to point out that the hydrogen bonding can be considerably more reactive than the acid dissociation for basic media. Both compounds IV and V were however far below the line plotted in Fig. 5, in spite of the stronger acids of them. This fact suggests that the proton-donating abilities level off at about 11 of the pK_a values in this selected area.

Acknowledgement The authors express their deep gratitude to Mr. M. Nakatomi, President of Hisamitsu Pharmaceutical Co., Inc., for the supply of several chemicals. The microanalyses were performed by Mrs. K. Shiraki of the Central Analysis Room of this Faculty ot whom they are also grateful.

[Chem. Pharm. Bull. 22(8)1927—1929(1974)]

UDC 547.571.04 : 547.556.7.04

Mechanism of the Color Reaction between Aldehyde and Diazotized Aromatic Amine. II.[1] Color Reaction Product of Benzaldehyde with Diazotized Sulfanilamide

Masaru Nakamura, Kimiyo Araki, Kunihide Mihashi,[2a] and Yosuke Ohkura[2b]

Faculty of Pharmaceutical Sciences, Fukuoka University[2a] and Faculty of Pharmaceutical Sciences, Kyushu University[2b]

(Received February 14, 1974)

In the previous paper,[1] the main coloring matters produced in the color reaction of formaldehyde with diazotized sulfanilamide and with diazotized *p*-aminobenzoic acid were isolated in crystalline forms and their chemical structures were characterized as 1,3,5-tri- and 1,5-diphenylformazan derivatives. This paper extends the study to the color reaction of benzaldehyde with diazotized sulfanilamide.

Isolation of Color Reaction Product

The color reaction of aldehyde, especially aromatic one, with diazotized aromatic amine in an alkaline medium, which has been utilized for its detection, usually required sodium amalgam as a reducing agent.[3] Recently, sodium hydrosulfite was found in our laboratories to be substituted for the amalgam, which was more conveniently used for the present purpose and gave more intense color.[4]

Benzaldehyde gave a reddish violet color when treated with diazotized sulfanilamide in the presence of sodium hydrosulfite at moderately high temperature. In the reaction, the maximum color intensity was observed in the molar ratio of the aldehyde to the diazotized amine approximately 1: 2. Therefore, this ratio was adopted to isolate the reaction product.

The reaction mixture thus obtained was neutralized. The resulting precipitate was collected and extracted with acetone. The extract was subjected to the column chromatographic separation on an alumina with acetone as developing solvent to give three fractions. The first and third fractions, which showed a yellow and violet colors on the alumina, respectively, were so small that they could not be treated successfully. The second and main fraction showed a red-brown color on the alumina and gave dark red needles of mp 236.5—237.5° (I).

1) Part I: M. Nakamura, K. Mihashi, and K. Egami, *Chem. Pharm. Bull.* (Tokyo), **20**, 35 (1972).
2) Location: *a*) *Nanakuma, Nishi-ku, Fukuoka*; *b*) *Katakasu Higashi-ku, Fukuoka.*
3) F. Penzolt and E. Fischer, *Ber.*, **16**, 657 (1883).
4) Details of this study will be published elsewhere in the near future.

NII-Electronic Library Service

I showed the absorption spectrum with the maximum at 525 nm when dissolved in 0.2N sodium hydroxide solution, which was almost identical in shape and maximum with that of the reaction mixture of benzaldehyde with diazotized sulfanilamide, as shown in Fig. 1. This observation indicated that I was the sole coloring matter produced in the reaction.

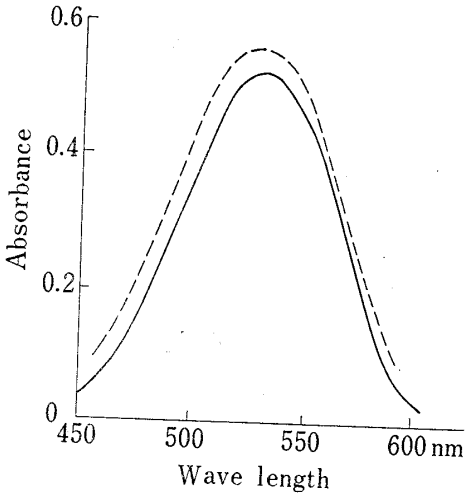

Fig. 1. Absorption Spectra of I and the Reaction Mixture of Benzaldehyde with Diazotized Sulfanilamide

------ : I[a] ——— : the reaction mixture[b]

a) 0.45 mg of I was dissolved in 100 ml of aqueous 0.2N NaOH. Measured against water.

b) The reaction mixture of benzaldehyde with diazotized sulfanilamide, described in Experimental, was diluted 100 times with aqueous 0.2N NaOH. Measured against the reagent blank.

Structure of I

The data of elemental analysis of I well agreed with the molecular formula of $C_{19}H_{18}O_4N_6S_2$. This elemental composition suggested that I might result from the reaction of one mole of benzaldehyde with two moles of diazotized sulfanilamide, whose molar ratio was consistent with the ratio which gave the maximum color intensity in the reaction.

The infrared (IR) spectrum of I showed the presence of *p*-sulfamoylphenyl group in the molecule by the characteristic absorption bands due to SO_2, NH_2, aromatic C=C, and CH of 1,4-disubstituted benzene ring at 1330 and 1145, 3340 and 3240, 1596, and 834 cm^{-1}, respectively. The nuclear magnetic resonance (NMR) spectrum of the compound dissolved in dimethylsulfoxide-d_6 had a virtual singlet at δ 7.42 with the integral intensity of 7 which decreased by 4 on adding heavy water. This fact indicated that the signal might be caused by four amide protons of two sulfamoyl groups and three aromatic protons of phenyl group which originally came from benzaldehyde. The NMR spectrum also showed another virtual singlet at δ 7.97 with the intensity of 10, which was assigned to eight aromatic protons of two *p*-sulfamoylphenyl groups and two *o*-protons of the phenyl groups.[5] Therefore, the three aromatic protons in the signal at δ 7.42 were ascribed to *m*- and *p*-protons of the phenyl group.

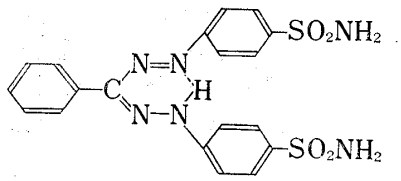

Chart 1. Structure of I

The absorption bands ascribable to the vibrations of C=N, N=N, C–N, and N–N at 1514, 1352, 1228, and 1019 and 1042 cm^{-1}, respectively, in the IR spectrum[6] indicated the presence of a red form of formazan moiety in the molecule.[7] Then, the presence of a strongly hydrogen bonding imino group in the formazan moiety was confirmed

5) The NMR spectra of the red form of 1,3,5-tri(*p*-sulfamoylphenyl)formazan and the yellow form of 1,5-di(*p*-sulfamoylphenyl)formazan dissolved in dimethylsulfoxide-d_6 showed the signals due to aromatic protons of *p*-sulfamoylphenyl groups at δ 8.02 and 7.75, respectively; Y. Ohkura, M. Yamaguchi, and T. Momose, *Chem. Pharm. Bull.* (Tokyo), **22**, 1414 (1974).

6) The IR spectrum of the red form of 1,3,5-triphenylformazan had the characteristic bands due to the vibrations of C=N, N=N, C–N and N–N at 1513, 1351, 1234, and 1018 and 1043 cm^{-1}, respectively, in the solid state, but the band due to NH was not observed; W. Otting and F.A. Neugebauer, *Chem. Ber.*, **102**, 2520 (1969).

7) Triphenylformazan derivative usually existed as its red form, and the yellow form gradually changed to the red one even on standing in the crystalline state; F. Foffani, C. Pecile, and S. Ghersetti, *Tetrahedron Letters*, **1959**, 16.

8) The NMR spectrum of the red form of formazan derivative showed the signal of imino proton at δ about 14; W. Otting and F.A. Neugebauer, *Z. Naturforsch.*, **23B**, 1064 (1968).

NII-Electronic Library Service

by the signal at δ 14.34 in the NMR spectrum, which disappeared on adding heavy water.[8]

From the evidences described above, I should be the red form of 1,5-di(*p*-sulfamoylphenyl)-3-phenylformazan, shown in Chart 1.

Experimental[9]

Isolation of I——To a solution of 1.7 g of sulfanilamide dissolved in a mixture of 5 ml of concentrated HCl and 40 ml of H_2O was added 1 g of $NaNO_2$ freshly dissolved in 5 ml of H_2O with stirring under ice-water cooling at 5°. To the resulting diazotized sulfanilamide solution were added successively 3.0 g of benzaldehyde freshly distilled and 6.0 g of $Na_2S_2O_4$ with shaking. To the mixture was added 50 ml of 3N NaOH and warmed at 60° for 20 min. After cooling, the reaction mixture was neutralized with dilute HCl, and separated precipitates were collected. The product was washed with H_2O, air-dried, and extracted with acetone.

The above procedure was repeated ten times, and the combined extract was dried over Na_2SO_4, concentrated *in vacuo*, poured onto a column packed with about 200 g of neutralized Al_2O_3,[10] and eluted with acetone to afford three fractions. The second and main fraction left I when its elutae was concentrated.

Dark red needles, mp 236.5—237.5° (from acetone). Yield 116 mg. *Anal.* Calcd. for $C_{19}H_{18}O_4N_6S_2$: C, 49.77; H, 3.96; N, 18.33. Found: C, 49.71; H, 3.97; N, 18.14. UV $\lambda_{max}^{0.2N\ NaOH}$ nm(log ε): 525 (4.77).

Acknowledgement The authors are grateful to Prof. Dr. T. Momose of Meiji Pharmaceutical College for his helpful advices and encouragement. Thanks are also due to Miss Y. Soeda, Mr. H. Matsui, Mr. Y. Tanaka, and Mr. M. Shido of Kyushu University for the IR and NMR measurements, and the elemental analysis.

9) UV spectrum was measured by a Shimadzu Double-40 Multiconvertible spectrophotometer in a cell of 10 mm optical length, IR spectrum by a Nihonbunko 701G infrared spectrophotometer in KBr tablet, NMR spectrum by a Nihondenshi PS-100 NMR spectrometer at 100 MHz with tetramethylsilane as an internal standard. The melting point is uncorrected.

10) Commercial activated alumina (Merck, 100 mesh) was dispersed in H_2O, neutralized with 10% HCl and filtered. After washing with H_2O, it was air-dried and activated at 120° for 10 hr.

[Chem. Pharm. Bull. 22(8)1929—1932(1974)]

UDC 547.466.1.09 : 615.36.033

Studies on the Passage of α-Chymotrypsin across the Intestine. III.[1] Quantitation of α-Chymotrypsin in the Mesenteric Perfusate by Single Radial Immunodiffusion

Chiaki Moriwaki, Keiko Yamaguchi, Toshio Kato, and Hiroshi Moriya

Laboratory of Physiological Chemistry, Faculty of Pharmaceutical Sciences, Science University of Tokyo[2]

(Received March 29, 1974)

In our preceding experiment,[1] an immunoreactive substance against the anti-chymotrypsin serum was found in the mesenteric perfusate of rats after administrating α-chymotrypsin (CT) in the intestinal lumen. Although this fact suggests the transmittance of CT into the circulatory system through the intestinal wall, it would be prefered to determine

1) Part II: C. Moriwaki, K. Yamaguchi, and H. Moriya, *Chem. Pharm. Bull.* (Tokyo), **22**, 1029 (1974).

2) Location: *Funakawara-cho, Ichigaya, Shinjuku-ku, Tokyo.*

NII-Electronic Library Service