  Bull. Chem. Soc. Jpn., 55, 3520—3522 (1982) [Vol. 55, No. 11

The Spectrofluorometric Determination of 9,10-Phenanthrenequinone

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(Received April 12, 1982)

9,10-Phenanthrenequinone (9,10-PQ) reacts with guanidine in an alkaline medium to form 2-amino-1*H*-phenanthro[9,10-*d*]imidazole. The reaction can be used for the fluorometric determination of 9,10-PQ, since the reaction product is fluorescent in an acidic solution. A dimethyl sulfoxide-methanol mixture is suitable as the solvent. A sample solution was allowed to stand for 15 min at room temperature after the additions of guanidine hydrochloride and sodium methoxide solutions, followed by the addition of hydrochloric acid. After the solution had been allowed to stand for 20 min, the fluorescence intensity of the solution was measured at 403 nm with excitation at 363 nm. The fluorescence intensity was stable for at least 8 h. The determination limit of 9,10-PQ was 50 ng.

For the determination of small amounts of 9,10-phenanthrenequinone (9,10-PQ), spectrophotometric methods[1-2] have been developed. In these methods, however, the determination limit of 9,10-PQ is approximately 1 μg.

In the present paper, a spectrofluorometric determination of 9,10-PQ will be described for the determination of minute amounts of 9,10-PQ. In order to convert 9,10-PQ into a fluorescent substance, the reaction of 9,10-PQ with guanidine[3] is useful. 9,10-PQ forms 2-amino-1*H*-phenanthro[9,10-*d*]imidazole with guanidine in an alkaline medium, and the product is fluorescent in an acidic solution.

Experimental

Reagents. Dimethyl sulfoxide (DMSO) was purified by using active aluminium oxide and a molecular sieve. To 500 g of commercial DMSO, 50 g of active aluminium oxide was added. The mixture was sufficiently shaken and then allowed to stand for several hours. After the DMSO had been separated by decantation, this process was repeated by using the molecular sieve instead of the aluminium oxide. Finally, the molecular sieve was filtered off.

9,10-PQ was purified by zone melting. Guanidine hydrochloride used was of a special-use reagent grade purchased from Tokyo Kasei Kogyo Co., Ltd. Methanol used was of a guaranteed reagent grade. Sodium methoxide was purchased from Kanto Chemicals Co., Inc.

Solutions. The guanidine solution was prepared by dissolving 50 mg of the hydrochloride in 100 ml of DMSO. The sodium methoxide solution was prepared by dissolving 1.00 g of the solid in 100 ml of a mixture of DMSO and methanol (4:1).

Apparatus. All the fluorometric measurements were carried out using a Hitachi 650-10S fluorescence spectrophotometer. A 150-W xenon lamp was used as the exciting source. A 10 mm × 10 mm × 45 mm quartz cell was used.

Procedure. Five milliliters of a sample solution containing less than about 1 μg of 9,10-PQ in a mixture of DMSO and methanol (4:1) was transferred into a 10-ml volumetric flask (amber). Then, 1 ml of the guanidine solution and 1 ml of the sodium methoxide solution were added. After the mixture had been allowed to stand for 15 min at room temperature, it was diluted to 10 ml with a 0.1 M (1 M= 1 mol dm^{-3}) solution of hydrochloric acid dissolved in DMSO. The resultant solution was allowed to stand for 20 min. The 9,10-PQ was determined by measuring the fluorescence intensity of the solution at 403 nm, with excitation at 363 nm. A solution containing 1.00 μg of 9,10-PQ was treated under the same conditions and at the same time; the resultant fluorescence was used as the standard.

Results and Discussion

Fluorescence Spectra. The fluorescence spectra of the reaction product of 9,10-PQ with guanidine are shown in Fig. 1. The excitation spectrum has maxima at 266 nm, 313 nm, and 363 nm. The emission spectrum exhibits a maximum at 403 nm. As the excitation wavelength, 363 nm was selected for the determination in order to minimize the interference of diverse substances. The fluorescence intensity of the reagent blank was negligibly small.

Effects of Sodium Methoxide and Methanol. DMSO was selected as the solvent considering the solubility of polynuclear aromatic compounds including 9,10-PQ. For the reaction of 9,10-PQ with guanidine, it is necessary to alkalify the solution. For this purpose, sodium methoxide was useful. To dissolve sodium methoxide in DMSO, the presence of methanol is necessary. The effect of sodium methoxide on the fluorescence intensity was studied by varying the amounts of sodium methoxide. The fluorescence intensities were almost constant over the range of 8—20 mg of

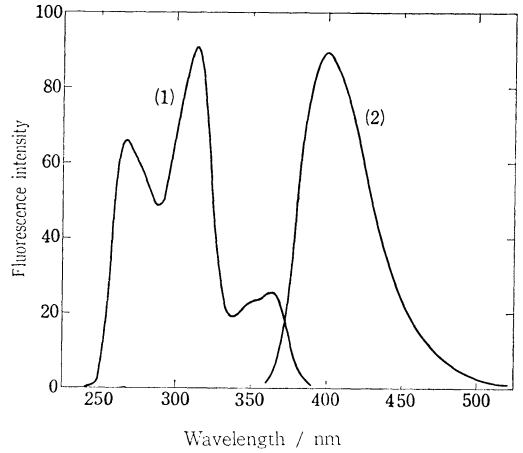

Fig. 1. Fluorescence spectra of the reaction product of 9,10-phenanthrenequinone (9,10-PQ) with guanidine. (1): Excitation spectrum, uncorrected; (2): emission spectrum, uncorrected.

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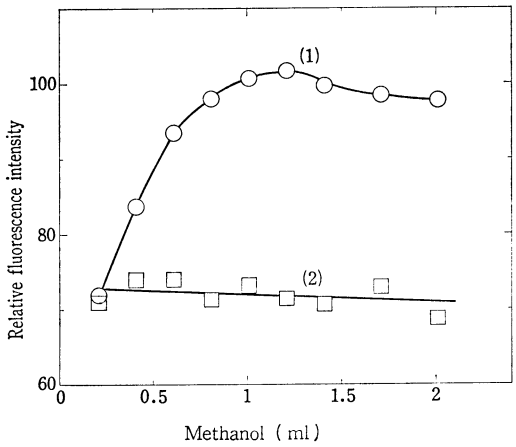

Fig. 2. Effect of the amounts of methanol on the fluorescence intensity.
Standard: fluorescence intensity of anthracene solution (40 ng/ml) in CH_3OH–DMSO (1:4) was taken as 100 div.; 9,10-PQ: 1.00 μg; (1): methanol was added before the reaction; (2): methanol was added after acidifying the solution of the reaction product (0.2 ml of methanol exists during the reaction).

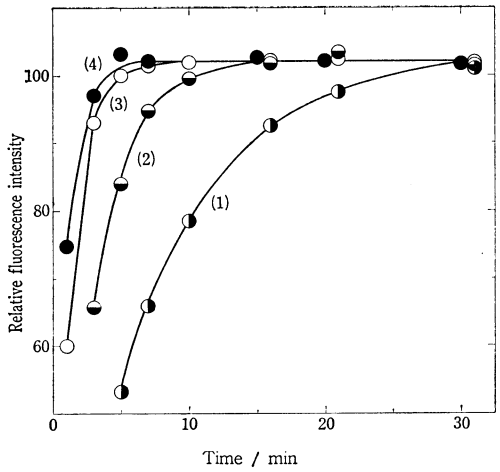

Fig. 3. Effect of reaction time on the fluorescence intensity in the presence of various amounts of guanidine hydrochloride.
Standard: fluorescence intensity of anthracene solution (40 ng/ml) in CH_3OH–DMSO (1:4) was taken as 100 div.; (1): 0.05 mg; (2): 0.15 mg; (3): 0.5 mg; (4): 1.5 mg; temp: 20 °C.

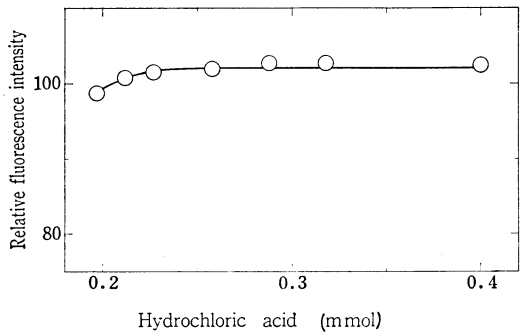

Fig. 4. Effect of the amounts of hydrochloric acid. 9,10-PQ: 1.00 μg; standard: fluorescence intensity of anthracene solution (40 ng/ml) in CH_3OH–DMSO (1:4) was taken as 100 div.

sodium methoxide. It is recommended, therefore, that 10 mg of sodium methoxide is added for the determination.

The reaction of 9,10-PQ with guanidine was carried out in the presence of various amounts of methanol. The fluorescence intensities measured are illustrated in Fig. 2 (1). For comparison, the effect of methanol was also studied in the case in which methanol was added after the solution of the reaction product had been acidified. These experimental results are shown in Fig. 2 (2). Figure 2 indicates that the reaction of 9,10-PQ with guanidine is influenced by the presence of methanol and that the maximum intensity is obtained when 1.2 ml of methanol exists during the reaction. Therefore, a mixture of DMSO and methanol (4:1) is suitable as the solvent for dissolving the sample and sodium methoxide.

Effects of Guanidine and Reaction Time. The hydrochloride of guanidine was used because of its high solubility in DMSO. The sulfate dissolves only slightly in DMSO. The amounts of guanidine and the reaction time are important variables for the determination. The effect of the reaction time on the fluorescence intensity was investigated at 20 °C in the presence of 0.05—1.5 mg of guanidine hydrochloride. The results of these experiments are shown in Fig. 3. Figure 3 indicates that the reaction of 9,10-PQ with guanidine is completed within 10 min at 20 °C when more than 0.5 mg of guanidine hydrochloride has been added. It is recommended, therefore, that the solution is allowed to stand for 15 min at room temperature in the presence of 0.5 mg of guanidine hydrochloride.

Effect of Hydrochloric Acid. The reaction product of 9,10-PQ with guanidine is not fluorescent in an alkaline solution, whereas it is fluorescent in an acidic solution. To acidify the solution, hydrochloric acid dissolved in DMSO was employed. The effect of the amounts of hydrochloric acid on the fluorescence intensity is illustrated in Fig. 4. The amounts of hydrochloric acid were varied from 0.20 to 0.40 mmol, because 0.19 mmol of hydrochloric acid was required to neutralize 10 mg of sodium methoxide. From the results in Fig. 4, the intensities are almost constant over the range from 0.23 to 0.40 mmol. In the determination procedure, about 3 ml of 0.1 M hydrochloric acid was used.

Stability of Fluorescence. In order to investigate the stability of the fluorescence intensity of the reaction product, a solution containing 1.00 μg of 9,10-PQ was operated according to the procedure. The fluorescence intensity was measured by varying the standing time from 2 min to 8 h. The intensity increased for a while after the solution had been acidified. The fluorescence intensity, however, remained highly constant for at least 8 h after 15 min. An amber volumetric flask was used in the procedure to shield it from light in some degree, since the intensity was relatively unstable under scattered sunlight.

Calibration Curve and Reproducibility. A calibration curve was prepared from 9,10-PQ solutions con-

TABLE 1. EFFECTS OF DIVERSE SUBSTANCES

Substances added (μg)		Relative fluorescence intensity[a)]	Difference
None		100.0	
Biphenyl	103	103.3	+0.3
Naphthalene	105	99.3	−0.7
Acenaphthene	104	100.0	±0.0
Dibenzofuran	106	99.4	−0.6
Dibenzothiophene	104	100.4	+0.4
Fluorene	5.3	99.2	−0.8
	21	96.6	−3.4
Carbazole	1.0	100.4	+0.4
	2.1	103.3	+3.3
Phenanthrene	21	100.9	+0.9
	103	102.1	+2.1
Anthracene	0.005	100.4	+0.4
	0.020	103.8	+3.8
Acridine	2.2	99.3	−0.7
	5.5	97.7	−2.3
Fluoranthene	0.11	100.8	+0.8
	0.21	102.6	+2.6
9-Fluorenone	20	99.3	−0.7
	102	96.2	−3.8
Acenaphthenequinone	107	99.2	−0.8
Anthraquinone	10	99.7	−0.3
	51	96.6	−3.4

a) The fluorescence intensity of the solution of the reaction product (1.00 μg 9,10-PQ per 10 ml) was taken as 100 div.

taining 0—1.00 μg per 5 ml according to the procedure. As the standard, the solution obtained by operating 1.00 μg of 9,10-PQ was used. The calibration curve thus prepared was linear between the fluorescence intensity and the concentration of 9,10-PQ. The coefficient of variation obtained in 10 measurements was 1.3% at 1.00 μg of 9,10-PQ. In these measurements, an anthracene solution was used as the standard.

Effect of Diverse Substances. The effects of 14 substances on the determination are summarized in Table 1. The determination of 9,10-PQ is not interfered with the presence of 100-fold amounts of biphenyl, naphthalene, acenaphthene, dibenzofuran, dibenzothiophene, and acenaphthenequinone and 20-fold amounts of phenanthrene, 9-fluorenone, and anthraquinone. Fluorene and acridine reduce the fluorescence intensity. Carbazole gives a positive error. Anthracene and fluoranthene give serious interferences

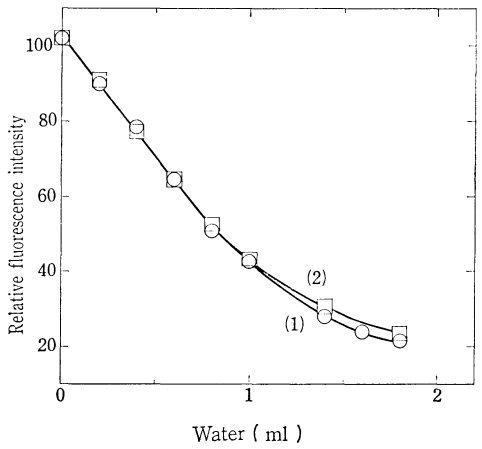

Fig. 5. Effect of water on the fluorescence intensity. 9,10-PQ: 1.00 μg; standard: fluorescence intensity of anthracene solution (40 ng/ml) in CH_3OH–DMSO (1:4) was taken as 100 div.; (1): water was added before the reaction; (2): water was added after acidifying the solution of the reaction product.

because they are fluorescent at the characteristic wavelength of the reaction product of 9,10-PQ.

Effect of Water. The effect of water on the fluorescence intensity was studied by varying the water content in the solvent. The fluorescence intensities measured are shown in Fig. 5 (1). For comparison, the additions of water were performed after the solution of the reaction product had been acidified. As is shown in Fig. 5 (2), these results are identical with that in Fig. 5 (1). Figure 5 indicates that the fluorescence intensity greatly decreases in the presence of water. Therefore, a nonaqueous solvent is desirable for a highly sensitive determination of 9,10-PQ. The identical solvent must be used during the determination to avoid the influence of water, which is contained in the solvent as an impurity.

The authors are indebted to Miss Sumiko Yamada for her technical assistance. The present work was partially supported by a Grant-in-Aid for Scientific Research from the Ministry of Education, Science and Culture.

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