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Structure Confirmation of Active Metabolite of Dicloxacillin in Man by Nuclear Magnetic Resonance Spectroscopy

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The active metabolite of dicloxacillin was isolated from human urine, and its structure was investigated. The ^{1}H-nuclear magnetic resonance (NMR) and ^{13}C-NMR spectra indicated that one proton had been lost from the 5-methyl group of dicloxacillin. Fast atom bombardment mass spectra showed that one oxygen atom had been added to dicloxacillin by the biotransformation. These results confirmed that hydroxylation of the methyl group at the 5-position of the isoxazolyl moiety of dicloxacillin occurs to yield the active metabolite.

Keywords——dicloxacillin; active metabolite; metabolism; urinary excretion; ^{1}H-NMR; ^{13}C-NMR; fast atom bombardment mass spectroscopy; isoxazolylpenicillin

Dicloxacillin is an isoxazolylpenicillin having broad-spectrum antibacterial activity along with β-lactamase inhibitory activity. This drug is biotransformed to a considerable extent in man to an active metabolite whose activity against certain bacteria is comparable to that of the parent penicillin.[1)]

In the previous papers[2–4)] we described the pharmacokinetic profiles of metabolism and excretion of isoxazolylpenicillins in man, taking account of the active metabolite as well as unchanged penicillin and two inactive metabolites. The structure of the active metabolite isolated from human urine following administrations of oxacillin[5)] or flucloxacillin[3)] was elucidated by gas chromatography-mass spectrometric (GC-MS) analysis as the 5-hydroxymethyl derivative of the parent pencillin, while Thijssen[6)] reported mass spectral data which indicated that the active metabolite in the rat is also the 5-hydroxymethyl derivative for four isoxazolylpenicillins. Van Harken *et al.*[7)] reported that the active metabolite of dicloxacillin is also the 5-hydroxymethyl derivative, but without providing spectral evidence.

A number of papers have dealt with nuclear magnetic resonance (NMR) spectroscopic investigations of the configuration and/or conformation of penicillins and cephalosporins, whereas few reports have described the spectral changes accompanying their biotransformation. This paper presents ^{1}H-NMR and ^{13}C-NMR spectral evidence for the structure of the active metabolite of dicloxacillin in man.

Experimental

Reagents and Materials——Dicloxacillin sodium was supplied by Banyu Pharmaceutical Co. (Tokyo). The active metabolite was isolated from human urine according to the procedure described below. Other chemicals used were commercial products of analytical-reagent grade.

Isolation of Active Metabolite——About 2000 ml of urine collected from volunteers at 1 to 4 h after administration of a dicloxacillin capsule (250 mg) was chromatographed in portions on a LiChroprep RP-18 column (31 cm × 2.5 cm i.d., 40 to 63 μm particle diameter, E. Merck) using 0.2 M acetate buffer (pH 5.2)/acetonitrile = 10/3

(v/v) as a developing solvent. The eluate was subjected to high performance liquid chromatography (HPLC)[4] with detection of ultraviolet (UV) absorbance at 220 nm, and the fraction containing the active metabolite was collected. After removal of the solvent the residue was dissolved in a small portion of water and rechromatographed on the same column with 0.03 M acetate buffer (pH 5.6)/acetonitrile = 40/9 (v/v) mixture. The metabolite fraction was concentrated and developed again on the same column with water/acetonitrile = 4/1 (v/v) mixture. The fraction following elution of the buffer salt was collected. Removal of the solvent followed by lyophilization finally gave a small amount (about 70 mg) of a fleecy white solid. HPLC analysis of the solid showed a single peak.

NMR Measurement——The ^{1}H-NMR and ^{13}C-NMR chemical shifts were measured on a JEOL FX-200 NMR spectrometer (JEOL, Tokyo) using 5 mm i.d. spinning tubes at ambient temperature and employing the deuterium field/frequency lock system. The samples were dissolved in D_2O. The chemical shift values are given in parts per million from internal sodium 3-(trimethylsilyl)propionate.

Results and Discussion

The NMR spectral data were consistent with the expected structures of dicloxacillin and the 5-hydroxymethyl derivative of the parent penicillin. The ^{13}C-NMR spectrum of the active metabolite is depicted in Fig. 1, and the observed chemical shifts and multiplicities are summarized in Table I together with those of dicloxacillin. The values of the chemical shifts for dicloxacillin are in good agreement with the literature values.[8] A comparison of the values for dicloxacillin and the active metabolite indicates that chemical shifts unrelated to the substituent group at the 5-position on the isoxazole ring remain almost unchanged, while a quartet signal of dicloxacillin at 15.4 ppm observed by off-resonance decoupling does not appear in the spectrum of the active metabolite, which shows instead a triplet signal at 57.9 ppm.

The ^{1}H-NMR spectrum of dicloxacillin gave a signal at 2.70 ppm with an intensity equivalent to three protons, assignable to the methyl group at the 5-position on the isoxazole ring. This signal was absent in the active metabolite, which instead exhibited a signal at 5.08 ppm with intensity equivalent to two protons. These results suggested that one proton

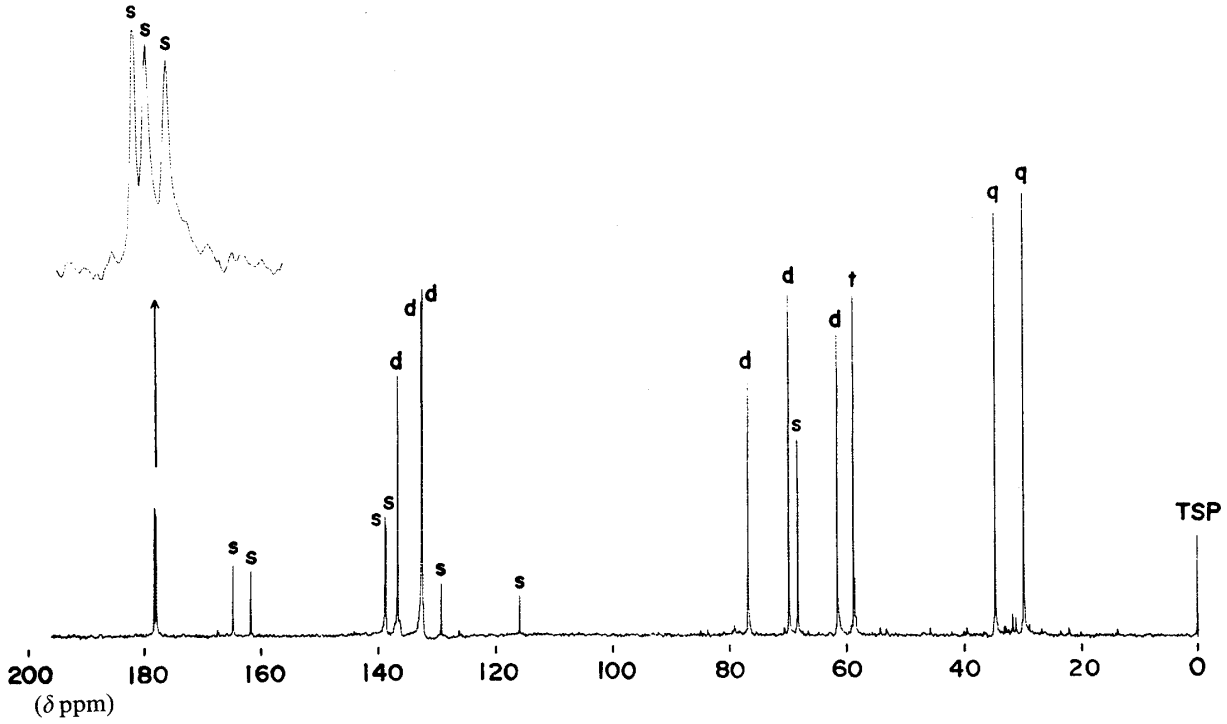
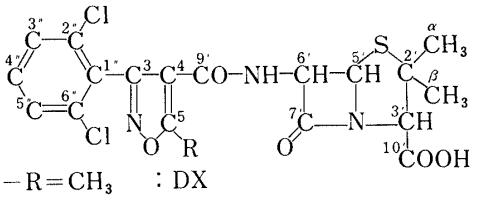


Fig. 1. ^{13}C-NMR Spectrum of the Active Metabolite of Dicloxacillin

TSP: sodium 3-(trimethylsilyl)propionate.

TABLE I. Observed Chemical Shifts (in ppm) and Multiplicities[a] of ^{13}C-NMR Signals of Dicloxacillin (DX) and Its Active Metabolite ($DX_{5\text{-}OH}$)



$-R=CH_3$: DX
$-R=CH_2OH$: DX_{5-OH}

Carbon number	DX[b]		$DX_{5\text{-}OH}$[b]	
	Chemical shift[c]	Multiplicity	Chemical shift[c]	Multiplicity
2′	67.5	s	67.4	s
3′	75.9	d	75.8	d
5′	68.7	d	68.9	d
6′	60.3	d	60.6	d
7′	178.3	s	177.0	s
9′	176.7	s	176.6	s
10′	177.0	s	176.9	s
3	159.4	s	160.4	s
4	113.9	s	114.8	s
5	164.3	s	163.5	s
1″	128.0	s	128.1	s
2″	138.0	s	137.7	s
3″	131.9	d	131.5	d
4″	136.0	d	135.6	d
5″	131.8	d	131.4	d
6″	137.9	s	137.6	s
α-CH_3	29.3	q	29.1	q
β-CH_3	32.9	q	34.0	q
R	15.4	q	57.9	t

a) Multiplicities determined by off-resonance decoupling (s=singlet, d=doublet, t=triplet, q=quartet),
b) In D_2O.
c) The assignments of 5′ and 6′, 3 and 5, 2″ and 6″, 3″ and 5″, and α- and β-CH_3 carbon atoms may be reversed.

was lost from the 5-methyl group of dicloxacillin. This was confirmed by the fact that selective decoupling (irradiation frequency offset 57.8279 kHz) resulted in a marked increase in the intensity of the ^{13}C-NMR signal at 57.9 ppm.

On the other hand, a preliminary investigation of the fast atom bombardment (FAB) mass spectrum indicated that the molecular ion peak of the active metabolite appeared at *m/z* 486 as $(M+H)^+$. This suggests that one oxygen atom was added to dicloxacillin molecule (MW 469) by the biotransformation.

These results lead to the conclusion that the active metabolite of dicloxacillin in man is 6-[3-(2,6-dichlorophenyl)-5-hydroxymethyl-4-isoxazolecarboxamido]penicillanic acid *i.e.* the 5-hydroxymethyl derivative of dicloxacillin.

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