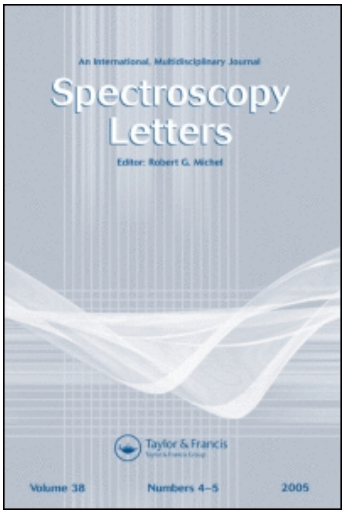


Spectroscopy Letters

Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597299

Structural Study of N-(1-Benzoyl-3-Pyrrolidinyl) Benzamide

Jongmin Park[a]; In Hwa Chung[bc]; Joong Hyup Kim[b]; Choong Sup Kim[d]; K. Hun Moks[e]; Yoongho Lim[a]
[a] Dept. of Applied Biology and Chemistry, Konkuk Univ., Seoul, Korea [b] Biochemicals Research Center, Korea Institute of Science and Technology, Seoul, Korea [c] Department of Chemistry, Korea University, Seoul, Korea [d] Korea Research Institute of Chemical Technology, Yusong, Taejon, Korea [e] Oxford University, Oxford, United Kingdom

To cite this Article Park, Jongmin , Chung, In Hwa , Kim, Joong Hyup , Kim, Choong Sup , Moks, K. Hun and Lim, Yoongho(2000) 'Structural Study of N-(1-Benzoyl-3-Pyrrolidinyl) Benzamide', Spectroscopy Letters, 33: 6, 777 — 793
To link to this Article: DOI: 10.1080/00387010009350157
URL: http://dx.doi.org/10.1080/00387010009350157

SPECTROSCOPY LETTERS, 33(6), 777–793 (2000)

STRUCTURAL STUDY OF N-(1-BENZOYL-3-PYRROLIDINYL) BENZAMIDE

Key words: NMR, Molecular Modeling, Rotamer

Jongmin Park,[1] In Hwa Chung,[2,3] Joong Hyup Kim,[2] Choong Sup Kim,[4] K. Hun Mok[5] and Yoongho Lim[1,*]

[1]Dept. of Applied Biology and Chemistry, Konkuk Univ., Seoul, Korea
[2]Biochemicals Research Center, Korea Institute of Science and Technology, Seoul, Korea
[3]Department of Chemistry, Korea University, Seoul, Korea
[4]Korea Research Institute of Chemical Technology, Yusong, Taejon, Korea
[5]Oxford University, Oxford, United Kingdom

ABSTRACT

Oxazolidinones show potent activity against vancomycin-resistant *Staphylococcus aureus* (VRSA) species, and are currently under active development. We present NMR spectroscopy and molecular dynamics calculation studies on N-(1-benzoyl-3-pyrrolidinyl) benzamide, an oxazolidinone derivative with substitution at the amine group of 3-pyrrolidinamine. The ^{1}H-NMR and ^{13}C-NMR spectra exhibited two sets of peaks, one major and one minor, giving rise to the existence of isomers at room temperature. In order to deduce the nature of its isomeric distribution, a series of derivatives were synthesized and analyzed using NMR spectroscopy and computer-aided molecular modeling (CAMM)

simulations. The results suggest that rotation of the benzoyl group attached to the secondary amine in N-(1-benzoyl-3-pyrrolidinyl) benzamide is responsible for conformational heterogeneity.

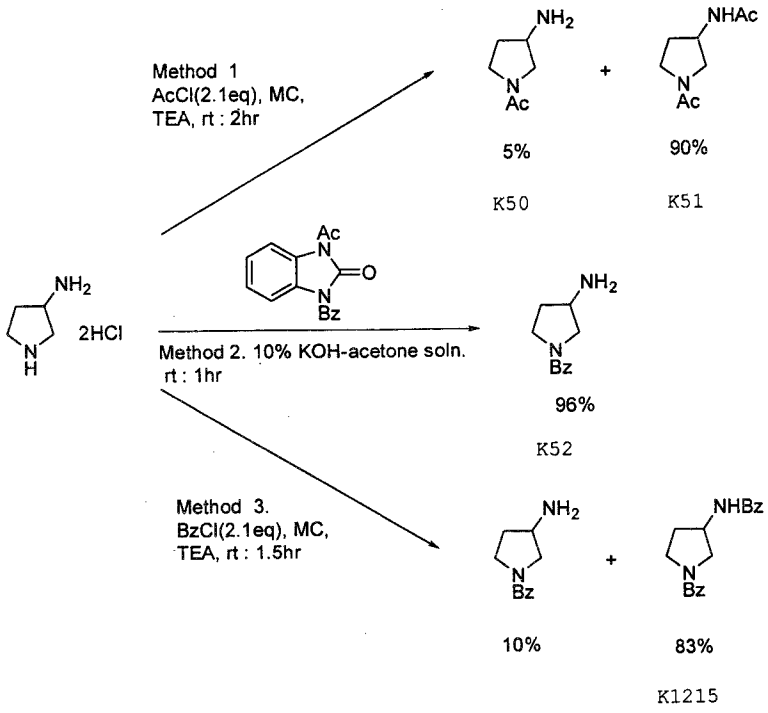
INTRODUCTION

Oxazolidinones have been recently gaining widespread attention due to their potent activities against vancomycin-resistant *Staphylococcus aureus* (VRSA),[1,2] a strain of bacteria which is posing alarming threats to the public health of both industrialized and developing countries alike. Of these oxazolidinones, linezolid and eperezolid have entered as lead compounds, and both of them contain the 1,3-oxazolidin-2-one moiety.[3-6] As part of a series of studies to discover potent oxazolidinones, we have synthesized N-(1-benzoyl-3-pyrrolidinyl) benzamide (K1215), a 3-pyrrolidinamine derivative (SCHEME 1). Interestingly, its ^{1}H-NMR spectrum showed major and minor peaks whose ratio was 1 : 0.8 (FIG. 1). Likewise, the ^{13}C-NMR spectrum showed major and minor peaks as well (FIG. 2). Possible reasons to explain these spectra include the stereospecific attack of 3-pyrrolidinamine in the acylation reaction by *N*-acetyl, *N'*-benzoyl-1,3-dihydro-2H-benzimidazol-2-one, or rotation of the product benzoyl group. We present the syntheses and conformational studies of a series of compounds to allow the accurate determination of the cause in the spectroscopic heterogeneity of K1215 as displayed in FIGs.1 and 2.[7,8]

EXPERIMENTAL

Synthesis

1-(3-Aminotetrahydro-1*H*-1-pyrrolyl)-1-ethanone (K50) and *N*1-(1-acetyl-tetrahydro-1*H*-3-pyrrolyl)acetamide (K51) : Acetyl chloride (0.98 ml, 2.2 eq) was slowly added to the solution of 3-aminopyrroline hydrochloride (1 g, 6.28 mmol) and Et_3N (4.38 ml, 5 eq) in 40 ml anhydrous CH_2Cl_2 under an ice bath.



SCHEME 1.

Synthesis of K1215, K50, K51 and K52.

The reaction mixture was stirred for about 5 hr at room temperature. After filtering off the resulting solids, the solvent was concentrated under reduced pressure. K50 (0.04 g, 5%) and K51 (0.96 g, 90%) were purified by column chromatography as yellow oils.

(3-Aminotetrahydro-1*H*-1-pyrrolyl)(phenyl)methanone (K52) : 3-Amino-pyrroline hydrochloride (0.5 g, 3.14 mmol) was dissolved in 80% THF solution, and NaOH (0.157 g, 2 eq) was added to the solution. *N*-Ac, *N'*-Bz benzimidazol-2-one was added to a clean solution under an ice bath, and stirred for about 1hr. THF was removed under reduced pressure, and the resulting solid was filtered off. Filtrates were concentrated under reduced pressure. Chilled CH_2Cl_2 was added to

the solution, and the resulting solid was filtered off once again. CH_2Cl_2 was removed to gain a yellow oily product with a good yield (0.57 g, 96%).

***N*1-(1-Benzoyltetrahydro-1*H*-3-pyrrolyl)benzamide (K1215)** : Benzoyl chloride (1.6 ml, 2.2 eq) was slowly added to the reaction mixture of 3-aminopyrroline hydrochloride (1 g, 6.28 mmol) and Et_3N (4.38 ml, 5 eq) in 50ml anhydrous CH_2Cl_2. After stirring for about 5 hr, the reaction mixture was concentrated under reduced pressure. K1215 (1.53 g, 83%) was gained as a pale yellow solid by column purification. K52 (0.11 g, 10%) could also be obtained as a minor component in this reaction.

***N*-Acetyl-(S)-(+) 2-pyrroline methanol (K15)** : *N,N'*-Diacetylbenzimidazol-2-one (0.218 g, 1 mmol) was dissolved in 5 ml anhydrous CH_2Cl_2, and (S)-(+) 2-pyrroline methanol (0.098 ml, 1 eq) was added to the solution. The reaction mixture was stirred for about 2 hr at room temperature. K15 was obtained by concentration of the solvent.

***N*-Benzoyl-(S)-(+) 2-pyrroline methanol (K16)** : K16 was obtained using identical procedures except for the addition of *N*-acetyl *N'*-benzoyl benzimidazol-2-one.

***N*-Acetyl-DL-2-pyrroline methanol (K15-1)** : 2-pyrroline methanol was synthesized from DL-proline. Acetyl chloride (0.79 ml, 1.3 eq) was added to the solution of DL-proline (1g, 8.6mmol), Et_3N (1.43 ml, 1.2 eq) and 20 ml CH_2Cl_2 under an ice bath. The mixture was refluxed for 4 hr. CH_2Cl_2 was washed with 10 ml H_2O, sat. 10 ml $NaHCO_3$, 10 ml 1 N HCl, and 20 ml brine. *N*-Acetyl DL-proline (1.49 g, 95%) was gained as pale yellow oil by concentration of the organic layer. $NaBH_4$ (1 g, 1.5 eq) was added to a solution containing *N*-Acetyl DL-proline dissolved in 10 ml THF and 2 ml MeOH. The mixture was stirred for 6 hr. *N*-Acetyl-DL-2-pyrroline methanol was gained in quantitative yield by washing the organic layer with H_2O.

***N*-Benzoyl-DL-2-pyrroline methanol (K16-1)** : *N*-Benzoyl-DL-2-pyrroline methanol was also obtained with quantitative yield by the same synthetic route of *N*-Acetyl-DL-2-pyrroline methanol (K15-1).

NMR and Molecular Dynamics calculation

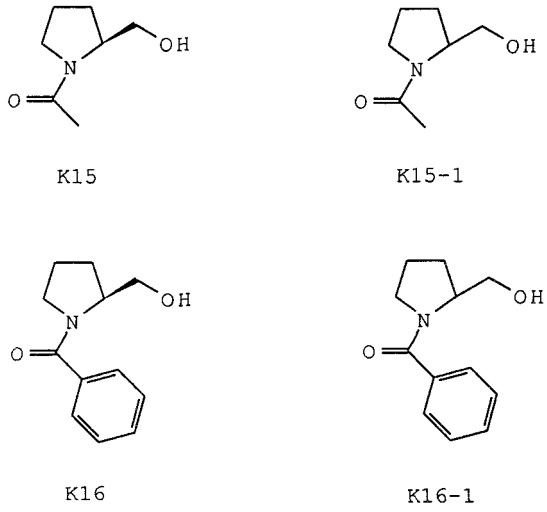
NMR spectra were obtained on a Bruker Avance 400 (9.4 T) instrument in $CDCl_3$. For the ^{1}H-NMR experiments, 32 transients were acquired with 1 sec relaxation delay using 32K data points, and the 90° pulse was 9.7 μsec, with spectral width of 4,000 Hz. For the ^{13}C-NMR and DEPT experiments, 3000 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 22,000 Hz. 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.[9] HMQC spectrum was collected using the methods as described by Bax.[10] All computational calculations were performed using Biosym/MSI software (San Diego, CA) on a Silicon Graphics INDY R4400 workstation. The dihedral angle was calculated with the Discover module of InsightII, where the consistent-valence forcefield (CVFF) was used for 500 psec.

RESULTS AND DISCUSSION

The 3-pyrrolidinamine used as starting material was a racemate. When a benzoyl group is added to the secondary amine, two sets of NMR signals can be expected assuming that the racemates are spectrally resolved. In order to test this assumption, K15, K15-1, K16, and K16-1 were synthesized as shown (SCHEME 2).

While K15 and K16 are R isomers, K15-1 and K16-1 are racemates. The ^{1}H-NMR spectrum of K15 (FIG. 3(a) and (b)) is the same as that of K15-1. Likewise, in the case of K16 and K16-1, their ^{1}H-NMR spectra are found to be identical (FIG. 4(a) and (b)). As a result, it was quite obvious, early on, that the major and minor peaks found in FIGs. 1 and 2 could not be explained through the existence of racemates.

An alternative explanation may be the existence of rotamers generating two sets of NMR signals. The full assignment of K1215 was carried out in order to



SCHEME 2. Structures of K15, K15-1, K16 and K16-1.

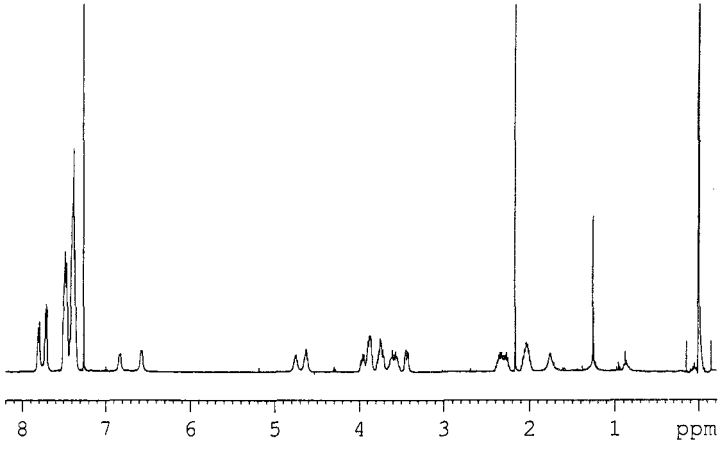


FIG. 1. The ^{1}H-NMR spectrum of K1215.

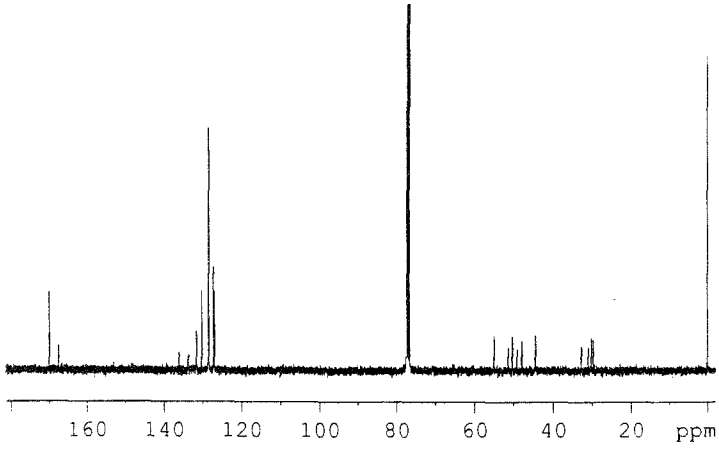


FIG. 2. The ^{13}C-NMR spectrum of K1215.

look into this possibility. TABLE 1 lists its total assignments based on ^{1}H-NMR, ^{13}C-NMR, DEPT, COSY and HMQC.

As shown in SCHEME 3, when the oxygen atom of the 1-benzoyl group is directed to the left side (K1215a), H5 of 3-pyrrolidinyl group is deshielded more than H2, resulting in a downfield shift of H5 with respect to that of H2. That is, the chemical shift of H5 is 3.76 ppm and that of H2 is 3.43 ppm. On the contrary, in the case of K1215b where the oxygen atom of 1-benzoyl group is directed to the right side, the chemical shift of H5, 3.58 ppm, is more upfield than that of H2, 3.73 ppm. Based on this, the major and minor peaks shown in FIG. 1 appear to be caused by the different directions of the ketone group of the 1-benzoyl group.[11]

To clarify this further, several ^{1}H-NMR experiments were carried out at various temperature conditions between 25°C and 45°C (FIG. 5). The major and minor peaks observed at 25°C were found to converge at 45°C. The higher temperature appears to allow the ketone group of 1-benzoyl to overcome its rotational energy barrier.

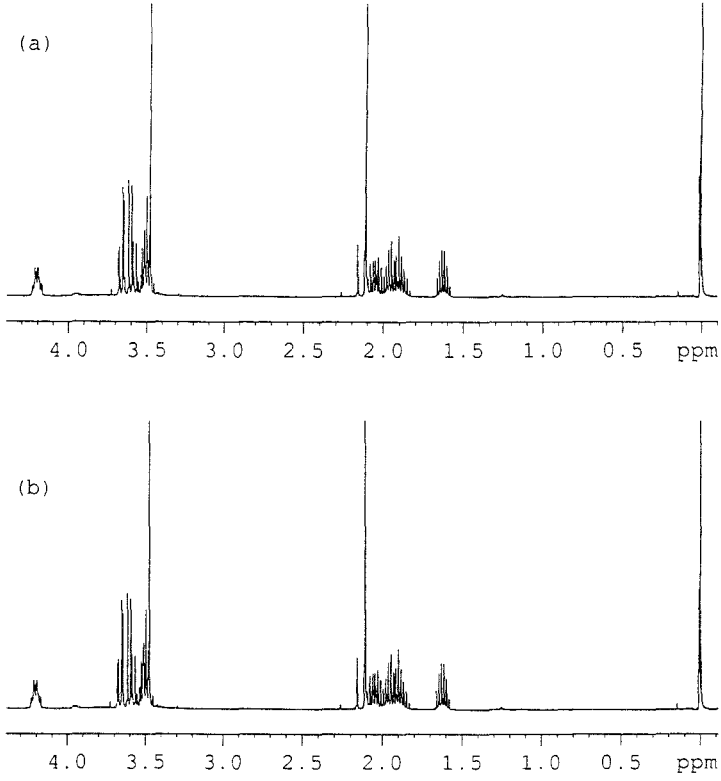


FIG. 3. ^{1}H-NMR spectra of K15 (a) and K15-1 (b).

As K1215 has two ketone groups, K50, K51, and K52 were further synthesized (SCHEME 1) in order to see which ketone group was responsible for the two sets of NMR signals. In the case of K50, only the secondary amine group is substituted by an acetyl group, but in K51, both of the two amine groups are substituted by acetyl groups. Because K1215 has a benzoyl group attached to a secondary amine, K52 possesses the same group substituted at the same position. As shown in FIG. 6(a), (b) and (c), however, all three compounds gave two sets of NMR signals whose ratios were identically 1 : 0.8. As a result, regardless of the

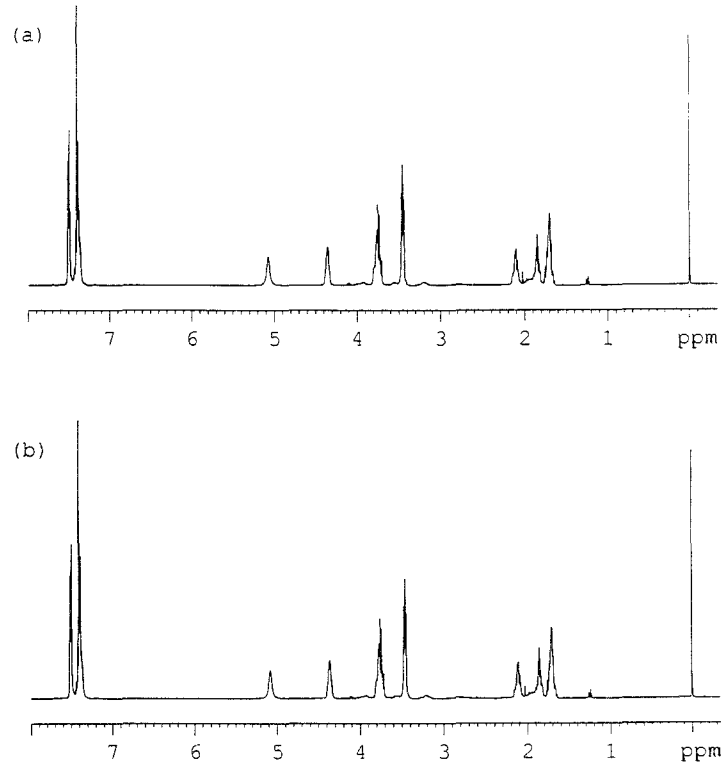


FIG. 4. ^{1}H-NMR spectra of K16 (a) and K16-1 (b).

substitution at the primary amine, the acetyl or benzoyl group of the secondary amine caused major and minor peaks.

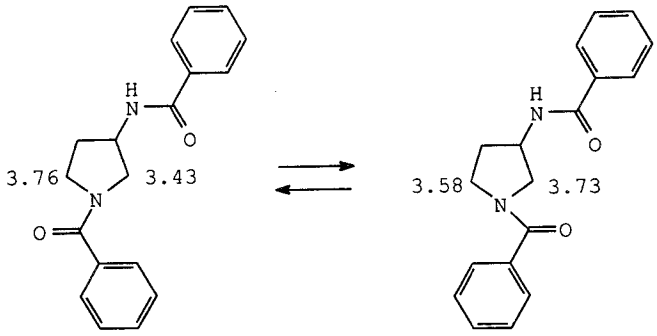
The reason for this result may be explained by free rotation of the primary amine group or a high rotational energy barrier at 25°C. Calculations of rotational energy barriers for K50, K52 and K1215 were carried out using CAMM in order to analyze this. The results are listed in TABLE 2. Since K50 and K52 do not have the functional group substituted at the primary amine, the changes in energy observed should be due to the rotations of the acetyl group of K50 and the benzoyl group of K52.

TABLE 1
NMR data assignments of K1215.

δ_C(ppm)	CH_n DEPT	δ_H(ppm) HMQC	COSY	Assignments
30.16	t	2.04, 2.35	H4a/H4b,5a,5b,3 H4b/H4a,5a,5b,3	C4
33.16	t	2.02, 2.27	H4a'/H4b',5a',5b',3 H4b'/H4a',5a',5b',3	C4'
44.80	t	3.75, 3.89	H5a/H5b,4a,4b H5b/H5a,4a,4b	C5
48.24	t	3.56, 3.60	H5a'/H5b',4a',4b' H5b'/H5a',4a',4b'	C5'
49.42	d	4.76	H3'/H2a',2b',4a',4b'	C3'
50.63	d	4.63	H3/H2a,2b,4a,4b	C3
51.67	t	3.72, 3.96	H2a'/H2b',3 H2b'/H2a',3	C2'
55.36	t	3.43, 3.88	H2a/H2b,3 H2b/H2a,3	C2
127.40	d	7.71	H17,19/H18	C17,19
127.49	d	7.80	H17',19'/H18'	C17',19'
127.58	d	-	-	C10,12,10',12' [a]
128.81	d	-	-	C8,13,8',13' [b]
128.98	d	-	-	C16,20,16',20' [c]
130.68	d	7.38	H18/H17,19	C18
130.68	d	7.39	H18'/ H17',19'	C18'
132.11	d	-	-	C11'
132.19	d	-	-	C11
134.19	s	-	-	C8
134.40	s	-	-	C8'
136.54	s	-	-	C15'
136.65	s	-	-	C15
167.97	s	-	-	C7,7'
170.51	s	-	-	C14,14'
			N6H/H3 N6H'/H3'	N6H(6.57ppm) N6H'(6.82ppm)

a) b) c) : may be interchanged ' : minor product

As shown in SCHEME 4(a) and (b), the energy barrier by the rotation of the acetyl group of K50 is 9 kcal/mol, and that of K52 is 6 kcal/mol. When the benzoyl group substituted at the secondary amine of K1215 is rotated, the rotational energy barrier is 12 kcal/mol. When the benzoyl group substituted at the secondary amine is fixed and that at the primary amine is rotated, the energy difference is greater than 20 kcal/mol, which is too high to rotate a benzoyl group.



K1215a K1215b

SCHEME 3.

Isomers determined by the direction of carbonyl group of benzoyl group substituted at secondary amine of K1215.

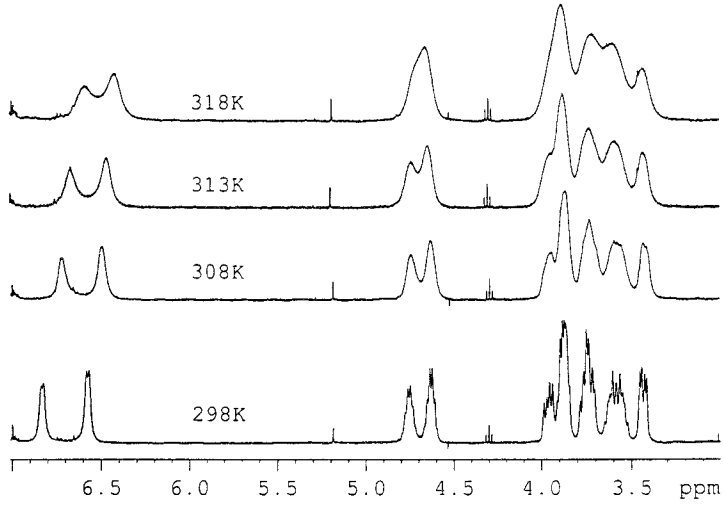


FIG. 5. ^{1}H-NMR experiments of K1215 carried out at various temperature conditions between 25°C and 45°C.

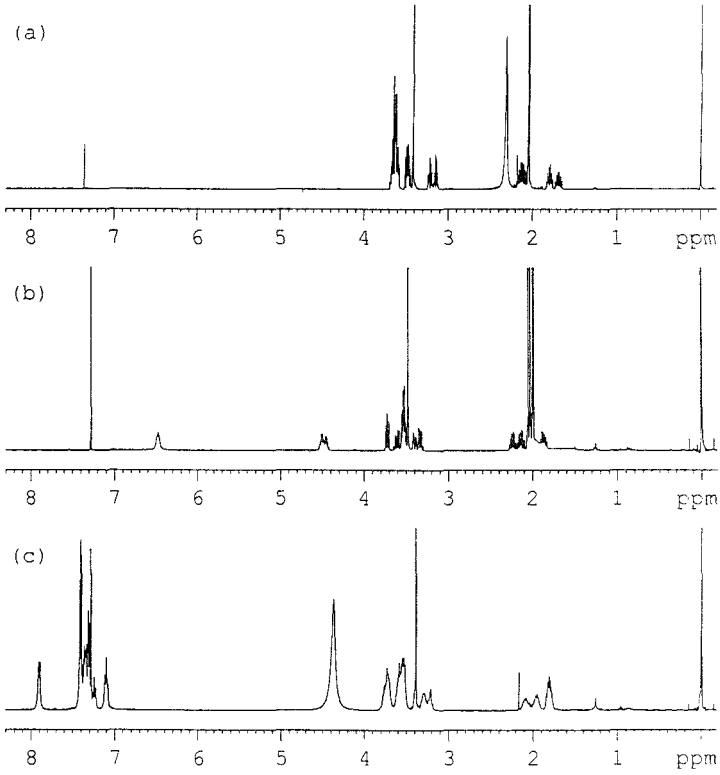


FIG. 6. ^{1}H-NMR spectra of K50 (a), K51 (b) and K52 (c).

The energy barrier can be calculated from the NMR data using Eyring's equation[12] :

Free energy = RT[23.76 – In(k/T)]

where k is the rate constant of internal rotation that can be calculated using the *Gutowsky-Holm equation.*[13]

$$k = \pi\delta\nu / \sqrt{2}$$

Here $\delta\nu$ is the difference between the frequencies of the NMR peaks separated by the rotation. In the case of H5 and H5', the free energy barrier at

TABLE 2

(a) The energy by the rotation of acetyl group of K50 using Molecular Dynamics caculation, (b) that of benzoyl group of K52, (c) that of benzoyl group substituted at primary amine of K1215 and (d) that of benzoyl group substituted at secondary amine of K1215.

(a)

Dihedral angle(°) C2-N1-C6=O	Energy(kcal/mol)
0	14-17
60	23-25
120	21-24
180	15-18
240(-120)	20-23
300(-60)	23-26
360(0)	14-17

(b)

Dihedral angle(°) C2-N1-C6=O	Energy(kcal/mol)
0	117-121
60	123-127
120	117-121
180	120-125
240(-120)	117-120
300(-60)	123-126
360(0)	117-121

(c)

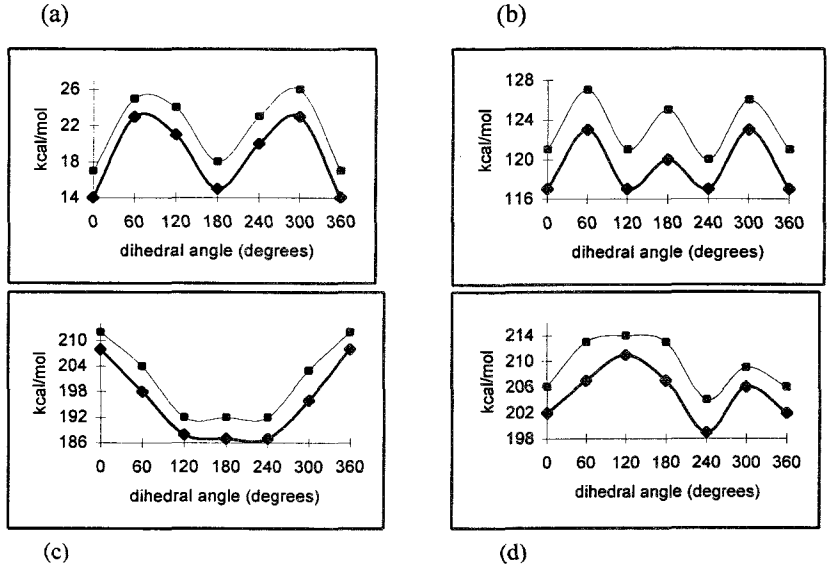
Dihedral angle(°)		Energy(kcal/mol)
C2-N1-14=O	H-N6-C7=O	
0	0	208-212
0	60	198-204
0	120	188-192
0	180	187-192
0	240(-120)	187-192
0	300(-60)	196-203
0	360(0)	208-212

(continued)

(TABLE 2 cont.)

(d)

Dihedral angle(°)		Energy(kcal/mol)
C2-N1-14=O	H-N6-C7=O	
0	0	202-206
60	0	207-213
120	0	211-214
180	0	207-213
240(-120)	0	199-204
300(-60)	0	206-209
360(0)	0	202-206



SCHEME 4.

(a) The energy diagram by the rotation of the acetyl group of K50 using Molecular Dynamics caculation, (b) that of the benzoyl group of K52, (c) that of the benzoyl group substituted at primary amine of K1215 and (d) that of the benzoyl group substituted at secondary amine of K1215. (black line : lowest energy, gray line : highest energy)

TABLE 3
The relationship of the change of the chemical shift ($\Delta\delta$) with temperature change (ΔT).

	298K	308K	313K	318K	$\Delta\delta/\Delta T$	R^2
N6H(ppm)	6.584	6.501	6.461	6.431	-0.0078	0.9959
H3(ppm)	4.639	4.646	4.653	-	0.0009	0.9643
N6H'(ppm)	6.835	6.730	6.659	6.600	-0.0118	0.9963
H3'(ppm)	4.765	4.757	4.747	-	-0.0011	0.9368

25°C is 14.4 kcal/mol ($\delta\nu$ = 76 Hz and thus k = 169 Hz), which is closer to the result obtained for the rotation of the benzoyl group substituted at the secondary amine than the primary amine. Hence, based on the NMR data and CAMM calculation, the two sets of NMR signals appear to arise from the rotation of the benzoyl group substituted at the secondary amine.

The existence of intramolecular hydrogen bonds in K1215 may also serve as the reason for the observed small chemical shift changes as found above. A plot of change in chemical shift with respect to temperature provides information into any existence of hydrogen bonds. As listed in TABLE 3, in the case of H3 the slope is 0.0009 ppm/K. In the case of N6H', which may possess the potential to be involved in a hydrogen bond, the slope is a higher value of 0.0118 ppm/K, thereby serving as evidence against hydrogen bonding.

Furthermore, the structure calculated using molecular dynamics also shows that the distance between the oxygen of the benzoyl group substituted at the secondary amine and the hydrogen atom of the primary amine is 5.84Å, negating the possibility of any hydrogen bond for K1215.

In conclusion, the major and minor peaks observed in the ^{1}H-NMR spectrum at 25°C of N-(1-benzoyl-3-pyrrolidinyl) benzamide are found to be caused by the rotation of the benzoyl group attached to the secondary amine.

ACKNOWLEDGEMENT

The authors acknowledge the financial support provided by the Korea Ministry of Science and Technology (grant No. EN18460).

REFERENCES

1. Rybak, M., Cappelletty, D., Moldovan, T., Aeschlimann, J., Kaatz, G. Comparative In Vitro Activities and Postantibiotic Effects of the Oxazolidinone Compounds Eperezolid (PNU-100592) and Linezolid (PNU-100766) versus Vancomycin against Staphylococcus aureus, Coagulase-Negative Staphylococci, Enterococcus faecalis, and Enterococcus faecium. Antimicrob. Agents Chemother. 1998; 42: 721.

2. Tucker, J., Allwine, D., Grega, K., Barbachyn, M., Klock, J., Adamski, J., Brickner, S., Hutchinson, D., Ford, C., Zurenko, G., Conradi, R., Burton, P., Jensen, R. Piperazynyl Oxazolidinone Antibacterial Agents Containing a Pyridine, Diazene, or Triazene Heteroaromatic Ring. J. Med. Chem. 1998; 41: 3727.

3. Noskin, G., Siddiqui, F., Stosor, V., Hacek, D., Peterson, L. In Vitro Activities of Linezolid Against Important Gram-positive Bacterial Pathogens Including Vancomycin-resistant Enterococci. Antimicrob. Agents Chemother. 1999; 43: 2059.

4. Shinabarger, D., Marotti, K., Murray R., Lin, A., Melchior, E., Swaney, S., Dunyak, D., Demyan, W., Buysse, J. Mechanism of Action of Oxazolidinones: Effects of Linezolid and Eperezolid on Translation Reactions. Antimicrob. Agents Chemother. 1997; 41: 2132.

5. Zurenko, G., Yagi, B., Schaadt, R., Allison, J., Kilburn, J., Glickman, S., Hutchinson, D., Barbachyn, M., Brickner, S. In Vitro Activities of U-100592 and U-100766, Novel Oxazolidinone Antibacterial Agents. Antimicrob. Agents Chemother. 1996; 40: 839.

6. Brickner, S., Hutchinson, D., Manninen, P., Ulanowicz, D., Garmon, S., Grega, K., Hendges, S., Toops, D., Ford, C., Zurenko, G. Synthesis and Antibacterial Activity of U-100592 and U-100766, Two Oxazolidinone Antibacterial Agents for the Potential Treatment of Multidrug-Resistant Gram-positive Bacterial Infections. J. Med. Chem. 1996; 39: 673.

7. Haddain, M., Jarrar, A. Conformation of 1,3-Diacyl and 1,3-Diaroylbenzimidazolones. Tetrahedron Lett. 1971; 20: 1651.

8. Brosius, A., Overman, L., Schwink, L. Total Synthesis of (+)-Aloperine. Use of a Nitrogen-Bound Silicon Tether in an Intramolecular Diels-Alder Reaction. J. Am. Chem. Soc. 1999; 121: 700.

9. Freeman, R., Morris, G. Correlation of Proton Chemical Shifts by Two-Dimensional Fourier Transform NMR. J. Magn. Reson. 1981; 42: 164.

10. Bax, A., Griffey, R., Hawkins, B. Correlation of Proton and Nitrogen-15 Chemical Shifts by Multiple Quantum NMR. J. Magn. Reson. 1983; 55: 301.

11. Novak, L., Hanania, M., Kovacs, P., Rohaly, J., Kolonits, P., Szantay, C. Synthesis of Novel Tryptamine and Azepinoindole Derivatives. Heterocycles 1997; 45: 2331.

12. Gunther, H. NMR Spectroscopy, 2nd ed., John Wiley & Sons, New York. 1992.

13. Kitamura, M., Tsukamoto, M., Takaya, H., Noyori, R. Conformational Study on 2-Acyl-1-alkylidene-1,2,3,4-tetrahydroisoquinolines. Bull. Chem. Soc. Jpn. 1996; 69: 1695.

Date Received: June, 30, 2000
Date Accepted: August 16, 2000