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Fluorescent Product in the Determination of Aromatic Aldehydes with 4,5-Dimethoxy-1,2-diaminobenzene

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The fluorescent product formed from benzaldehyde or *p*-hydroxybenzaldehyde with 4,5-dimethoxy-1,2-diaminobenzene has been characterized by structural analysis as the corresponding 2-aryl-5,6-dimethoxybenzimidazole. The product fluoresces most intensely in alkaline solution.

Keywords——4,5-dimethoxy-1,2-diaminobenzene; benzaldehyde; *p*-hydroxybenzaldehyde; fluorescent product; 2-phenyl-5,6-dimethoxybenzimidazole; 2-(*p*-hydroxyphenyl)-5,6-dimethoxybenzimidazole

A sensitive and selective fluorimetric method for the determination of aromatic aldehydes has been reported, based on their reactions in dilute hydrochloric acid with 4,5-dimethoxy-1,2-diaminobenzene (DDB) monohydrochloride to give a compound which fluoresces in alkaline solution.[1] This paper describes the isolation and characterization of the fluorescent compounds produced from benzaldehyde and *p*-hydroxybenzaldehyde.

Experimental

Reagents——All chemicals were of reagent grade, unless otherwise noted. Double-distilled water was used throughout. DDB·HCl was prepared as described previously.[1]

Apparatus——Corrected fluorescence spectra and intensities were measured with a Hitachi 650-60 spectrofluorimeter in 10 × 10 mm quartz cells; spectral bandwidths of 10 nm were used in both the excitation and emission monochromators. Ultraviolet (UV) spectra were taken with a Hitachi 200-20 spectrophotometer in C_2H_5OH solution in 10 mm quartz cells. Infrared (IR) spectra were recorded with a Nihonbunko A-302 spectrophotometer in KBr pellets. Proton nuclear magnetic resonance (^{1}H-NMR) spectra were obtained with a Hitachi R-22 spectrometer at 90 MHz for about 5% solutions in CH_3OH-d_4 or $(CH_3)_2SO$-d_6 containing $(CH_3)_4Si$ as an internal standard. Mass spectra (MS) were taken with a JEOL D-300 spectrometer. pH was measured with a Hitachi-Horiba M-7 pH meter at about 25 °C. Melting points are uncorrected.

Isolation of the Product from Benzaldehyde and Its Spectral Properties——DDB·HCl (3.0g, 14.6 mmol) dissolved in a mixture of 10 ml of 0.5 M HCl and 190 ml of C_2H_5OH was mixed with 2.0 g (18.8 mmol) of freshly distilled benzaldehyde. The mixture was refluxed for about 30 h and then concentrated *in vacuo*. Yellowish crystals that separated on cooling were collected and recrystallized from C_2H_5OH to give colorless prisms (I hydrochloride), mp 228 °C (dec.). Yield 480 mg. *Anal.* Calcd for $(C_{15}H_{14}N_2O_2 \cdot HCl)_4 \cdot H_2O$: C, 61.02; H, 5.29; N, 9.49. Found: C, 60.98; H, 5.09; N, 9.32.

The hydrochloride of I (250 mg) was dissolved in about 50 ml of hot H_2O, then the solution was made alkaline with 3 ml of 10% NH_4OH and chilled. The separated crystals were filtered off and washed successively with small portions of cold 10% NH_4OH and H_2O to give colorless prisms (I), mp 98 °C, slightly hygroscopic. Yield, 200 mg. *Anal.* Calcd for $(C_{15}H_{14}N_2O_2)_2 \cdot 3H_2O$: C, 64.04; H, 6.09; N, 9.96. Found: C, 64.23; H, 5.96; N, 9.79. UV $\lambda_{max}^{C_2H_5OH}$ nm (log ε): 206 (4.51), 326 (4.41). IR ν_{max}^{KBr} cm^{-1}: 3310 (NH), 1585 (aromatic C=C), 770 and 696 (CH, monosubstituted benzene), 886 (isolated CH in benzene ring). ^{1}H-NMR (in CH_3OH-d_4) δ (ppm): 3.90 (s, 6H, methyl protons), 7.12 (s, 2H, isolated aromatic protons), 7.38—7.54 (m, 3H, aromatic protons), 7.94—8.05 (m, 2H, aromatic protons). MS *m*/*z*: 254.1051 (M^+ of anhydrous I, base peak; calculated value for $C_{15}H_{14}N_2O_2$, 254.1050), 239 ($M^+ - CH_3$), 211

(M^+-CH_3-CO).

Isolation of the Product from *p*-Hydroxybenzaldehyde and Determination of Its Spectral Properties——DDB·HCl (3 g, 14.6 mmol) and 2.5 g (20.5 mmol) of *p*-hydroxybenzaldehyde (recrystallized from H_2O) were treated in the same way as described for the mixture of DDB·HCl and benzaldehyde to obtain slightly yellowish prisms (II hydrochloride), mp 251 °C (dec.). Yield, 1040 mg. *Anal.* Calcd for $(C_{15}H_{14}N_2O_3\cdot HCl)_2\cdot H_2O$: C, 57.06; H, 5.11; N, 8.87. Found: C, 57.28; H, 5.02; N, 8.68.

The hydrochloride of II (500 mg) was treated as described for I hydrochloride to give colorless prisms (II), mp 153 °C, slightly hygroscopic. Yield, 420 mg. *Anal.* Calcd for $C_{15}H_{14}N_2O_3\cdot 2H_2O$: C, 58.82; H, 5.92; N, 9.15. Found: C, 58.74; H, 5.76; N, 8.99. UV $\lambda_{max}^{C_2H_5OH}$ nm (log ε): 208 (4.53), 325 (4.41). IR ν_{max}^{KBr} cm^{-1}: 3320 (NH and OH, br), 1609 and 1548 (aromatic C=C and/or C=N), 885 (isolated CH in benzene ring), 838 (adjacent two CHs in benzene ring). ^1H-NMR (in $(CH_3)_2SO$-d_6) δ(ppm): 3.82 (s, 6H, methyl protons), 6.89 (d, $J=8.5$ Hz, 2H, aromatic protons), 7.07 (s, 2H, isolated aromatic protons), 7.93 (d, $J=8.5$ Hz, 2H, aromatic protons), 9.80 (br s, 1H, hydroxyl proton, exchangeable with deuterium). MS *m/z*: 270.1008 (M^+ of anhydrous II, base peak; calculated value for $C_{15}H_{14}N_2O_3$, 270.1000), 255 (M^+-CH_3), 227 (M^+-CH_3-CO).

Results and Discussion

Corrected fluorescence excitation and emission spectra of compounds I and II, dissolved in the reagent blank for the method of determination, were identical with those of the reaction mixtures of benzaldehyde and *p*-hydroxybenzaldehyde, respectively. The excitation and emission maxima were at 336 and 409 nm for both I solution and the reaction mixture of benzaldehyde, and 340 and 391 nm for both II solution and the reaction mixture of *p*-hydroxybenzaldehyde, respectively. This suggests that the fluorescence reaction affords a single fluorescent product from an aromatic aldehyde.

The most intense fluorescence of the compounds dissolved in Britton–Robinson buffers occurs at higher pHs, while the fluorescence of DDB almost disappears at pHs exceeding 6.8 (Fig. 1). These pH dependences are analogous to that of the reaction mixture in the method of determination.

It was reported previously that the fluorescence of the reaction mixture in the analytical procedure was unstable in the absence of β-mercaptoethanol.[1] However, the fluorescence of I and II solutions in 0.04 M Britton–Robinson buffers of pH 4.10—11.58 is stable in daylight for more than 1 h even in the absence of β-mercaptoethanol. The reason for this discrepancy is not clear.

The molecular ions in the MS and the elemental analysis data of the compounds indicate that the anhydrous free bases of I and II have the molecular formulae $C_{15}H_{14}N_2O_2$ and $C_{15}H_{14}N_2O_3$, consistent with those of 2-phenyl- and 2-(*p*-hydroxyphenyl)-5,6-dimethoxybenzimidazoles, the expected reaction products for benzaldehyde and *p*-hydroxybenzaldehyde, respectively (Chart 1).

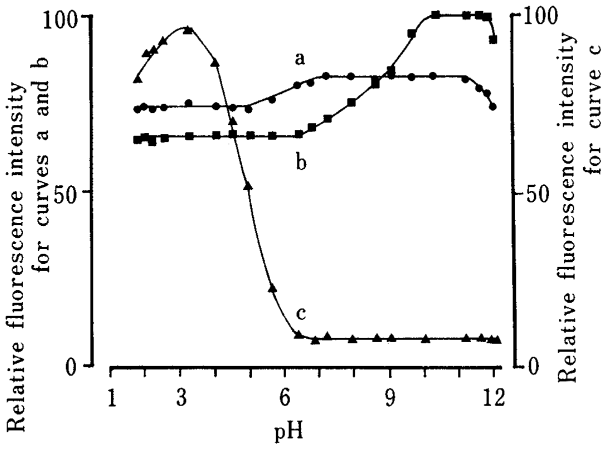

Fig. 1. Effect of pH on the Intensity of Fluorescence from I, II and DDB

Samples were dissolved in 0.04 M Britton–Robinson buffers of various pHs, and corrected fluorescence intensity was measured at the emission maximum with irradiation at the excitation maximum in each case. The intensity for I at pH 11.2 was taken as 100.

a, I (1×10^{-7} M); b, II (1×10^{-7} M); c, DDB (1×10^{-4} M).

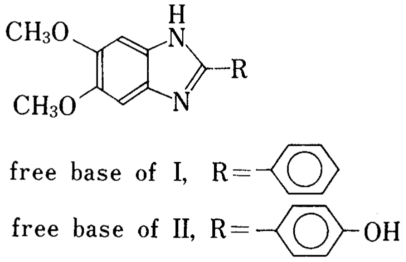

Chart 1

The presence of a phenyl group in I and a *p*-hydroxyphenyl group in II is suggested by the presence in the ^{1}H-NMR spectra of two multiplets, and two doublets and a broad singlet which disappeared on adding heavy water, respectively. The ^{1}H-NMR, mass and IR spectral patterns of I and II are in agreement with the 5,6-dimethoxybenzimidazole structures.

From these observations, we conclude that the fluorescent product of a given aromatic aldehyde is the corresponding 2-aryl-5,6-dimethoxybenzimidazole; the carbon atom of the aldehyde group is incorporated into the five-membered heterocyclic moiety of the fluorescent compound under the analytical reaction conditions. This is analogous to the fluorescence reactions of aromatic aldehydes with 2-aminothiophenol,2) with 2,2′-dithiobis-(1-aminonaphthalene)3) and with 1,2-diaminonaphthalene4) to yield 2-arylbenzthiazole,2,5) 2-arylnaphtho[1,2-*d*]thiazole6) and 2-arylnaphtho[1,2-*d*]imidazole,7) respectively.

References and Notes

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