Co.). A 5—50 μl portion of the filtrate was used for HPLC analysis. The specimens remained unchanged for at least one week when kept frozen at −20°.

Results

1. HPLC Separation of Urinary Metabolites

Recently, Lee *et al.*[9] described a method for the simultaneous determination of AM–PC and AM–PA excreted in human urine by using HPLC combined with fluorometric detection of their fluorescamine derivatives, but they did not refer to any new metabolite. The precise separation of urinary metabolites after administration of an amino-penicillin allowed us to detect the peak due to the new metabolite.

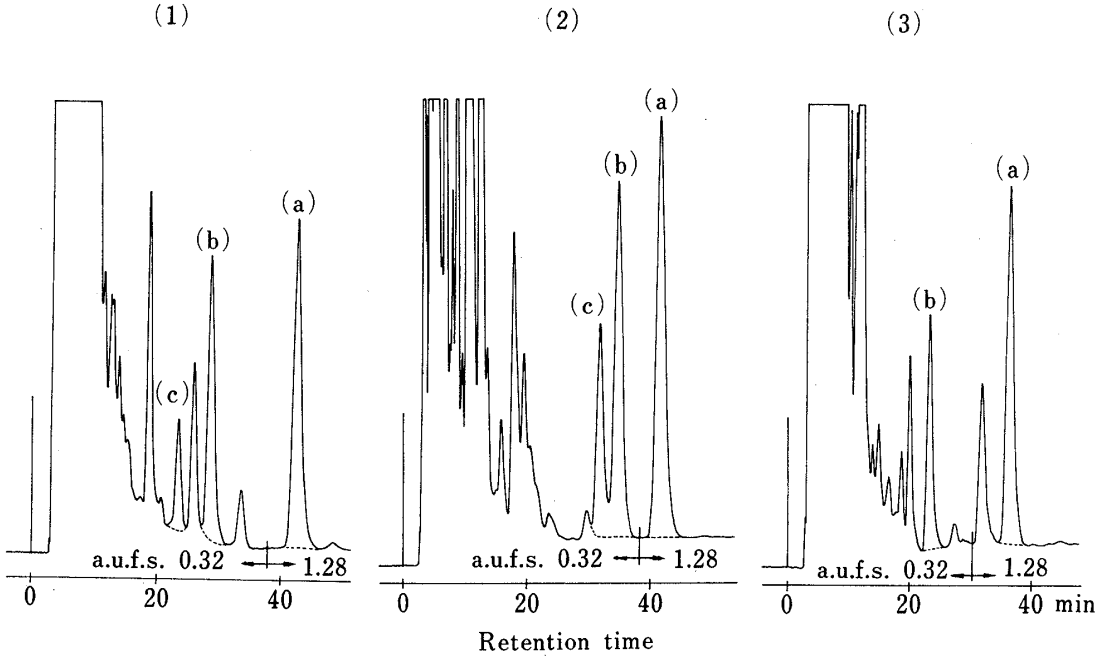


Fig. 2. Chromatogram of Human Urine Specimens after Oral Administration of 500 mg of Amino-penicillins ((1) AB–PC, (2) AM–PC, and (3) AC–PC)

Control urine (------) was obtained before drug administration. Peak: (a) unchanged penicillin, (b) penicilloic acid, (c) new metabolite.
Conditions: see the text.

Figure 2 shows chromatograms of human urine collected 2—3 hr after administration of AB–PC, AM–PC, and AC–PC; the broken line indicates a background chromatogram of control urine taken just before drug administration. It was found, as expected, that peaks of unchanged penicillins and penicilloic acids were common to all the penicillins examined, but the new metabolite peak (peak (c) in Fig. 2) was found only after administration of AB–PC and AM–PC. In spite of careful searching under various HPLC conditions, AC–PC did not

show any trace of a peak due to a corresponding new metabolite.

The metabolite peaks from AB–PC and AM–PC have longer retention times than that of 6-APA, which overlaps with the background peak. Therefore, the excretion of 6-APA after oral administration of amino-penicillins was investigated under different HPLC conditions. Figure 3 shows chromatograms of human urine specimens after oral administration of 500 mg of amino-penicillicins; no peak corresponding to 6-APA can be seen. This indicates that the excretion of 6-APA, if it occurs, is less than 2 μg/ml, the limit of detection by the present method.

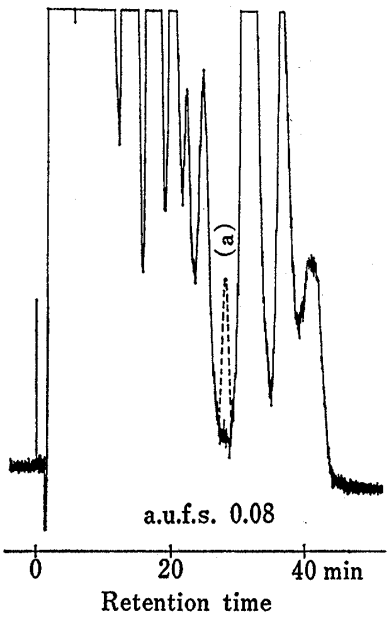


Fig. 3. Chromatogram of Human Urine Specimens after Oral Administration of 500 mg of Amino-penicillins (AB–PC, AM–PC, and AC–PC)

(a) indicates the retention time of authentic 6-APA dissolved in the urine specimens. Conditions: see the text.

In order to investigate the origin of this metabolite, some *in vitro* experiments were performed. When penicilloic acids of amino-penicillins were kept either in NaH_2PO_4 solution or in $HgCl_2$ solution, a degradation product having the same retention time as the new metabolite was obtained in the cases of AB–PC, AM–PC, and AC–PC (Fig. 4). The peak intensity of the degradation product (peak (c) in Fig. 4) increased with a simultaneous decrease in that of the penicilloic acid (peak (b)). The rate of degradation in 0.00125% $HgCl_2$ solution was so rapid that the amount reached a maximum at 3 min after the initiation of the reaction, while the amount of degradation product in NaH_2PO_4 solution continued to increase for 6 hr, followed by a gradual decrease with the formation of other by-products. Higher concentrations of $HgCl_2$ and higher reaction temperatures induced the increasing formation of by-products. The rate profile was essentially common to AB–PC, AM–PC, and AC–PC. The parent penicillin itself did not undergo such degradation upon similar treatment.

In order to confirm that the metabolite is identical with the degradation product of the penicilloic acid, the urine specimens of Fig. 2 were treated with 0.00125% $HgCl_2$ solution. Figure 5 shows the resulting chromatograms. The decrease in the peak of penicilloic acid (peak (b) in Fig. 5) is again accompanied by an increase in the new metabolite peak (peak (c) in Fig. 5). No formation of other degradation products was observed, and the background chromatogram was unchanged by the same treatment of the control urine. A similar result was obtained upon NaH_2PO_4 treatment of the same urine specimens. It was confirmed by varying the HPLC conditions that the metabolite peak consisted of a single substance. These results made it possible to deduce the structure of the new metabolite without its isolation from the urine specimens.

2. Detection of SH Group

1) Visible Spectra——It is well known that the C–S bond in the thiazolidine ring of a penicilloic acid is cleaved by Hg^{2+}.[21,22] Therefore, we examined the coloration reaction of the new metabolite with Feigl's disulfide (disulfide of 4,4′-bis(dimethylamino)thiobenzophenone), which has been used for the detection of thiol proteins.[23] As shown in Fig. 6, the

21) J.P. Hou and J.W. Poole, *J. Pharm. Sci.*, **60**, 503 (1971).
22) B. Bleivine, *Arch. Biochem. Biophys.*, **93**, 50 (1961).
23) L. Jirousek and M. Soodak, *Biochem. Biophys. Res. Commun.*, **59**, 927 (1974).

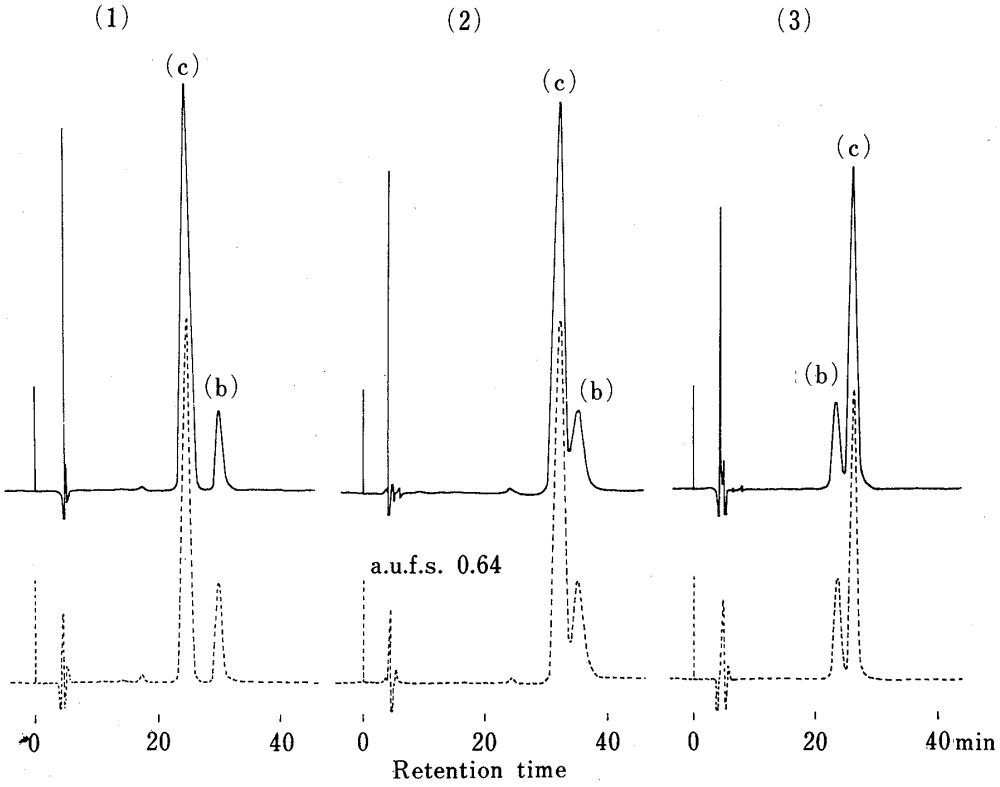


Fig. 4. Chromatograms of (1) AB–PA, (2) AM–PA, and (3) AC–PA after Standing at 30° for 6 hr in Aqueous 1 M NaH_2PO_4 Solution (----) and at Room Temperature for 3 min in Aqueous 0.00125% $HgCl_2$ Solution (——)

Peak: (b) penicilloic acid, (c) degradation product of penicilloic acid.
Conditions: see the text.

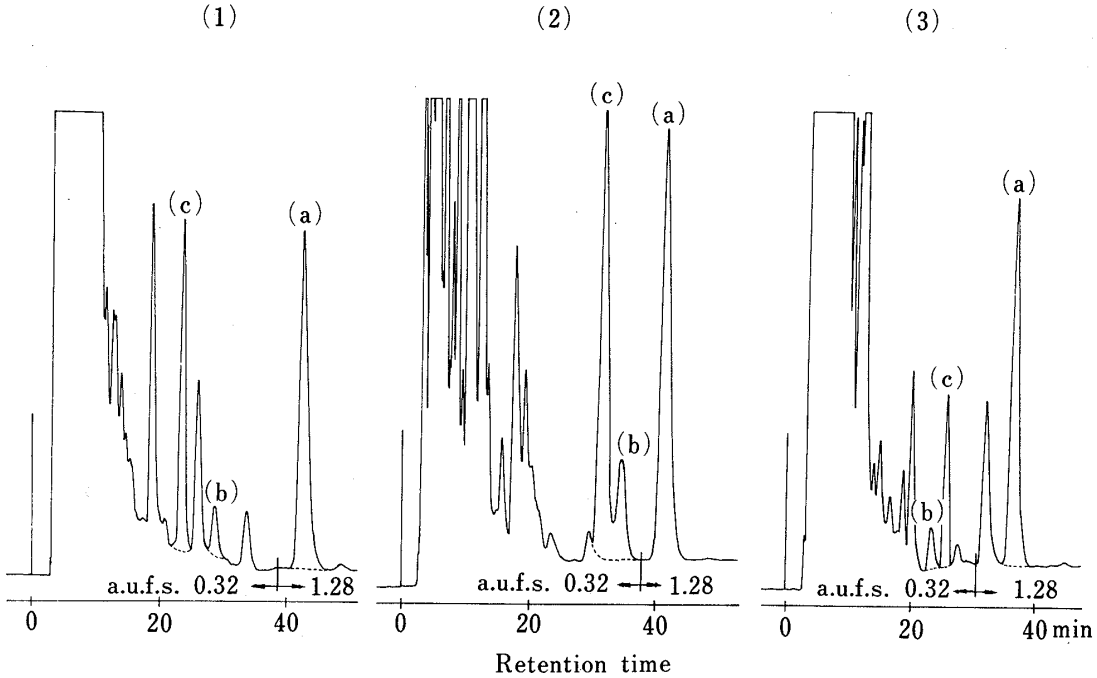


Fig. 5. Chromatogram of Human Urine Specimens obtained after Oral Administration of 500 mg (1) AB–PC, (2) AM–PC, and (3) AC–PC, and of Control Urine obtained before Drug Administration (----), All treated with Aqueous 0.00125% $HgCl_2$ Solution

Peak: (a) unchanged penicillin, (b) penicilloic acid, (c) new metabolite.
Conditions: see the text.

spectrum of the colored degradation product of the penicilloic acid treated with 1 M NaH_2PO_4 solution is quite similar to that of the penicillamine, which has an SH group (λ_{max}: 385, 450, 630 nm), but is different from those of the penicillin, the penicilloic acid and the reagent blank (λ_{max}: 325, 530, 640 nm).

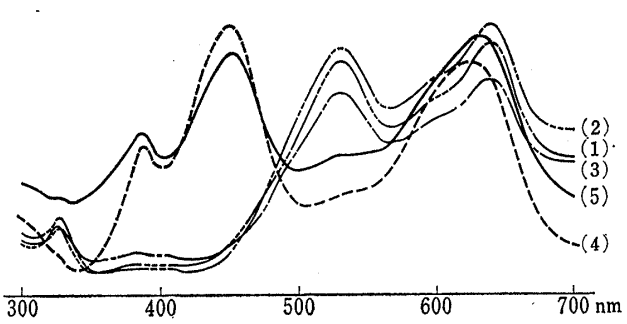


Fig. 6. Absorption Spectra of (1) Coloration Reagent (Feigl's disulfide), (2) Penicilloic Acids+Coloration Reagent, (3) Authentic Penicillins+Coloration Reagent, (4) Penicillamine+Coloration Reagent, and (5) 1 M NaH_2PO_4 Degradation Product of Penicilloic Acid+Coloration Reagent

2) **Raman Spectra**——Since it was thus expected that the new metabolite contained an SH group, we measured the Raman spectrum of the degradation product of the penicilloic acid. Raman spectroscopy permits the use of an aqueous sample solution, and gives a stronger ν_{SH} band than infrared spectroscopy.

The Raman spectrum of the degradation product of penicilloic acid treated with aqueous 1 M NaH_2PO_4 solution shows a strong Raman band at 2480 cm^{-1} which is characteristic of SH stretching vibration. The SH group of the penicillamine also gave a strong band at 2530 cm^{-1}. This indicates cleavage of the C–S bond of the thiazolidine ring of the penicilloic acid to form an SH group.

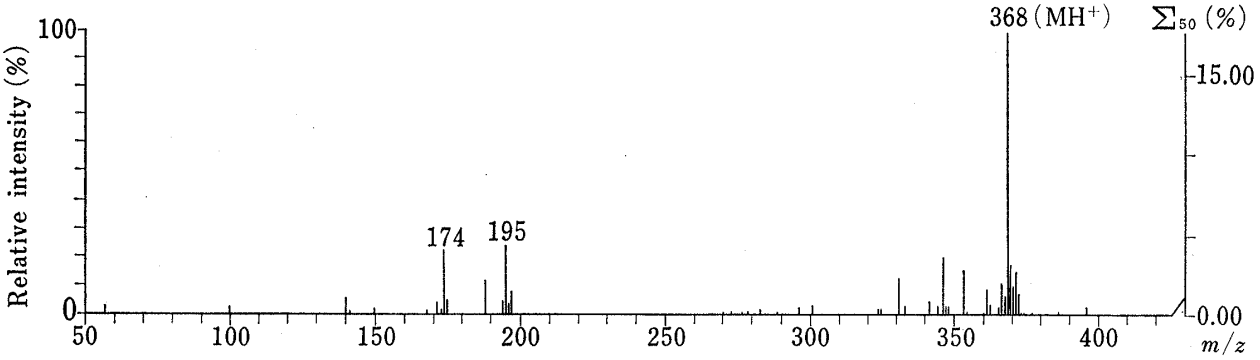


Fig. 7. FD Mass Spectrum of the 1 M NaH_2PO_4 Degradation Product of AB–PA

3. FD Mass Spectra

The degradation product of AB–PA in NaH_2PO_4 solution was isolated and the FD mass spectrum was measured. The spectrum (Fig. 7) gave a base peak at m/z 368 which is assignable to $(M+1)^+$ of $C_{16}H_{21}O_5N_3S$. This molecular formula corresponds to AB–PC ($C_{16}H_{19}O_4N_3S$)+H_2O, indicating that the degradation of AB–PA caused no change in molecular weight. The fragment peaks at m/z 174 and 195 are unidentified.

4. NMR Spectra

Although many papers have described NMR spectral features of various penicillins[24–27] and cephalosporins,[28,29] there have been no reports on the spectral changes accompanying

24) R.D.G. Cooper, P.V. Demarco, J.C. Cheng, and N.D. Jones, *J. Am. Chem. Soc.*, **91**, 1408 (1969).
25) B.C. Carlstedt, H.L. Crespi, M.I. Blake, and J.J. Katz, *J. Pharm. Sci.*, **60**, 1661 (1971).
26) W.L. Wilson, H.W. Avdovich, and D.W. Hughes, *J. Assoc. Off. Anal. Chem.*, **57**, 1300 (1974).
27) C.M. Dobson, L.O. Ford, S.E. Summers, and R.J.P. Williams, *J. Chem. Soc.*, Faraday II, **1975**, 1145.
28) R.J. Warren, J.E. Zarembo, D.B. Staiger, and A. Post, *J. Pharm. Sci.*, **67**, 1481 (1978).
29) G.F.H. Green, J.E. Page, and S. Staniforth, *J. Chem. Soc.*, **1965**, 1595.

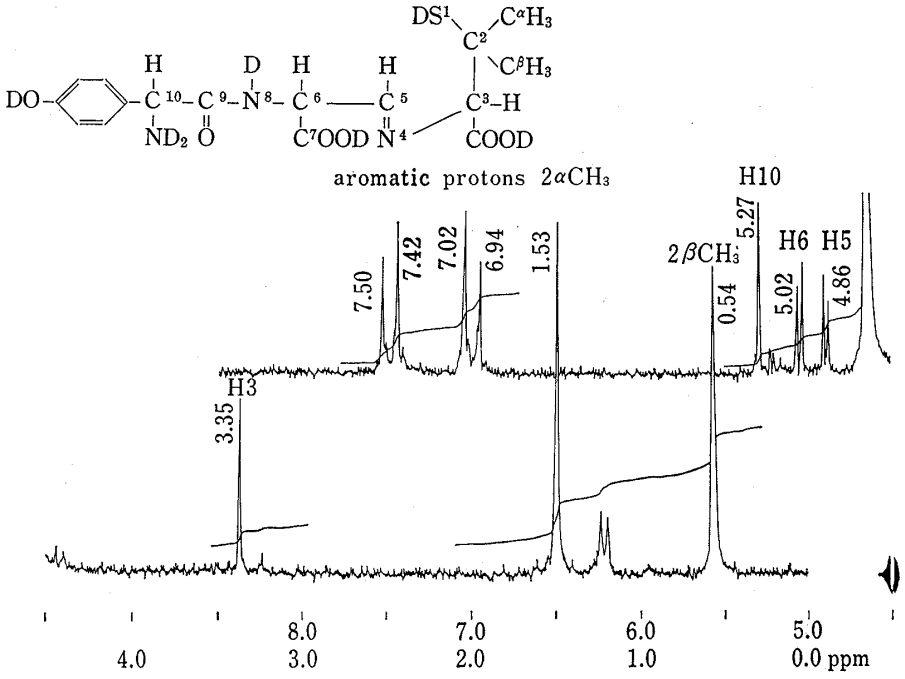


Fig. 8. The 100 MHz ^{1}H NMR Spectrum of the 1 M NaH_2PO_4 Degradation Product of AM–PA

the degradation of penicillins. In order to investigate the molecular structure of the new metabolite, we measured the NMR spectra of amino-penicillins, penicilloic acids, and the degradation products of penicilloic acids. Figure 8 shows a 100 MHz ^{1}H NMR spectrum of the degradation product of AM–PA obtained by treatment with NaH_2PO_4 solution. The peak assignments were made by taking into account spin-spin coupling, nuclear Overhauser effect, and the expected chemical shift values mentioned below. Among the peaks observed, the signals due to the C5 and C6 protons (H5, H6) on the β-lactam ring and the 2α-, 2β-CH_3 protons on the thiazolidine ring were noted in order to characterize the degradation of penicillins. Figure 9 is a schematic illustration of the dependence of chemical shifts on the structures of penicillins, penicilloic acids, and degradation products of penicilloic acids. As expected, the signals of the H5 and H6 protons (Fig. 9 (A)) appear as an AB type quartet for AM–PC (J= 3.7 Hz), AC–PC (J=3.8 Hz), and 6-APA (J=4.0 Hz), while these β-lactam protons of AB–PC appear as a singlet at 5.43 ppm. The corresponding signals of penicilloic acids, however, show larger differences in AB chemical shift than the parent penicillins in all cases. The subsequent degradation of penicilloic acids leads to a decrease of the chemical shift difference, though it is still larger than in the case of the parent penicillins. Figure 9 (B) illustrates the chemical shifts of 2α- and 2β-CH_3 protons; AB–PC and AB–PA each give an apparent single peak at almost the same resonance position, but the degradation product of AB–PA shows a remarkable peak separation with an unexpected upfield shift of one methyl signal at 0.69 ppm. Similar behavior is observed with the degradation product of AM–PA, which shows an upfield shift at 0.54 ppm. The β-lactam ring opening of AC–PC and 6-APA, unlike that of AB–PC and AM–PC, causes a wider separation of the signals than subsequent cleavage of the C–S bond does. These observations imply that the upfield shifts are due to the so-called aromatic ring current effect. Indeed, we can easily construct a molecular model locating one methyl group above the benzene ring. This structure is possible only when the C–S bond in the thiazolidine ring of the penicilloic acid is cleaved.

Thus, it follows that if a penamaldic acid exists as a metabolite of AM–PC in human urine, it might be expected to show the signal due to the methyl group at about 0.5 ppm in the 200 MHz ^{1}H NMR spectrum. This is in fact observed, as shown in Fig. 10, where the samples

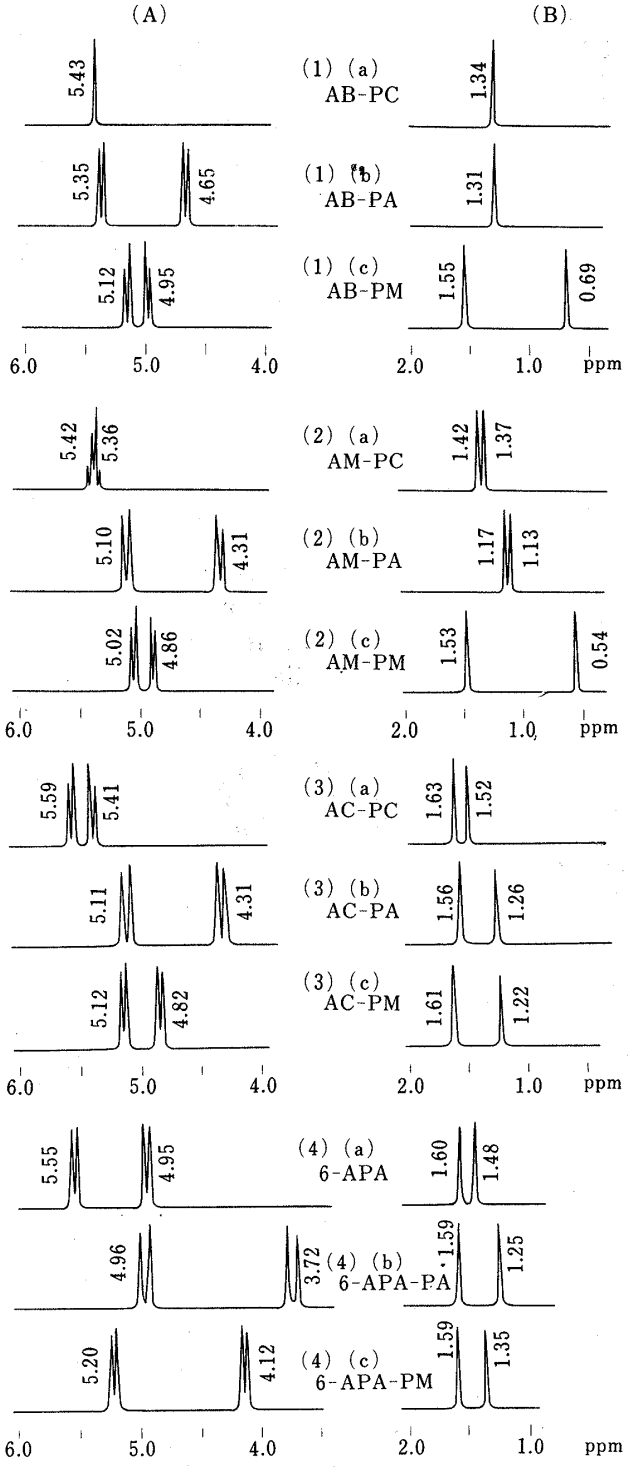


Fig. 9. Schematic Illustration of the Chemical Shifts of (A) H5 and H6 of the β-Lactam Ring and (B) $2\alpha CH_3$ and $2\beta CH_3$ on the Thiazolidine Ring

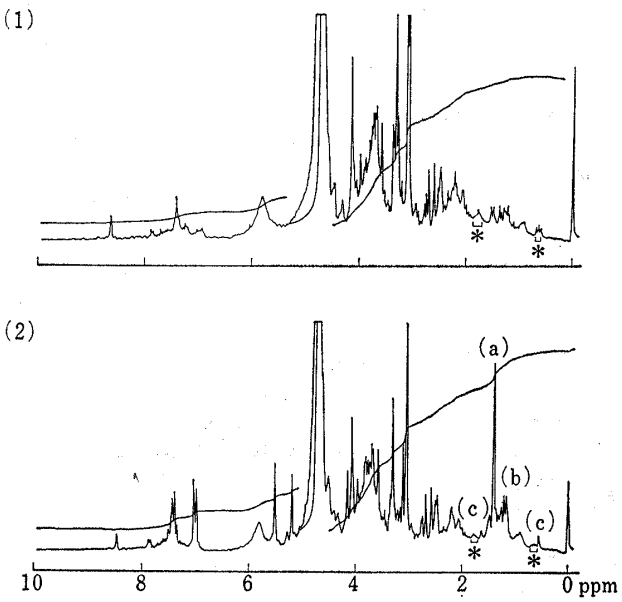


Fig. 10. The 200 MHz ^1H NMR Spectra of (1) Control Urine and (2) Human Urine after Oral Administration of 1 g of AM–PC

The indicated signals are assigned to the 2α-, 2β-methyl protons of (a) AM–PC, (b) AM–PA, and (c) the new metabolite, and * DSS.

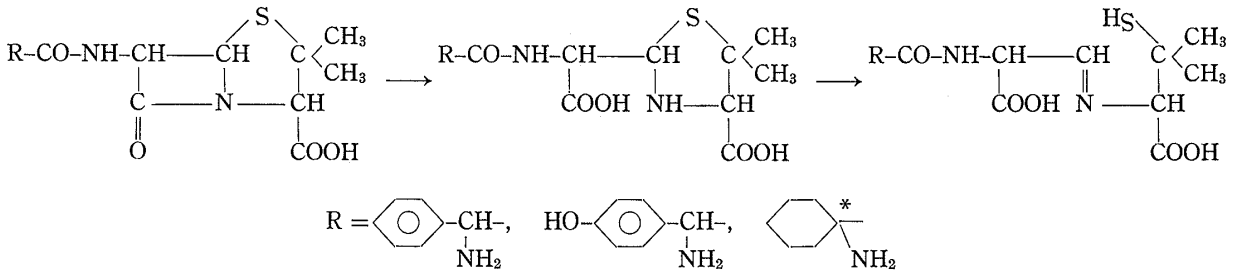
Conditions: temp., 25.3°; spectral width, 400 Hz; acquisition time, 4 sec; flip angle, 90°; number of transients, 200.

consisted of intact urine dissolved in deuterium oxide after freeze-drying in order to avoid interference by the water signal. Figure 10 (1) shows the spectrum due to control urine, with no signal at around 0.5 ppm. Figure 10 (2) shows the spectrum due to urine specimens collected after oral administration of 1 g of AM–PC. The signals in the spectrum of Fig. 10 (2) can be assigned to the 2α- and 2β- CH_3 protons of AM–PC (δ=1.42, 1.40 ppm), AM–PA (δ=1.20, 1.16 ppm), and AM–PM (δ=1.58, 0.54 ppm), Comparison of these spectra clearly shows the excretion of AM–PM in the urine together with AM–PC and AM–PA.

Discussion

There is controversy regarding cleavage of the C–S bond of the thiazolidine ring in the human body. Harris *et al.*[30] reported that the ring of thiazolidine-4-carboxylic acid was cleaved by liver mitochondria to form N-formylcysteine, while Williams[31] and Walkenstein *et al.*[32] showed that the thiazolidine

30) J. Harris and C.G. Mackenzie, *Fed. Proc.*, **14**, 223 (1955).
31) R.T. Williams, "Detoxication Mechanism," 2 nd, Chapman & Hall Ltd., London, 1959, p. 618.
32) S.S. Walkenstein, N. Chumakov, and J. Seifter, *Antibiot. Chemother.*, **4**, 1245 (1954).



* The penamaldic acid of cyclacillin (AC–PM) was not detected.

Fig. 11. Metabolic Pathways of Amino-penicillins in Man

ring of penicilloic acid was probably stable in the human body.

The present work leads to the conclusion that the C–S bond of the thiazolidine ring in the penicilloic acid derivatives of AB–PC and AM–PC is cleaved in the human body, while that of AC–PA remains intact. The new metabolites of AB–PC and AM–PC were confirmed to be penamaldic acids by the chromatographic and spectroscopic results described above. These results imply that the metabolic pathway of amino-penicillins in man is as given in Fig.11. The results of HPLC determinations of the new metabolite along with the unchanged penicillin and penicilloic acid, and a re-evaluation of the pharmacokinetic profile of amino-penicillins in man will be presented shortly.

Acknowledgement The authors wish to thank Dr. K. Machida of Kyoto University for helpful advice. Thanks are also due to Dr. M. Sano and the staff of Daiichi Seiyaku Co., Ltd., Tokyo, for the measurements of 200 MHz ^{1}H NMR and FD mass spectra. This work was supported in part by a Grant-in-Aid for Scientific Research from Ministry of Education, Science and Culture, Japan.