Tautomeric Equilibrium of Salicylidene Schiff Base. UV-Visible Absorption and Raman Spectroscopic Studies

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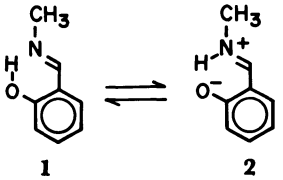
Synopsis. The solvent effect and temperature dependence on the Raman and absorption spectra demonstrated the existence of a tautomeric equilibrium for the title compound between the enol form and the protonated Schiff base form in methanol; the latter form is more stable by ca. 850 cal mol^{-1}(1 cal=4.184 J) than the former. Characteristic Raman bands for the two tautomers have been identified.

An intramolecular proton transfer has been considered to be a major factor for controlling the activity of some biological molecules.[1] Aspartate aminotransferase, having a pyridoxal Schiff base as a cofactor, is an example for which the enzymic activity has been associated with the protonation state of the cofactor,[2] although the detailed mechanism has not yet been established. Salicylidene Schiff bases are expected to serve as a good model for the active site of the pyridoxal enzyme. Previous IR and UV-visible absorption studies[3-5] suggested that the Schiff base exists as a tautomeric mixture in a polar solvent; however there has been no firm spectroscopic evidence for this idea. Accordingly, in this study, the UV-visible and Raman spectra were investigated for the simplest Schiff base of this class, namely, *N*-salicylidene methylamine (SAL-MA) and *N*-(*o*-methoxybenzylidene)methylamine (ANIS-MA) (Fig. 1), in which the vibrational contribution from the amine moiety is minimized. SAL-MA has a proton that can be transferred whereas ANIS-MA does not. Thus, ANIS-MA can serve as a model of tautomer **1** in Fig. 1.

Experimental

SAL-MA and ANIS-MA were synthesized by a direct condensation of salicylaldehyde or *o*-anisaldehyde with methylamine in alkaline methanol (MeOH), respectively. The absorption spectra were recorded on a Hitachi 220S spectrometer. For Raman measurements, the off-resonance condition was chosen so that Raman signals from both tautomers could be observed simultaneously. The 514.5 nm line of an Ar^+ ion laser (NEC GLG3200) was used for the excitation. Excitation at shorter wavelengths brought about an intense fluorescence which hindered us from measuring the resonance Raman spectrum of each tautomer. Raman spectra were recorded on a JEOL-400D Raman spectrometer equipped with a cooled RCA-31034a photomultiplier. Low-temperature measurements for absorption and Raman spectra were carried out in a specially designed glass Dewar. A quartz cell with a 1-mm path length was placed inside the Dewar and cooled with cold ethanol. The temperature of the sample was monitored by a thermocouple attached to the sample cell.



1 2

Fig. 1. Tautomeric structures of SAL-MA. For ANIS-MA the OH group is replaced by the OCH_3 group in **1**.

Results and Discussion

The UV-visible absorption spectrum of SAL-MA in pure MeOH is shown in the inset of Fig. 2. Two bands were observed around 300 and 400 nm. For convenience, the bands at 300 and 400 nm are designated as Band A and B, respectively. When the solvent was changed from pure MeOH to pure $CHCl_3$, the 400-nm band disappeared and in a mixed solvent of MeOH/$CHCl_3$ SAL-MA gave an intermediate spectrum (not shown). On the other hand, ANIS-MA gave a single peak near 300 nm both in MeOH and $CHCl_3$. As the temperature was lowered, the relative intensity of Band B to Band A for SAL-MA increased. Isosbestic points were obtained at 283, 338, and 435 nm. The solvent effect and the temperature dependence suggest that there are two species in a MeOH solution of SAL-MA; one absorbs light at 300 nm and the other at 400 nm. The latter species should have a more polar structure than the former. In Fig. 2 the logarithm of the ratio of the peak intensities are plotted against the reciprocal absolute temperature. A fairly good straight line was obtained; its slope indicates that the species giving rise to Band B is more stable than the other by ca. 850 cal mol^{-1}. This is comparable to the value for *N*-(2-hydroxy-1-naphthylmethylene) aniline reported by Dudek.[6]

Figure 3 shows the temperature dependence and solvent effect on the Raman spectra of SAL-MA,

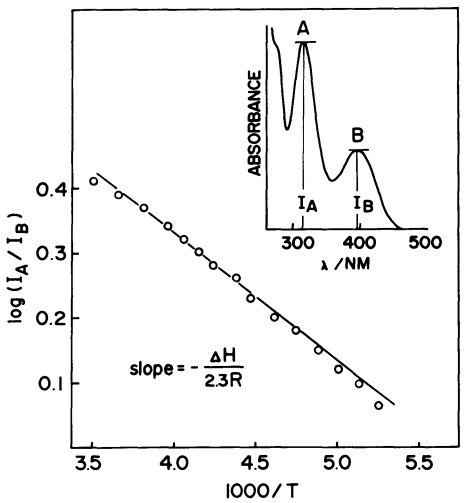


Fig. 2. Plot of the logarithm of the ratio of peak intensities versus the reciprocal absolute temperature. The inset is UV-visible absorption spectrum of SAL-MA in MeOH at 295 K.

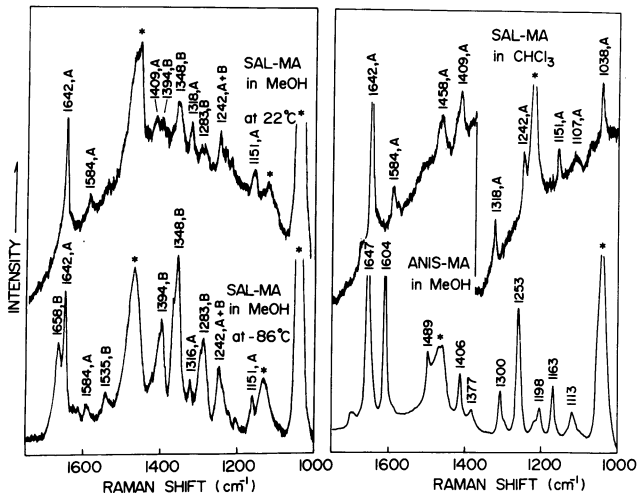


Fig. 3. Raman spectra of SAL-MA and ANIS-MA. left panel; Raman spectra of SAL-MA in MeOH at 22 °C (top) and at −86 °C (bottom), right panel; Raman spectra of SAL-MA in $CHCl_3$ (top) and ANIS-MA in MeOH (bottom) at room temperature. Excitation 514.5 nm, 150 mW, spectral slit-width 5 cm^{-1}. Solvent peaks are denoted by asterisks.

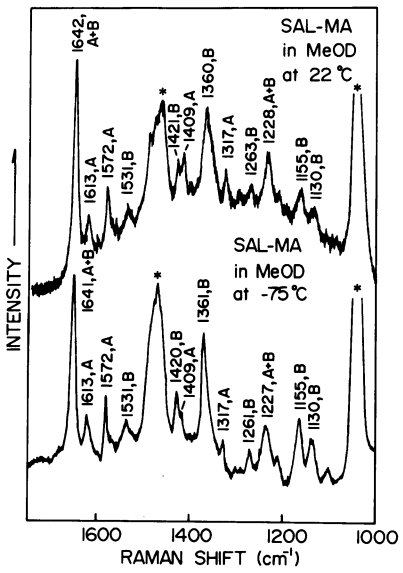


Fig. 4. Temperature dependence of Raman spectra of SAL-MA in MeOD. Excitation 514.5 nm, 100 mW, spectral slit-width 5 cm^{-1}.
Solvent peaks are denoted by asterisks.
The 1228 cm^{-1} and 1155 cm^{-1} bands contain marginal intensity contributions from the solvent.

together with the spectrum of ANIS-MA in MeOH. As the temperature of the MeOH solution of SAL-MA is lowered, the intensities of the Raman bands marked by B increase at the expense of intensities of the Raman bands marked by A. Most noticeable are the doublet features around 1650 cm^{-1} which fall in the C=N stretching region. This temperature dependence for Raman bands A and B is comparable with that observed for Bands A and B in the UV-visible absorption spectrum. In $CHCl_3$ SAL-MA shows only Raman bands marked by A. Consequently, it follows that the species yielding Bands A and B in the UV-visible spectra give rise to Raman bands A and B, respectively. It is noteworthy that the Raman spectrum of ANIS-MA is much closer to the Raman spectrum of A than to that of B.

The A bands of SAL-MA at 1642 and 1242 cm^{-1} correspond to the bands of ANIS-MA at 1647 and 1253 cm^{-1}, respectively. In MeOD the 1642 cm^{-1} band remained unshifted but the 1242 cm^{-1} band was shifted to lower frequency by 14 cm^{-1} (as shown in Fig. 4). The spectrum of O-deuterated SAL-MA in $CHCl_3$, which gives exclusively A peaks, showed the unshifted 1642 cm^{-1} band but lacked the 1242 cm^{-1} band, although the lower-frequency side of the 1242 cm^{-1} band was masked by the solvent band. The C-OH stretching mode of phenol shifts from 1252 to 1241 cm^{-1} upon O-deuteration.[7] Therefore, the 1642 and the 1242 cm^{-1} bands can be assigned to the -C=N- and C-OH stretching modes, respectively. The bands of SAL-MA at 1584, 1151, and 1038 cm^{-1} are assigned to the benzene ring modes (ν_{8a}, ν_{9a}, and ν_{18a}, respectively). These results suggest that species A corresponds to the enol form (**1** in Fig. 1). With regard to the Raman spectrum of species B, on the other hand, a comparison of Fig. 3 with Fig. 4 suggests that the 1658 cm^{-1} band undergoes a low-frequency shift by 15 cm^{-1} and the 1283 cm^{-1} band seems to disappear when the solvent is changed from MeOH to MeOD. The former can be assigned to the -C=NH^+-stretching mode because the higher frequency for the -C=NH^+- stretch compared to the -C=N- stretch and the proximity of the -C=ND^+- stretching frequency to the -C=N- one are consistent with the results for retinal Schiff base derivatives.[8] The latter is a candidate for the NH bending mode of the -C=NH^+- group, although the frequency seems to be too low for this mode. The effects of *N*-deuteration on the other B peaks are rather complicated; this prevents us from giving consistent band assignments. However, the data shown here strongly suggest that the B species takes a protonated Schiff base, structure **2**.

In conclusion, clear evidence for the tautomerism of a salicylidene Schiff base between **1** and **2** in Fig. 1 was obtained for the first time from the Raman spectrum; this will help in an interpretation of the resonance Raman spectrum of the pyridoxal enzyme.

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