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Received November 6, 1979

[Chem. Pharm. Bull. 28(1) 362—364 (1980)]

GC-MS Identification of Active Metabolite of Oxacillin in Man

The structure of active metabolite of oxacillin excreted in human urine was identified as 5-hydroxymethyl derivative by means of trimethylsilylation followed by GC–MS analysis.

Keywords——gas chromatography-mass spectrometry; metabolite; oxacillin; isoxazolylpenicillin; trimethylsilyl derivative; high performance liquid chromatography; β-lactam antibiotics

Oxacillin is one of isoxazolylpenicillins effective in treating infections caused by β-lactamase producing S. aureus. In the course of pharmacokinetic studies on this drug, we have found an unknown metabolite peak appearing on the high performance liquid chromatogram of human urine excreted after oral administration of oxacillin (peak 2 in Fig. 1). This peak had the retention time between those of unchanged oxacillin and penicilloic acid, the latter being known as an inactive metabolite common to most of penicillins.

It has been known that isoxazolylpenicillins undergo biotransformation to active metabolites,[1] the structure of the metabolite for dicloxacillin being identified as 6-[3-(2,6-dichlorophenyl)-5-hydroxymethyl-4-isoxazolecarboxamido]-penicillanic acid.[2] It has been left uncertain, however, if the active metabolite of oxacillin may have a similar structure, although the retention behavior on a reversed phase thin layer chromatography implied the possibility of analogy.[3]

While we were preparing this paper, Thijssen manifested by means of mass spectrometry that the active metabolites for isoxazolylpenicillins in the rat are 5-hydroxymethyl analogues common to oxacillin, cloxacillin, dicloxacillin, and flucloxacillin.[4] This paper describes gas chromatographic–mass spectrometric (GC–MS) identification of the unknown metabolite of oxacillin found from human urine.

Oxacillin tablets were orally dosed to human subjects (250 mg×2 for each), and urine was collected 2—2.5 hours after administration. The urine specimen was acidified to pH 2.5 with use of ammonium sulfate/surfuric acid solution and extracted with dichloroethane.

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2) D.R. Van Harken, C.W. Dixon, and J.M. Essery, *Pharmacologist*, **12**, 220 (1970).
3) H.H.W. Thijssen, *Arzneim. Forsch.*, **28**, 1065 (1978).
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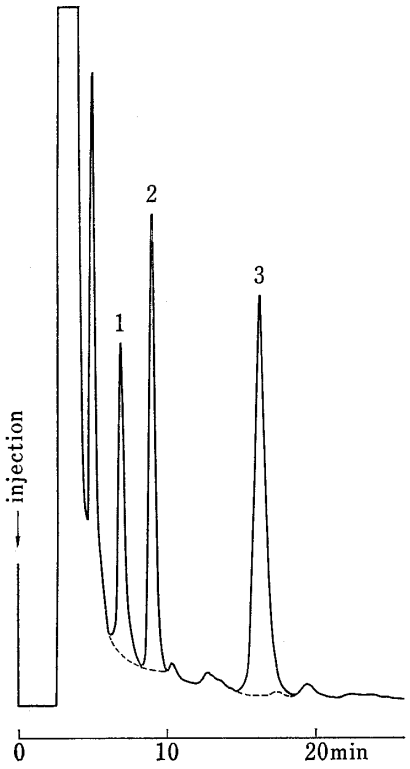

Fig. 1. HPLC Separation of Oxacillin and Its Metabolites from Endogenous Urinary Substances

peak 1 penicilloic acid, 2 active metabolite, and 3 unchanged form of oxacillin
conditions: apparatus, Waters Assoc. ALC/GPC 204; detection, UV 254 nm; stationary phase, LiChrosorb RP-18 packed in a 250 mm × 4.6 mm i.d. stainless steel tubing; mobile phase, acetate buffer solution (pH 5.1)/methanol=4/3 (v/v); flow rate, 1.0 ml/min at ambient temperature; sample size, 5.0 μl (neat urine); 0.02 a.u.f.s.

After removing the solvent, the dried extracts were trimethylsilylated in hexamethyldisilazane/pyridine solution and subjected to GC–MS analysis under the conditions described in the legend of Fig. 2. Figure 2 depicts the mass spectrum for the peak of the metabolite with the elapsed time of 11.8 min on TIM-chromatogram, and the mass chromatograms monitored at *m/e* 561, 546, 473, and 458. The spectrum indicates molecular ion ($M^{+\cdot}$) peak at *m/e* 561 as di-TMS derivative of 5-hydroxymethyl oxacillin and the peaks for the characteristic fragment ions at *m/e* 546, 315, 274, 232, 114, 103, and 73 (base peak). The peak at *m/e* 546 is due to $(M-CH_3)^+$ as often observed with TMS derivatives, and the peak at *m/e* 232 is characteristics of the protonated ion of TMS-derived thiazolidine resulted from cleavage of the β-lactam ring, which corresponds to the protonated ion at *m/e* 174 found with methylester of thiazolidine.[4,5] The peak at *m/e* 114 is assignable to $(232-COOTMS)^+$, *m/e* 103 to $TMS-OCH_2^+$, and *m/e* 73 to TMS^+. The peaks at *m/e* 315 and 274 (assigned by Thijssen[4] to the fragment ions A and B, respectively) are characteristic of TMS-derived 5-hydroxymethylisoxazole moiety. The mass chromatograms monitored at *m/e* 561 and 546 show each single peak whose retention time (11.8 min) agreed exactly with that of TMS-derivative obtained from the HPLC fraction of the unknown metabolite peak, although the fractionation did not give enough amount to measure the intensive mass spectrum. The mass chromatograms monitored at *m/e* 473 and 458 ($M^{+\cdot}$ and $(M-CH_3)^+$ for TMS-oxacillin) indicate that the peak of unchanged oxacillin on the TIM-chromatogram overlaps with those of endogenous urinary substances.

Thus, it follows that the metabolite found from human urine is 5-hydroxymethylisoxazole derivative of oxacillin as is the case of the rat. The subsequent studies now proceeding, however, suggest the absence of corresponding metabolite for flucloxacillin. This differs from the case of the rat. The time courses for the urinary excretion of this metabolite indicates the maximum rate at 1—2 hours after dosing and the cumulative amount until 8 hours comparable to those of unchanged oxacillin. The detailed pharmacokinetic considerations on isoxazolylpenicillins in man based on simultaneous HPLC determinations of the metabolites and unchanged form will follow in near future.

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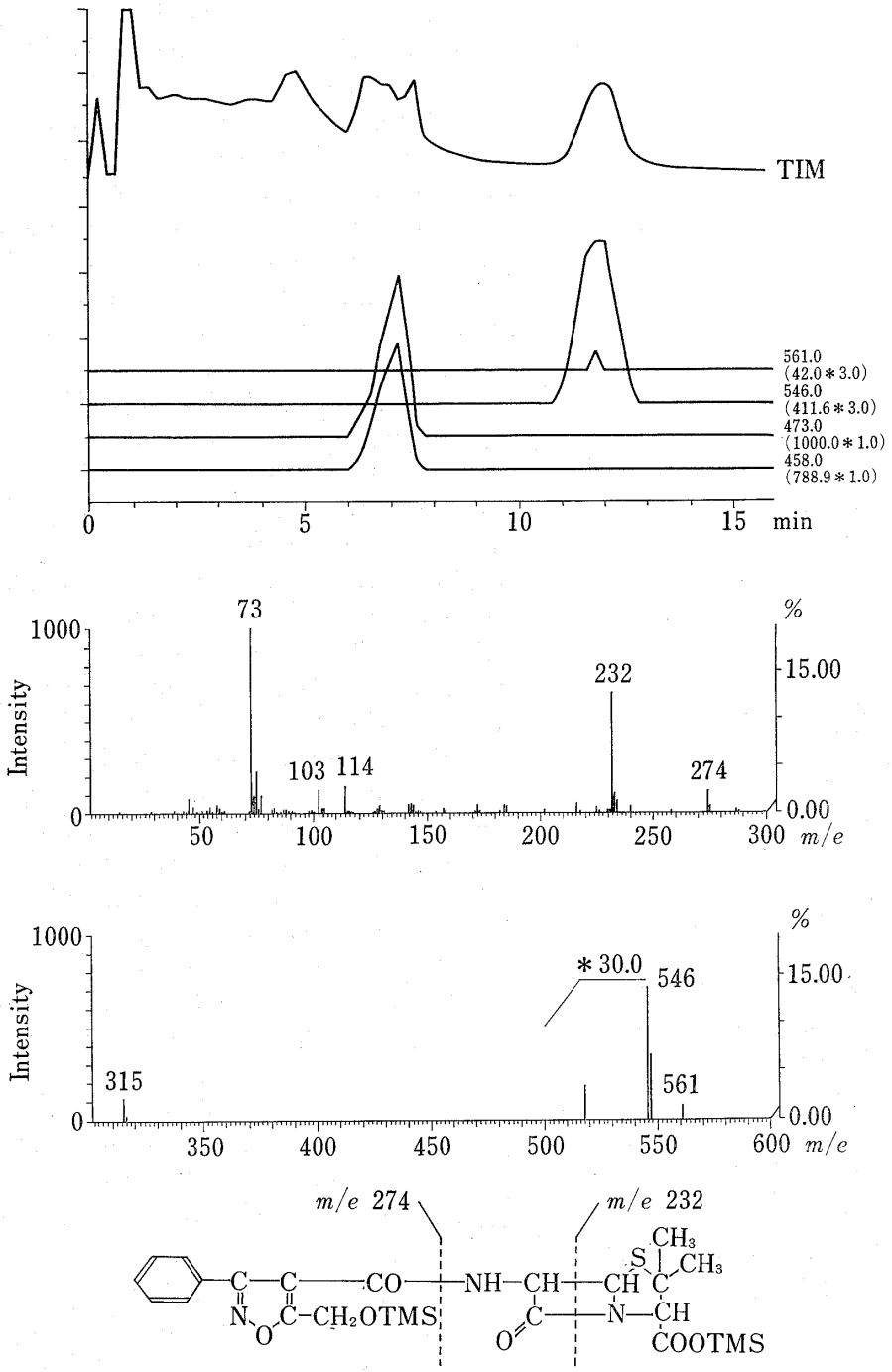

Fig. 2. Mass Spectrum and Mass Chromatogram of Active Metabolite of Oxacillin

Condition: apparatus (MS) JMS-OlSG-2, (GC) JGC-20K, (computer) JMA-2000; stationary phase, 2% OV-17 coated on Chromosorb W (80/100 mesh) packed in a 100 cm × 2 mm i.d. stainless steel tubing; column temperature, 260°; ionization voltage, 50 eV; acceleration voltage, 8 kV

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Received November 12, 1979