Note

Structural Changes of N-Methylmyosmine Based on pH

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N-Methylmyosmine is an important intermediate in a biological degradation of nicotine. The authors previously reported the structure of N-methylmyosmine 1 in an organic solvent and its reversible change to pseudooxynicotine 2 dihydrochloride, however, its structure under the physiological pH condition was not determined. When N-methylmyosmine was dissolved in buffers of various pHs, their UV spectra were different from one another as shown in Fig. 1. The figure indicates that the molecular species in acidic solutions is different from that in strongly alkaline solutions and that the third molecular species is present at physiological pHs. The molecular species were thought to be N-methylmyosmine and pseudooxynicotine under the strongly alkaline and strongly acidic conditions, respectively, while at the physiological pH range, nicotine-1'-iminium ion 3 was estimated to exist in an equilibrium.

We further examine the estimation with NMR.

The pHs of N-methylmyosmine and pseudooxynicotine dihydrochloride 10% D2O solution were 10.5 and 1.6, respectively.

The 1H-NMR spectrum of pseudooxynicotine in D2O is shown in Fig. 2-A. The solution was gradually made alkaline and the 1H-NMR spectra of the solution at each pH were recorded, and are shown in Fig. 2-B~E. When the pseudooxynicotine D2O solution was made alkaline up to pH 10.5, the 1H-NMR spectrum (Fig. 2-E) agreed with that of N-methylmyosmine (Fig. 2-F). When N-methylmyosmine D2O solution was acidified gradually, the same spectra were also obtained at the corresponding pHs, except the 3.43 ppm signals (d) in the acidic range, which were not observed this time. Since the C-3' olefinic proton of N-methylmyosmine is exchanged with deuterium, it is expected that the signals of C-2' protons of pseudooxynicotine formed in D2O from N-methylmyosmine do not appear.

When the pH of the solution was changed from 10.5 to 9.0, the methyl signals (a') of N-methylmyosmine shifted from 2.30 to 2.85 ppm according to pH values, and it did not shift below pH 9.0. The methyl signals (a) of pseudooxynicotine were at 2.82 ppm at pH 1.6 but at 2.85 ppm above pH 4.1. The signals of C-4' (c) and C-5' (b') protons of N-methylmyosmine are not to be distinguished from the signals of C-3' (c) and C-4' (b) protons of pseudooxynicotine, respectively. Namely, N-methylmyosmine and pseudooxynicotine at the physiological pH range can be distinguished from each other only on the basis of C-2' (d) proton signals according to H-NMR spectra. Between pH 1.6 and pH 4 the C-2' proton signals of pseudooxynicotine at 3.43 ppm were observed, and they disappeared when pH was above 5.

According to the 13C-NMR spectra of pseudooxynicotine, the 197.5 ppm signals, C-1', disappeared when the pH of the D2O solution was changed up to 5.0. This results also support the above-mentioned findings.

N-Methylmyosmine CD3OD solution was added to an anhydrous HCl CH3OD solution, and its 1H-NMR spectrum was measured (Fig. 2-G). In this case, an enammonium structure is formed. However, N-protonation takes place rapidly and is followed by a transfer of the proton to the C-3' carbon. The rearrangement of the enammonium structure to an iminium structure is known kinetically. In addition to these information, when the temperature of the solution was raised up to 50°C, the signals shown in Fig. 2-C did not change except solvent signals. These facts show that the
synthesized compound has the iminium structure. The signals at 3.74 (3H, singlet), 2.54 (2H, multiplet), and 4.54 ppm (2H, triplet) which were observed between pH 2 and 9.5 (Fig. 2) were respectively assigned to methyl, C-4' and C-5' proton signals of nicotine-1'-iminium ion; the spectrum is shown in Fig. 2-G.

As shown in Fig. 1, the peak of UV spectra at shorter wavelength slightly shifted between pH 6 and 10, and largely shifted at pH 4 and 5 toward longer wavelength. These results indicate that the iminium ion exists in some quantities at pH 4 and 5. The pK1 and pK2 of N-methylmyosmine were 5.5 and 8.6, respectively, supporting the pyrrole structure.

To sum up, molecular species in the aqueous solution are pseudoxynicotine below pH 4 and N-methylmyosmine above pH 5, and nicotine-1'-iminium ion exists in an equilibrium with the other two species between pH 2 and 9.5 (Fig. 3).

N-Methylmyosmine and pseudoxynicotine dihydrochloride were synthesized as described in previous papers.1,2 Nicotine-1'-iminium ion was synthesized by the addition of anhydrous HCl CD3OD solution into N-methylmyosmine CD3OD solution.1H-NMR spectra were measured with a JEOL JNM-PS-100 (100 MHz) NMR spectrometer.13C-NMR spectra were recorded by a JEOL JNM-FX100 Fourier transformation NMR spectrometer. Both compounds were dissolved in D2O at the concentration of 10%, and the pH was adjusted with conc NaOD, DCl and/or dried Na3PO4 and measured with a Toko combination electrode CE-103 (for NMR). None of the pH measurements was corrected for pD. The references for H-NMR were 2,2-dimethyl-2-silapentane-5-sulfonate sodium salt and tetramethylsilane for D2O and organic solvent solutions, respectively, and the reference for 13C-NMR was methanol. UV spectra were measured with a Hitachi EPS-3T spectrometer, at the concentration of 0.1 mM in 10 mM potassium phosphate buffer, and pKa were measured with a Toa Electronic auto burette HS-2A.

REFERENCES

2) T. Kisaki, M. Iida and E. Wada, Bull. Agric. Chem.
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