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^{1}H NMR and NOE Studies of 6α-Bromopenicillanates

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The ^{1}H NMR signals of three 6α-bromopenicillanates have been assigned and the Nuclear Overhauser Effect(NOE) study of these compounds was undertaken.

Key words: Penicillanates, ^{1}H NMR, NOE

INTRODUCTION

In our search for β-lactamase inhibitors, we have synthesized penicillin derivatives and studied their biological activity (Im *et al.*, 1994 in press). During the course of our investigations, 6α-bromopenicillanates (Figure 1) were prepared as intermediates and their ^{1}H NMR spectra were illuciclated by NOE studics in order to make key structural assignments, especially for the 2α-CH_3 and 2β-CH_3 groups.

^{1}H NMR and NOE studies of penicillin derivatives have been reported (Flynn, 1972). In this paper, we report the ^{1}H NMR assignments of the title compounds and also discuss their NOE spectra.

MATERIALS AND METHODS

The ^{1}H NMR spectra were recorded on a Bruker AC-200E spectrometer at 25℃ by using 10 mM solutions in $CDCL_3$ with tetramethylsilane as an internal standard. Coupling constants(J) of the ^{1}H NMR spectra are reported in Hertz(Hz). NOE data were collected using the multiple irradiation method (Neuhaus, 1983) with preirradiation times varying from six to ten seconds. NOE data are reported as percentage increases over the nonirradiated peak integration values.

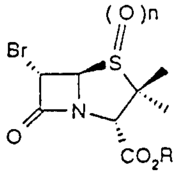
RESULTS AND DISCUSSION

The ^{1}H NMR assignments and NOE data of the title compounds are given in Table I and II respectively. The coupling constants($J_{5,6}$) between the C5-H and C6-H in these compounds (1, 2 and 3) are 1.2, 1.3, and 1.3 Hz, respectively. These coupling constants indicated that these protons are *trans* to each other, i.e., C5α-H and C6β-H.

From the NOE experimental data, the following conclusions can be drawn. In compounds (1 and 2),the C3-H would be closer to the low field methyl (C2α-CH_3) than to the high field methyl(C2β-CH_3). C5-H would be close to the low field methyl, but not to the high field methyl. The C6-H would be close to the high field methyl but not to the low field methyl. These observations suggest that the conformation of compounds (1 and 2) may be similar to conformation [A] in Figure 2.

The relative assignment of the two methyl singlets in compounds (1 and 2) is based on the fact that the C6-H should be closer to the C2β-CH_3 than to C2α-CH_3 in conformation[A]. The NOE between the C6-H and the high field methyl suggest that the high field methyl would be C2β-CH_3. But this assignment is the reverse of that of previously reported ones in 6α-aminopenicillanates (Flynn, 1972 and Cooper *et al.*, 1969), even though the conformation is the same.

Demarco and Nagarajan (Flynn, 1972) compared the NOE between the C3-H and the two C2-CH_3 and assigned the high field methyl to C2α-CH_3 and the low field methyl to C2β-CH_3. But the C6β-H is a better criteria for this assignment. The 3% NOE between the C6-H and the high field methyl and no NOE between



n = 0	R = H	(1)
n = 0	R = PMB	(2)
n = 2	R = PMB	(3)

PMB : p-methoxybenzyl

Fig. 1. Structure of 6α-bromopenicillanates.

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Table I. ^{1}H NMR assignment of 6α-bromopenicillanates(δ)[a]

Compounds	2α-CH_3	2β-CH_3	C3-H	C5-H	C6-H	OCH_3	CH_2 of PMB[b]
1	1.65	1.57	4.59	4.84 (d, 1.2 Hz)	5.41 (d, 1.2 Hz)	Nil	Nil
2	1.58	1.37	4.54	4.79 (d, 1.3 Hz)	5.40 (d, 1.3 Hz)	3.81	5.13
3	1.24	1.54	4.41	4.67 (d, 1.3 Hz)	5.15 (d, 1.3 Hz)	3.82	5.12, 5.26 (two d, 11.5 Hz)

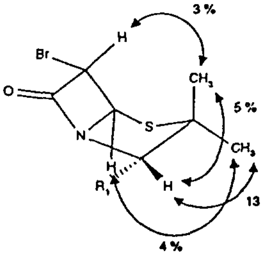
[a] Recorded in $CDCL_3$, [b] PMB: p-methoxybenzyl

Table II. NOE in 6α-bromopenicillanates[a]

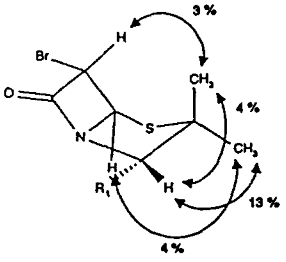
Compounds	Protons irradiated(δ)	Proton observed	Intensity increased(%)
1	Low-field methyl(1.65)	C3-H	13
		C5-H	4
		C6-H	Nil
	High-field methyl(1.57)	C3-H	5
		C5-H	Nil
		C6-H	3
2	Low-field methyl(1.58)	C3-H	13
		C5-H	4
		C6-H	Nil
	High-field methyl(1.37)	C3-H	4
		C5-H	Nil
		C6-H	3
3	Low-field methyl(1.54)	C3-H	10
		C5-H	Nil
		C6-H	1
	High-field methyl(1.24)	C3-H	2
		C5-H	10
		C6-H	Nil

[a] Sample concentration was 5% w/v with TMS as internal field frequency lock in $CDCl_3$

[b] Given as the percentage increase in integrated intensity on irradiation

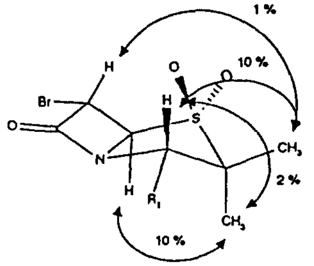


R_1 = CO_2H (1)



R_1 = CO_2PMB (2)

Conformation [A]



R_1 = CO_2PMB (3)

Conformation [B]

Fig. 2. Thiazolidine ring conformations and NOE in 6α-bromopenicillanates.

the C6-H and the low field methyl strongly suggests that the high field methyl is the C2β-CH_3.

In compound(3), the low field methyl would be closer to the C3-H and C6-H than the high field methyl. The high field methyl would be closer to the C5-H than the low field methyl, as shown in Figure 2 (conformation[B]). Thus, the low field methyl would be assigned to C2β-CH_3 and the high field methyl to C2α-CH_3. Similar NOE observations and methyl assignments were reported by Cooper(Cooper *et al.*, 1969).

In compound(2), the CH_2 protons of the p-methoxybenzyl(PMB) ester appeared as a singlet, but compound(3) showed two doublets for these protons. This may be explained as follows. Compound(2) has the conformation[A], in which the C2α-CH_3 is equatorial. In this model, the PMB ester has some space for free rotation and these CH_2 protons are magnetically equivalent to give a singlet. Compound(3), however, has the conformation[B],in which the C2α-CH_3 is in an axial position. When the PMB ester rotates around, it would be faced by steric hindrance from the C2α-CH_3 and would have restricted rotation. Without free rotation, these two protons are not magnetically equivalent and would not give a singlet. This observation also supports the chair form conformation[B] for compound(3).

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