TAUTOMERISM OF CLITIDINE, A PYRIDINE NUCLEOSIDE FROM THE POISONOUS MUSHROOM *CLITOCYBE ACROMELALGA*†

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Abstracts-Tautomerism of clitidine (**1a**), a toxic pyridine nucleoside from the poisonous mushroom *Clitocybe acromelalga*, was investigated by ^{15}N NMR spectroscopy in aqueous solution and by X-Ray crystallographic analysis in the solid state, which showed the tautomer (**1d**) to be predominant in both states.

Clitidine (**1a**) is a pyridine nucleoside isolated from the poisonous mushroom *Clitocybe acromelalga* as a toxic principle.[1] The 5′-mononucleotide (**1b**) has also been isolated from the same fungus recently.[2] The analyses of the ^{1}H NMR and UV spectra suggested the 1,4-dihydropyridine form (**1a**) rather than the pyridinium form **1c** for the structure of the pyridine moiety,[3] but nevertheless tautomerism of clitidine has not been settled because there could be another 1,4-dihydropyridine form (**1d**). We therefore reinvestigated tautomerism of clitidine by ^{15}N NMR spectroscopy in aqueous solution and by X-Ray crystallographic analysis in solid state, and found the tautomer (**1d**) to be predominant in both states. Reported herein are our findings.

1a, R=H
1b, R=PO₃H₂
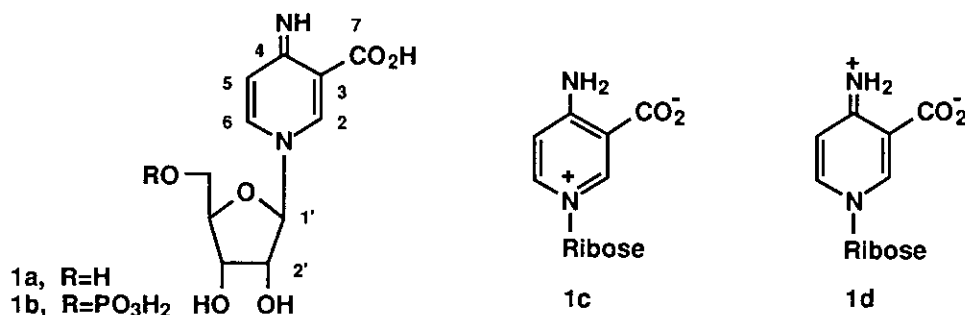
^{15}N NMR spectroscopy should be a valuable means for studying tautomerism of heterocyclic compounds, but it has not been practical because of poor sensitivity.[4] The use of a novel technique, pulsed field gradients (PFG), in combination with inverse-detection has overcome this drawback and made it possible to acquire ^{15}N NMR spectra at natural abundance.[5,6] Accordingly, we applied this novel method of ^{15}N NMR spectroscopy to investigate tautomerism of clitidine in aqueous solution.

There were two nitrogen signals at 81.6 and 153.9 ppm in the PFG ^{1}H-^{15}N HMBC spectrum in D$_2$O.[7] All the two- and three-bond correlation peaks were observed as shown in Figure 1. Thus, the signal at 153.9 ppm was unambiguously assigned to the ring nitrogen [N(1)] since it correlated to the proton signals at 4.20 [H(2')], 5.54 [H(1')], 6.79 [H(5)], 7.96 [H(6)] and 8.58 [H(2)] ppm. The chemical shift value (81.6 ppm) assigned to the N(4) is lower than those of amino nitrogen and higher than those of imino nitrogen, indicating restricted rotation and partial double bond character of the C-N bond.[8]

The PFG ^{1}H-^{15}N HMQC spectrum in 90% H$_2$O-D$_2$O at 4 °C clearly showed that N(4) bore two protons since the N(4) signal correlated to the proton signals at 7.91 and 9.02 ppm, assigned to NH (H$_b$ and H$_a$, respectively) because both of them did not appear in the ^{1}H NMR spectrum in D$_2$O. When irradiated the 9.02 ppm signal (H$_a$), NOEs were observed for the ^{13}C singlet signals at 117.4 [C(3)], 162.5 [C(4)] and 172.5 [C(7)] in the C{H}NOE difference spectrum as shown in Figure 2, which suggested that H$_a$ was close to the carbonyl group and, hence, formed a hydrogen bond with the carbonyl oxygen. Without decoupling, the PFG ^{1}H-^{15}N HMQC spectrum showed both of the N-H coupling constant ($^1J_{NH}$) to be 96 Hz. This value is larger than ordinary values for amines,[4] indicating the increased s-character of the C(4)—N(4) bond. Additionally, the H-D exchange experiment and variable temperature study at from 4 to 50 °C also suggested the restricted rotation of the C(4)—N(4) bond. When measured at 50 °C in 90% H$_2$O-D$_2$O, only the signal of H$_b$ disappeared by coalescence into H$_2$O signal whereas the H$_a$ signal remained almost unchanged, which implied that the exchange rate of H$_b$ with bulk water was faster than that of H$_a$ and could be rationalized by restricted rotation of the C(4)—N(4) bond. All these data led to a conclusion that the tautomer (1 d) is the predominant and most contributed form of clitidine in aqueous solution.[9]
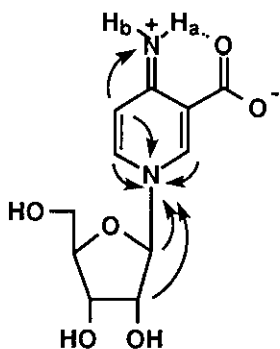

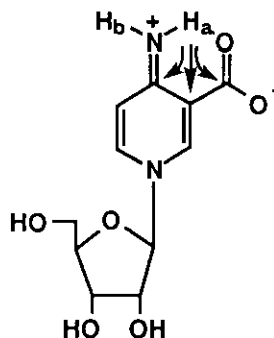Figure 1 PFG ^{1}H-^{15}N HMBC correlations.

Figure 2 C{H}NOEs irradiated at H$_a$

An X-Ray crystallographic analysis of clitidine[10] showed the tautomer (1 d) to be predominant in the solid state as well. An ORTEP drawing and the selected data are shown in Figure 3 and Table 1, respectively. Usually, when a pyridine ring is in pyridinium form, all the C-C bond lengths in the ring are nearly equal in a range of 1.35-1.39 Å, whereas in 1,4-dihydropyridine form, the length of C(β)—C(γ) is significantly longer than that of C(α)—C(β).[11] In the case of clitidine, the bond lengths of the C(3)—C(4) and C(4)—C(5) were much longer than those of the C(2)—C(3) and C(5)—C(6), which apparently indicated the pyridine ring to be in 1,4-dihydropyridine form. Another feature of the bond distances was the considerably

short distance of the C(4)—N(4) bond. It was shorter than any C-N bond in 2-, 3-, and 4-aminopyridines (1.35-1.38 Å),[12] and was even shorter than the ring C-N bonds [N(1)—C(2) and N(1)—C(6)], suggesting the highly double bond character of this bond. As for the bond angles, the angle of C(3)—C(4)—C(5) is nearly 120 °in pyridinium form, whereas it is significantly reduced (2-4°) in 1,4-dihydropyridine form.[11] In the case of clitidine, the angle of C(3)—C(4)—C(5) was 116.8°, implying 1,4-dihydropyridine structure. In addition, the atoms in the pyridine ring were almost coplanar including the iminium group and the carboxyl group, which suggested the hydrogen bonding between H_a and the carbonyl group, N(4)---O(7)= 2.668(4) Å, as seen in aqueous solution. Taking these evidences into considerations, the base moiety of clitidine adopts the 4-iminium-1,4-dihydropyridine form (**1 d**) in the solid state.

In conclusion, the tautomer (**1 d**) is the predominant and most contributed form of clitidine both in aqueous solution and in the solid state. This unusual predominance of 4-iminium-1,4-dihydropyridine form may arise from the stabilization of this form by the hydrogen bond between H_a and the carboxyl group, and the proximity of the iminium and the carboxylate groups.

Table 1. *Selected bond distances and angles (Å, °).*

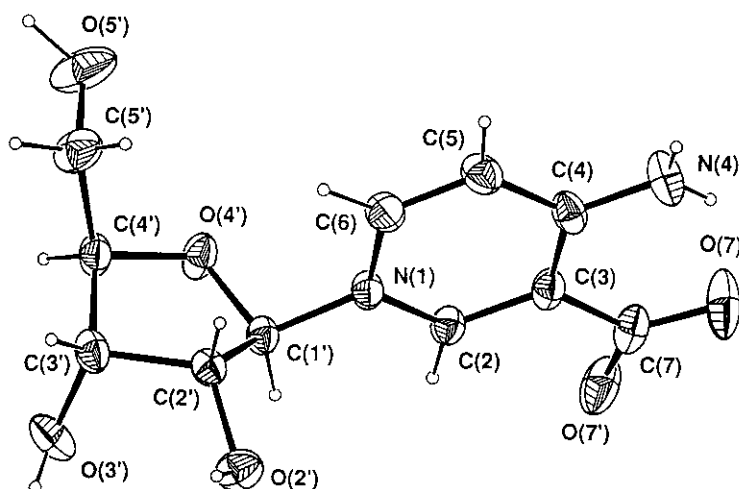| N(1)—C(2)	1.350(3)	C(2)—N(1)—C(6)	119.3(2)
C(2)—C(3)	1.370(3)	N(1)—C(2)—C(3)	122.8(2)
C(3)—C(4)	1.431(3)	C(2)—C(3)—C(4)	118.5(2)
C(4)—C(5)	1.418(3)	C(3)—C(4)—C(5)	116.8(2)
C(5)—C(6)	1.345(3)	C(3)—C(4)—N(4)	122.5(2)
C(6)—N(1)	1.368(2)	C(5)—C(4)—N(4)	120.6(2)
C(4)—N(4)	1.330(3)	C(4)—C(5)—C(6)	121.0(2)
N(1)—C(1')	1.478(3)	C(5)—C(6)—N(1)	121.3(2)
C(3)—C(7)	1.508(3)	C(2)—N(1)—C(1')	119.7(1)
C(7)—O(7)	1.242(4)	C(6)—N(1)—C(1')	121.0(2)
C(7)—O(7')	1.262(3)	C(2)—C(3)—C(7)	118.8(2)
		C(4)—C(3)—C(7)	122.7(2)

Figure 3   ORTEP drawing of the crystallographic structure of clitidine.

REFERENCES AND NOTES

† This paper is dedicated to Professor Koji Nakanishi on the occasion of his 75th birthday.

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7.   Clitidine (10 mg) was dissolved in D_2O or 90% H_2O-D_2O (0.3 mL). The ^{1}H-^{15}N HMBC and HMQC spectra were recorded on a 600 MHz NMR spectrometer (JEOL JNM α600). $^{15}NH_4NO_3$ in DMSO-d_6 was used as an external standard at 0 ppm.

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9.   This study demonstrated for the first time that PFG ^{1}H-^{15}N HMBC and HMQC spectroscopy be useful for investigation of tautomerism of heterocyclic compounds at natural abundance and may have wide applicability to such investigations.

10. Crystal data of clitidine: $C_{11}H_{14}N_2O_6$·1/2H_2O (recrystallized from water), monoclinic, space group $C2$, a=20.286(5) Å, b=6.684(2) Å, c=9.360(2) Å, β=108.59(2)°, V=1202.8(6) Å^3, Z=4, R=0.040, Rw=0.054, Number of reflections used was 1374.

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