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Studies on the Phosphorimetric Determination of Amines with Halonitrocompounds. I. Phosphorimetric Determination of 4-Homosulfanilamide with 4-Fluoronitrobenzene

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4-Homosulfanilamide was found to react with 4-fluoronitrobenzene to form N^4-(4′-nitrophenyl)-4-sulfamoylbenzylamine which strongly phosphoresced in ethanol-dimethyl sulfoxide solution (9: 1, v/v). This observation was successfully used to determine microamounts of 4-homosulfanilamide. A linear relationship between the phosphorescence intensity and amine concentration was observed in the range of 2×10^{-6}—2×10^{-4}M.

This phosphorimetric method was 5—25 times more sensitive than colorimetric methods.[2]

Keywords——phosphorimetry; microdetermination; 4-homosulfanilamide; 4-fluoronitrobenzene; N^4-(4′-nitrophenyl)-4-sulfamoylbenzylamine

Several colorimetric methods for the determination of homosulfamine have been reported,[2] but they require at least several micrograms of the amine. No fluorometric or phosphorimetric method has been published, although they are generally more sensitive and selective. We successfully used a phosphorimetric method for the determination of 4-homosulfanilamide (homosulfamine free base). In dimethyl sulfoxide (DMSO), 4-homosulfanilamide (4-HS) reacts with 4-fluoronitrobenzene (4-FNB) to give a strongly phosphorescent compound in ethanol in determining phosphorescence and fluorescence properties of 4-nitroaniline derivatives. This observation was successfully applied to the phosphorimetric microdetermination of 4-HS and a procedure was established.

Experimental

Apparatus——Phosphorescence excitation and emission spectra and intensity were measured with a Hitachi MPF-2 spectrofluorimeter equipped with a Hitachi phosphoroscope attachment. After being frozen at 77°K in liquid nitrogen, the sample solution was held in a fused quartz microsampletube of 2 mm inner diameter. The resolution time of the phosphorimeter was kept constant at 3.0 msec and the lifetimes were measured by the same apparatus equipped with a Hitachi synchroscope V-104. Decay curves were recorded by using a Polaroid camera attachment.

Reagents——4-HS: To a solution of 10 g of homosulfamine[3] in 50 ml of H_2O, 25 ml of 10% NH_4OH was added dropwise with stirring at room temperature. After filtering, the colorless needles obtained were washed with H_2O, then dried *in vacuo* in a desiccator; mp 152—153°,[3] yield 6.7 g (84%).

4-HS Stock Standard Solution: 4-HS (186 mg) was dissolved in DMSO to make 500 ml (2×10^{-3}M). The solution was stable for at least 2 weeks when stored at room temperature protected from light.

4-HS Working Standard Solutions: These were prepared by diluting the stock standard solution with DMSO to the designated concentrations before use.

4-FNB: This was prepared by distilling 50 ml of reagent grade 4-FNB at 62—64°/4 mmHg.

4-FNB Solution: 4-FNB (4.23 g) was dissolved in 500 ml of DMSO (6×10^{-2}M). The solution was stable for at least a month when stored at room temperature protected from light. It was diluted with DMSO to the designated concentration before use.

1) Location: *Sagisu, Fukushima-ku, Osaka, 553, Japan.*
2) T. Momose and T. Yasumura, *Yakugaku Zasshi*, **70**, 672 (1950); T. Tsukamoto and K. Yuhi, *ibid.*, **79**, 1294 (1959); K. Kakemi, T. Kawamura, and M. Sezaki, *ibid.*, **82**, 1579 (1962); M. Kagawa, *Bunseki Kagaku*, **16**, 671 (1967).
3) The homosulfamine listed in "Pharmacopoeia Japonica Editio Octava VIII" was used.

Ethanol: Reagent grade EtOH (1000 ml) was distilled after 10 g of metal Na had been dissolved in it.
DMSO: Reagent grade DMSO (500 ml) was distilled at 53—55°/4 mmHg.

Procedure——Test solution (1.0 ml) containing 4-HS (2×10^{-6}—2×10^{-4}M) was pipetted into a glass-stoppered flask having a 20 ml mark, 1.0 ml of 4-FNB solution was added, and the solution was mixed throughly. The mixture was heated at 150° for 1 hr, and cooled under running water. Ethanol was added to the 20 ml mark and mixing was done by inversion. At the same time, a reagent blank and a phosphorescence standard solution were prepared by treating 1.0 ml of DMSO and 1.0 ml of a working standard solution of 4-HS, respectively, as described above.

The phosphorescence intensity was measured at 523 nm with excitation at 387 nm, setting the intensities of the reagent blank and the phosphorescence standard solution to zero and an arbitrary unit, respectively. The value of 4-HS in the test solution was obtained from the calibration curve described below.

Calibration Curve——Two—20×10^{-6}M or 2—20×10^{-5}M working standard solutions of 4-HS were treated in the same way as given above. The calibration curves thus obtained were straight lines, as shown in Fig. 1.

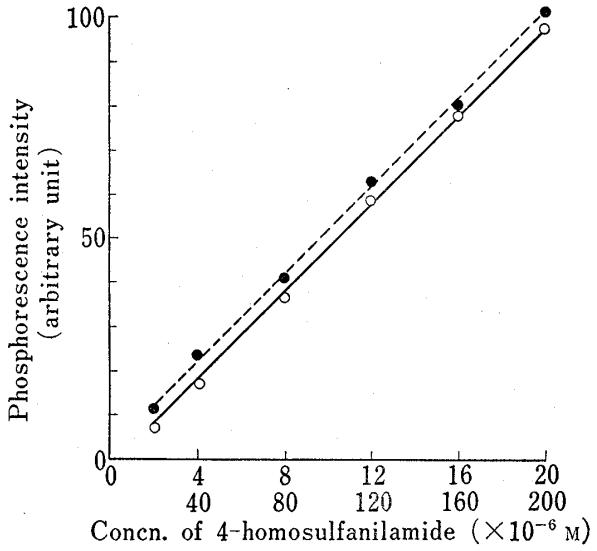

Fig. 1. Calibration Curves for 4-Homosulfanilamide

Mixtures of 4-HS and 6×10^{-2}M 4-FNB were heated at 150° for 60 min.
●-----●: 2—20×10^{-5} M ○——○: 2—20×10^{-6} M

Preparation of Phosphorescent Compound——N^4-(4′-Nitrophenyl)-4-sulfamoylbenzylamine(I)[4]: To a solution of 0.28 g 4-HS in 2 ml DMSO, 0.07 g 4-FNB was added. The mixture was heated in an oil bath at 120° for 4 hr. The reaction mixture was poured onto 80 ml of cold water, then the precipitates were filtered off and recrystallized from EtOH to red-brown needles; mp 173—174°, yield, 0.1 g (67%). *Anal.* Calcd. for $C_{13}H_{13}O_4N_3S$: C, 50.81; H, 4.26; N, 13.67; S, 10.43. Found: C, 50.92; H, 4.24; N, 13.87; S, 10.46. IR ν_{max}^{Nujol} cm^{-1}: 3328, 3257 (NH_2), 1529, 1337 (NO_2), 1318, 1171 (SO_2). NMR (δ in DMSO-d_6) ppm: 4.53 (2H, doublet, J=6.1 Hz, $-C\underline{H}_2NH-$), 6.68 (2H, doublet, J=9.0 Hz, aromatic protons at 2′ and 6′ positions), 7.32 (2H, singlet, $-SO_2N\underline{H}_2$), 7.52—8.01 (7H, multiplet, aromatic protons and $-CH_2N\underline{H}-$).

Results and Discussion

Solvent Selection

A clear rigid glass is usually prerequisite to measure the phosphorescence intensity at 77°K. The reaction of 4-HS with 4-FNB proceeded well in DMSO which did not form a clear rigid glass when chilled at 77°K. On the other hand, the reaction did not occur in ethanol which formed a clear rigid glass and did not affect the phosphorescence measurement when tested with the phosphorescent compound (I). Ethanol containing 8—10% (v/v) DMSO formed a clear rigid glass in which a constant phosphorescence intensity was observed, but 12% (v/v) DMSO in the mixture interfered with the intensity by forming a snowy rigid glass. Therefore, the reaction was performed in DMSO and then the reaction mixture was diluted with ethanol to bring the DMSO content to 10% (v/v).

Phosphorescent Compound

The phosphorescence excitation and emission spectra of the reaction mixture were observed at 387 and 523 nm (Fig. 2), respectively. The phosphorescent compound produced in the procedure was prepared in crystalline form and was determined to be I

4) Melting point was determined with a Yanagimoto Micromelting Point Apparatus and is uncorrected. The infrared (IR) spectrum was taken in Nujol with JASCO DS 403G; and nuclear magnetic resonance (NMR) spectrum in DMSO-d_6 solution with a Varian A-60 using tetramethylsilane as the internal reference, chemical shifts being shown as δ.

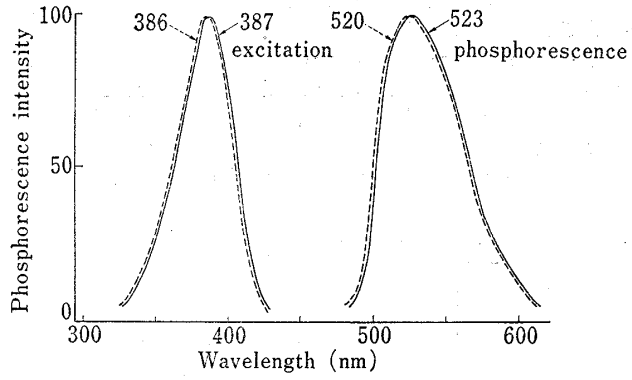

Fig. 2. Uncorrected Excitation and Emission Spectra of the Reaction Mixture and I

reaction mixture: 1.0 ml of 2.0×10^{-4} M 4-HS solution was treated according to the procedure.
——: excitation and emission spectra
mean lifetime: 0.4 sec
I: dissolved in the same solvent system as the reaction mixture in a concentration of 1.0×10^{-5} M
------: excitation and emission spectra
mean lifetime: 0.4 sec

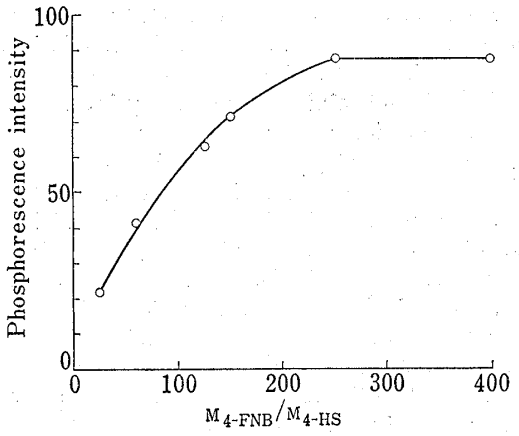

Fig. 3. Effect of 4-FNB Concentration on the Phosphorescence Development

A fixed concentration of 2.0×10^{-5} M 4-HS was treated as in the procedure with varying amounts of 4-FNB.

with data from elemental analysis and infrared and nuclear magnetic resonance spectroscopy. Fig. 2 shows the phosphorescence excitation and emission spectra of I dissolved in ethanol containing 10% (v/v) DMSO. These spectra coincided with those of the final reaction mixture, indicating that I was the sole phosphorescent compound produced in the procedure.

Effect of 4-FNB Concentration

The concentration of 4-FNB affected the phosphorescence intensity. Fig. 3 clearly shows that the concentration of 4-FNB should be maintained at more than 250-fold molar excess over 4-HS to obtain a constant intensity.

Correlation between Reaction Temperature and Time

The reaction temperature and time were interdependent in the development of phosphorescence as shown in Fig. 4. A constant phosphorescence intensity was obtained for the reaction time of 45—120 min at 150°, but not at 125°. The reaction time of 60 min and temperature of 150° were selected as the optima for the procedure.

Phosphorescence Stability

Phosphorescence intensity of the final solution was stable for 2 hr at 24—25° under room light.

Effect of Foreign Substances

Some anilines, sulfanilamides, and aliphatic amines other than 4-HS were tested with respect to the quenching effect. As shown in Fig. 5, the phosphorescence development of the reaction mixture was quenched by adding *p*-toluidine at a concentration of 100-fold molar excess over 4-HS, though the phosphorescence of I was not quenched on adding it at 500-fold moles over I. The above facts indicated that *p*-toluidine affected the phosphorescence development by consuming 4-FNB in the reaction. Similar phenomena were also observed in the reaction of 4-HS with coexistence of sulfisoxazole, sulfisomesole, sulfadimethoxine, sulfiodizole, ethanolamine, histamine, and norepinephrine.

Namely, these compounds quenched the phosphorescence of the reaction mixture at a concentration of equivalent molar excess over 4-HS, but not that of I even at a concentration of 500-fold moles over I. On the other hand, sulfanilamide and *p*-chloroaniline did not affect the phosphorescence development of the reaction mixture and did not quench the

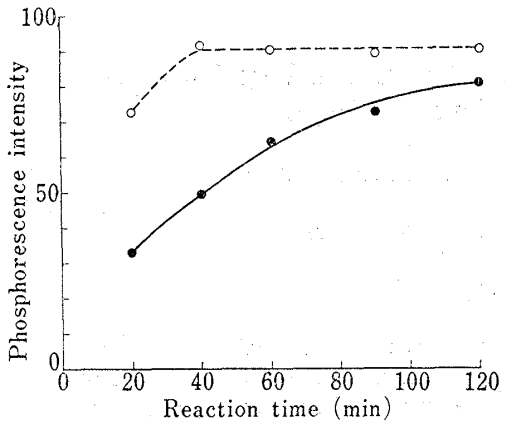

Fig. 4. Effect of the Reaction Temperature and Time on the Phosphorescence Development

1.0 ml of 2.0×10^{-4} M 4-HS solutions were treated as in the procedure.
○-----○: 150°
●——●: 125°

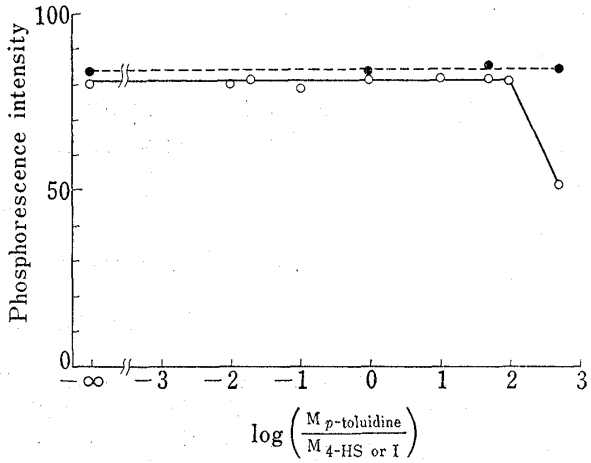

Fig. 5. Quenching Effect of *p*-Toluidine on the Reaction of 4-HS and the EtOH–DMSO Mixture of I

○——○: A 2.0×10^{-5} M of 4-HS was treated as in the procedure in the coexistance of various amounts of *p*-toluidine.
●-----●: A 1.0×10^{-6} M solution of I prepared by dissolving in the EtOH-DMSO mixture (9:1) with various amounts of *p*-toluidine.

TABLE I. Regression Analysis for the Determination of 4-Homosulfanilamide in the Presence of *p*-Chloroaniline and Sulfanilamide

Sample No.	Components in 1 ml of mixed sample solution ($\times10^5$ M)			Found ($\times10^5$ M)
	4-Homosulfanilamide (x)	*p*-Chloroaniline	Sulfanilamide	4-Homosulfanilamide (y)
1	2.00	200	200	2.65
2	4.00	200	200	4.10
3	6.00	200	200	5.92
4	8.00	200	200	7.80
5	10.00	200	200	10.58
6	12.00	200	200	11.74
7	14.00	200	200	13.53
8	16.00	200	200	15.38
9	18.00	200	200	17.64
10	20.00	200	200	21.12

regression equation: $y=0.9899x+0.157$, $s=0.59$, C.V.$=5.37\%$

phosphorescence of I when examined under the same conditions as in those of *p*-toluidine.

Regression Analysis

Regression analysis for the determination of 4-HS in the concentration range of 2—20 $\times10^{-5}$ M was examined using a mixed sample solution in which *p*-chloroaniline and sulfanilamide were present at 10- to 100-fold moles over 4-HS. As shown in Table I, the calculated results between the theoretical (x) and experimental (y) values indicated that the present method correctly determined 4-HS with a coefficient of variation of about 5%.

Advantage of This Method

This method is somewhat more troublesome than colorimetric method.[2] However, it is 5—25 times more sensitive, and the calibration curve is linear for a wide range of 4-HS concentrations.