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A New Metabolite of Ampicillin in Man

A new metabolite was found from human urine collected after oral administration of ampicillin. The chromatographic behavior, chemical properties, and spectral characteristics suggested that this metabolite is due to cleavage of C–S bond in thiazolidine ring, possible structure being α-aminobenzyl penamaldic acid.

Keywords——ampicillin; α-aminobenzyl penicilloic acid; metabolism; high performance liquid chromatography; coloration reaction; α-aminobenzyl penamaldic acid

Previous studies on the fate of ampicillin (AB-PC) in man have revealed that α-aminobenzyl penicilloic acid (AB-PA) is the major metabolite found in urine,[1,2] although the conjugation of benzylamino group has been suggested.[3,4] The excretion of 6-aminopenicillanic acid (6-APA) as a minor metabolite of AB-PC seems suspicious.[2] In the course of our studies on chromatographic determination and pharmacokinetic analysis of β-lactam antibiotics, we found an unknown peak due to a new metabolite appearing on the chromatogram (Fig. 1-(1)) of human urine collected after oral administration of AB-PC. The peak is completely separated from those of AB-PA, unchanged AB-PC, and endogenous components, and shows appreciable intensity of ultraviolet spectrum (UV) response. Standard material of 6-APA had shorter retention time than the unknown peak under this condition.

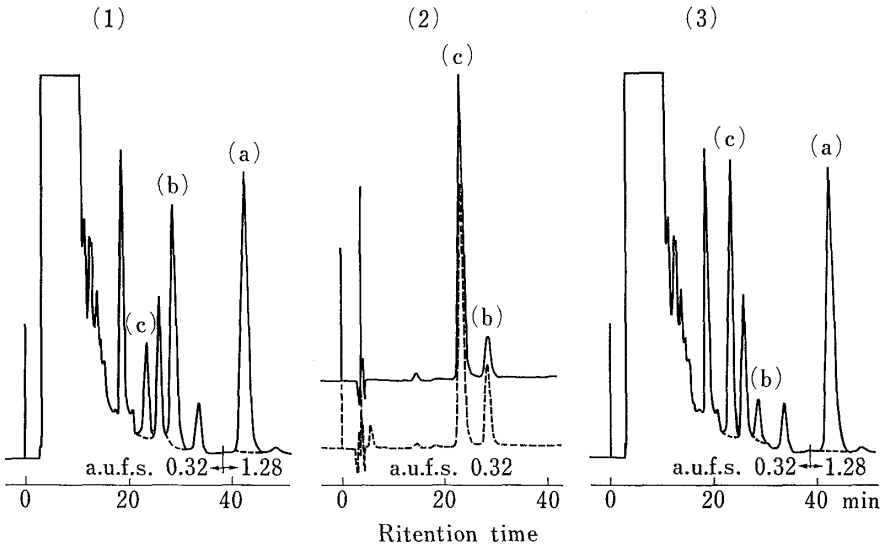

Fig. 1. Chromatogram of (1) Urine after Oral Administration of 500 mg Ampicillin (——) and Control Urine (------), (2) α-Aminobenzyl Penicilloic Acid kept at Room Temperature for 3 min in 0.00125% $HgCl_2$ Solution (——) and at 30° for 6 hr in 1M NaH_2PO_4 Solution (------), and (3) Urine after Administration of AB-PC treated with 0.00125% $HgCl_2$ (——) and Control Urine (------)

HPLC conditions: stationary phase, Nucleosil $10C_{18}$ packed in 250×4.6mm i.d. stainless steel column; Mobile phase, water/methanol (5/2, v/v) containing 0.01M sodium *n*-heptylsulfonate, 0.005M NaH_2PO_4, and 1.3% 0.5N HCl; flow rate, 0.8ml/min; detection, UV 218 nm.
peak (a) ampicillin, (b) α-aminobenzyl penicilloic caid, (c) new metabolite (α-aminobenzyl penamaldic acid).

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In order to evident the origin of this peak, we achieved some *in vitro* experiments. When AB-PA was kept either at room temperature for 3 min in aqueous 0.00125% $HgCl_2$ solution or at 30° for 6 hr in aqueous 1 M NaH_2PO_4 solution, a product having the same HPLC retention time with the unknown peak was obtained (Fig. 1-(2)), and when the urine sample of Fig. 1-(1) was treated with 0.00125% $HgCl_2$, the peak of AB-PA decreased with simultaneous increase in the unknown metabolite peak (Fig. 1-(3)). It was confirmed by varying HPLC conditions that the unknown peak comprises a single substance. These results suggest that the new metabolite is identical with the $HgCl_2$-degradation product of AB-PA. It has been known, on the other hand, that the C-S bond in thiazolidine ring of AB-PA can be cleaved in the presence of Hg^{2+}.[5,6] Thus, we tried to see if the new metabolite may lack the C-S bond of AB-PA by examining the coloration reaction presented by Jirousek *et al.*[7] which has been used for the detection of thiol proteins. Figure 2 shows the absorption spectra of some colored species, indicating that the spectrum for the degradation product of AB-PA is quite similar to that for penicillamine (which has SH group and shorter retention time than the unknown peak), but different from those for AB-PC, AB-PA and reagent blank (Feigl's disulfide).

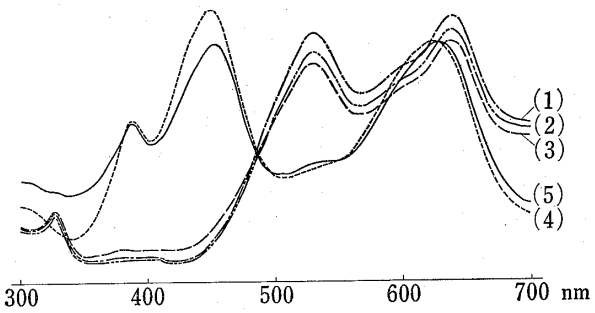

Fig. 2. Absorption Spectra of (1) Coloration Reagent (Feigl's disulfide), (2) α-Aminobenzylpenicilloic Acid+Coloration Reagent, (3) Ampicillin+Coloration Reagent, (4) Penicillamine+Coloration Reagent, and (5) Degradation Product of α-Aminobenzylpenicilloic Acid+Coloration Reagent

It follows consequently that the new metabolite is due to biological cleavage of C-S bond of thiazolidine ring, possible structure being α-aminobenzyl penamaldic acid. Amoxicillin also exhibited a similar metabolic behavior. Since this type of metabolism has not appeared in literature, we are trying to isolate this metabolite for further confirmation of chemical structure, and to examine other penicillins.

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