*Agric. Chem. Biotechnol.* **43**(2), 115-116(2000)

# Determination of the Substituted Position of Piperazine in Substitution Reaction of Oxazolidinones

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Received April 17, 2000

**Key words :** *oxizolidinones, piperazine.*

Oxazolidinones, a new class of synthetic antibacterial agents, are active against only gram-positive aerobic bacteria. In order to overcome this limitation, we tried to attach quinolone moiety to oxazolidinones. As a "spacer" molecule, piperazine was introduced. When piperazine was added to 4,6-dichloro-ethylnicotinate, piperazine was substituted at only one position at C4 or C6. Reaction scheme of synthesis is described in Scheme 1.[1,2] 1D, 2D NMR and computer aided molecular modeling experiments were carried out for the identification of the position of the substitution. NMR spectra were obtained on a Bruker Avance 400 (9.4 T) instrument in DMSO-$d_6$. For the $^1$H-NMR experiments, 32 transients were acquired with a 1 sec relaxation delay using 32K data points, and the 90° pulse was 9.7 μsec, with spectral width of 4,000 Hz. For the $^{13}$C-NMR and DEPT experiments, 1,024 transients were acquired with a 2 sec relaxation delay using 64K data points, and the 90° pulse was 9.8 μsec with spectral width of 21,000 Hz.

Since 1D experiments alone cannot give complete structural information on chemical shifts, 2D NMR experiments such as COSY, HMQC, and NOESY were carried out, and their results are listed in Table 1.

Two-dimensional spectra were acquired with 2048 data points in t2 and 256 in t1 increments. The COSY spectrum was collected with the magnitude method.[3] HMQC spectrum was collected using the methods described by Bax.[4] NOESY[5] spectra were collected with time proportional phase incrementation technique (TPPI). Phase sensitive NOESY spectrum was measured using a 1 sec mixing time.

**Table 1. NMR data and assignments of the product 1.**

| $\delta_c$ | CHn DEPT | $\delta_H$ ($J_{HZ}$) HMQC | Assignments |
|---|---|---|---|
| 14.2 | q | 1.39(t, 7.1) | ethyl-$CH_3$ |
| 45.0 | t | 3.13(m) | C3', C5' |
| 50.7 | t | 3.28(m) | C2', C6' |
| 61.5 | t | 4.36(q, 7.1, 5.9) | ethyl-$CH_2$ |
| 111.4 | d | 6.79 | C5 |
| 115.8 | s | | C3 |
| 153.1 | d | 8.57 | C2 |
| 155.2 | s | | C6 |
| 158.2 | s | | C4 |
| 165.9 | s | | C=O |

q, quartet; t, triplet; d, doublet; s, singlet.

Distances between ethyl-$CH_2$ and H2' or H6' were measured based on the 2D NOESY experiment. Because slow correlation time was needed in the experiment, DMSO-$d_6$ was used as a solvent instead of $CDCl_3$. In order to calculate distances from observed nOe peaks, row slice of ethyl-$CH_2$ signal at 4.36 ppm was collected and all peaks were integrated (Fig. 1). When the integrated value of ethyl-$CH_3$ is defined as 21, that of H2' or H6' is 1.

According to the following equation, the distance between ethyl-$CH_2$ and H2' or H6' can be calculated based on the distance between ethyl-$CH_2$ and ethyl-$CH_3$ being 2.37Å.

$$\frac{\eta_{ref}}{\eta_{samp}} = \frac{(r_{samp})^6}{(r_{ref})^6}$$

where η and r denote nOe and distance, respectively. Ethyl-$CH_3$ is considered as the center of the methyl group and ethyl-$CH_2$ that of the methylene group. The distance

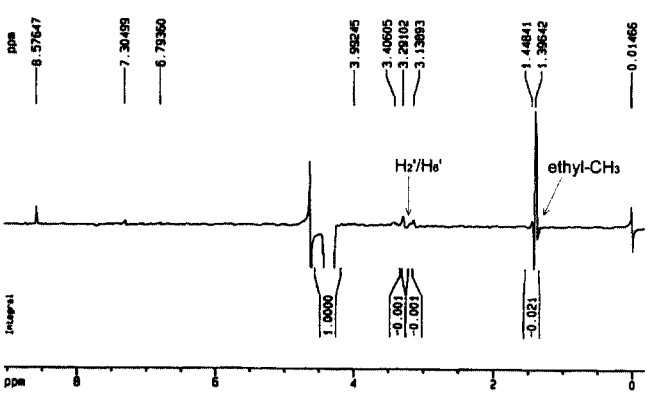


**Fig. 1. Row slice of ethyl-$CH_2$ signal at 4.36ppm obtained from the NOESY spectrum of compound 1.**

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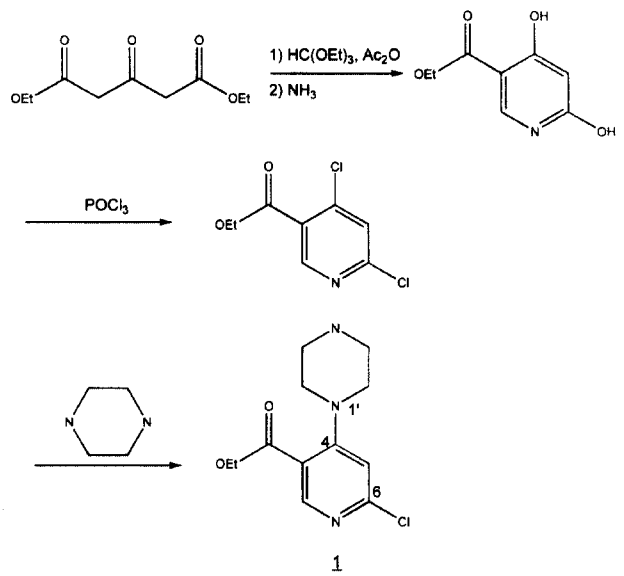


**Scheme 1. Synthesis of ethyl-6-chloro-4-(1-piperazinyl) nicotinate.**[1,2]

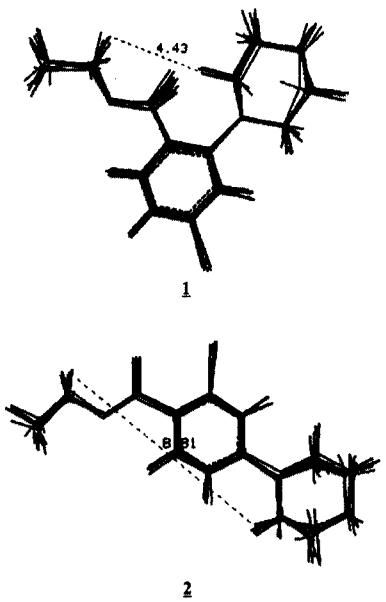


**Fig. 2. The superimposed structures of compounds 1 and 2 obtained from molecular dynamics, and distances (unit: Å) between ethyl-$CH_2$ and H2' or H6'.**

calculated from the nOe peak is 3.93Å. As shown in Scheme 1, the distance between ethyl-$CH_2$ and H2' or H6' in **1** is shorter than that in **2**. In order to determine the distance in the molecule with low energy state, Computer Aided Molecular Modeling was introduced. In this study, all computational calculations were performed using Biosym/ MSI software (San Diego, CA, USA) on a Silicon Graphics INDY R4400 workstation. The distance was calculated with the discover module of InsightII. Firstly, energy minimization and molecular dynamics on each compound were carried out. Conformers with low energy were selected and superimposed. In these conformers, the distance between ethyl-$CH_2$ and H2' or H6' in compound **2** and that in compound **1** were approximately 8.81Å and 4.43Å, respectively (Fig. 2). Therefore, the result obtained from the NMR experiment agrees with the value calculated for compound **1**. In conclusion, when piperazine is added to 4,6-dichloro-ethylnicotinate, position 4 is substituted.

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