# Fluorometric Determination of 1-Naphthol and Carbaryl with 3-Amino-2(1*H*)-quinolinethione. IV[1,2]

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**A fluorometric method for the determination of 1-naphthol (NP) with 3-amino-2(1*H*)-quinolinethione (AQT) was developed. The method is based on the oxidation of NP by Fremy's salt and the reaction with AQT in an acid medium, followed by extraction of a red fluorescent product (excitation maxima, 535 and 575 nm; emission maximum, 600 nm) with carbon tetrachloride after making the reaction mixture strongly basic. The calibration curve was linear in the range from 0.01 to 1.2 μg/ml of NP. This method was applied for the fluorometric determination of Carbaryl (NAC, 1-naphthyl *N*-methylcarbamate) (0.01—1.8 μg/ml), which was hydrolyzed with potassium hydroxide and determined by the same method as NP. The coefficient of variation was 1.3% ($n=10$) or 1.5% ($n=10$) for 0.3 μg/ml of NP or NAC, respectively.**

**Keywords** organic reagent; 3-amino-2(1*H*)-quinolinethione; fluorometry; 1-naphthol; 1-naphthyl *N*-methylcarbamate; Carbaryl

Several colorimetric methods have been reported for the analysis of 1-naphthol (NP).[3-7] Very few methods[8,9] are available for the selective determination of NP in the presence of 2-naphthol. Carbaryl, 1-naphthyl *N*-methylcarbamate (NAC), which is an insecticide used on a large scale to protect field crops against a broad spectrum of insects, has also been determined similarly.[10-12]

On the other hand, a fluorometric method for NP was reported, based on the measurement of its intrinsic fluorescence in a basic medium.[13] NAC was also determined by measurement of its intrinsic fluorescence[14] or of that of NP formed by hydrolysis of NAC.[15,16]

We have already reported the selective fluorometric determination of 1,4-naphthoquinone (NQ) with 3-amino-2(1*H*)-quinolinethione (AQT).[17] In this paper, we describe a sensitive and selective fluorometric method for determination of NP, based on oxidation with Fremy's salt [potassium nitrosodisulfonate (PNS)] and reaction with AQT, followed by extraction of the fluorescent product with carbon tetrachloride ($CCl_4$) after making the reaction mixture strongly basic. This method was also applied for the determination of NAC.

## Experimental

**Reagents and Materials** NP solution: NP (recrystallized from benzene and then from $CCl_4$, mp 94—95 °C) was dissolved in redistilled water at a concentration of 100 μg/ml. The solution was diluted with 0.1 M potassium dihydrogenphosphate ($KH_2PO_4$) solution to an appropriate concentration. NAC solution: NAC (purchased from Wako Pure Chemical Industries Ltd.) was dissolved in EtOH at a concentration of 10 μg/ml, and the solution was diluted with redistilled water to an appropriate concentration before use. PNS solution : PNS (synthesized according to the previous paper[18]) (50 mg) was dissolved in 100 ml of 0.1 M $KH_2PO_4$ solution before use. AQT solution: AQT (synthesized by the method described previously[17]) (0.2 g) was dissolved in 100 ml of EtOH. Uranine solution was used to adjust the sensitivity of the fluorophotometer. $CCl_4$ was purified by the method described in the previous paper.[2] All other chemicals and solvents were of reagent grade.

**Apparatus** Fluorescence spectra were taken on a Hitachi 650-10S spectrofluorophotometer equipped with a Hitachi 050 recorder, and routine fluorescence readings were made on a Hitachi FPL-2 fluorophotometer. Infrared (IR) spectra were obtained with a Hitachi 270-30 spectrometer. Mass spectra (MS) were taken on a JEOL JMS-D300 spectrometer. Proton nuclear magnetic resonance ($^1$H-NMR) spectra were taken on a JEOL PS-100 spectrometer with tetramethylsilane as an internal standard.

**Determination of NP** An aliquot of the sample solution (5 ml) containing 0.01—1.2 μg/ml of NP and 0.05% PNS solution (1 ml) were placed in a test tube fitted with a screw cap. The mixed solution was heated at 50 °C for 30 min, then 5% (w/v) $H_2SO_4$ (1 ml) solution and 0.2% AQT solution (3 ml) were added, followed by heating at 75 °C for 90 min. The reaction mixture was transferred into a separatory funnel by rinsing with $H_2O$. A 20% NaOH solution (5 ml) was added to the funnel to make the mixture strongly basic. After cooling to room temperature, the basic solution was extracted with 10 and 5 ml portions of $CCl_4$ for 5 min each. Each extract was filtered through a small cotton plug and washed with $CCl_4$. The organic layers were combined and diluted with $CCl_4$ to 20 ml. The relative fluorescence intensity of the $CCl_4$ solution was measured with a Hitachi FPL-2 fluorophotometer (primary filter, No. 546; secondary filter, No. 61).

The sensitivity of the fluorophotometer was adjusted to give a reasonable fluorescence intensity with a uranine solution (primary filter, No. 436; secondary filter, No. 53).

**Determination of NAC** An aliquot of the sample solution (5 ml) containing 0.01—1.8 μg/ml of NAC and 0.5 M KOH solution (1 ml) were placed in a test tube fitted with a screw cap. The mixture was left to stand for 20 min at room temperature, then 0.5 M $H_3PO_4$ (1 ml) and 0.05% PNS solution (1 ml) were added, followed by heating at 50 °C for 30 min. The subsequent experiments were carried out by the same method as described above.

**Condensation Product of AQT and NP** An EtOH solution (40 ml) containing AQT (62.4 mg) was mixed with NQ (47.4 mg) and 5% (w/v) $H_2SO_4$ (10 ml). The mixture was heated at 75 °C for 90 min, then the reaction solution was concentrated *in vacuo* to afford a dark violet precipitate. The precipitate obtained was extracted with hot EtOH. The EtOH extract was evaporated and the residue was dissolved in a small amount of MeOH, then submitted to column chromatography on silica gel with $C_6H_6$–cyclohexane–ethyl acetate (7:2.5:0.5). The yellow-orange eluate was collected and the solvent was removed. The residue was recrystallized from EtOH to give 7-thia-8,14-diazabenzo[*a*]naphthacen-5-one (**1**) (34 mg) as reddish brown needles, mp 263—264 °C. *Anal.* Calcd for $C_{19}H_{10}N_2OS$: C, 72.59; H, 3.21; N, 8.91. Found: C, 72.59; H, 3.37; N, 8.84. IR $\nu_{max}^{KBr}$ cm$^{-1}$: 1627 (C=O), 1304 (C=N). MS *m/z*: 314 ($M^+$), 286 ($M^+-CO$). $^1$H-NMR ($C_5D_5N$): 7.08 (1H, s, C-6), 7.55—8.88 (9H, m, Ar-H).

The above residue, insoluble in hot EtOH, was subjected to column chromatography on silica gel. The red fraction eluted with $C_6H_6$–ethyl acetate (95:5) was collected and the solvent was removed. The residue was recrystallized from dimethylformamide (DMF) to give 6,11,17,20-tetraaza-18,19-dithiabenzo[*j*]heptaphene (**2**) (23 mg) as a black-violet amorphous solid, mp 360 °C above. IR $\nu_{max}^{KBr}$ cm$^{-1}$: 1302 (C=N), 773 (C–H). MS *m/z*: 470 ($M^+$).

## Results and Discussion

**Determination of NP A. Fluorescence Spectra** The shapes of the uncorrected fluorescence excitation and emission spectra of the adduct formed from the reaction of NP with AQT under the standard conditions of the recommended procedure were the same as described previously.[2,17] The fluorescence-emission maximum was observed at 600 nm with excitation at 535 or 575 nm.

**B. Oxidation of NP** NP was oxidized by conventional oxidants under the standard conditions of the procedure described above. Table I shows that PNS is the most suitable oxidizing agent for NP in this procedure. Constant fluorescence intensity was observed over the concentration range of 0.02—0.07% PNS solution. After having been dissolved in 0.1 M $KH_2PO_4$ solution, PNS was stable for at least 24 h.

The effect of pH on the oxidation was examined in the pH range from 0.1 to 9.5. As constant fluorescence intensity was observed in the pH range 2—7, the oxidation was carried out in 0.1 M $KH_2PO_4$ solution. Taking account of the stability of PNS solution and the optimum pH for the oxidation of NP, the sample solution was prepared in 0.1 M $KH_2PO_4$ solution.

TABLE I. Effect of Oxidants on the NP Determination

| Oxidant[a] | R.F.I.[b] |
|---|---|
| $ON(SO_3K)_2$ | 100.0 |
| $NaIO_4$ | 32.0 |
| $Ce(SO_4)_2 \cdot 2(NH_4)_2SO_4 \cdot 4H_2O$ | 9.8 |
| $K_2S_2O_8$ | 3.3 |
| $FeCl_3$ | 3.1 |
| $K_3Fe(CN)_6$ | 2.0 |
| $K_2Cr_2O_7$ | 0.3 |
| $KIO_3$ | 0.3 |

*a*) A 0.05% solution was used. *b*) Relative fluorescence intensity.

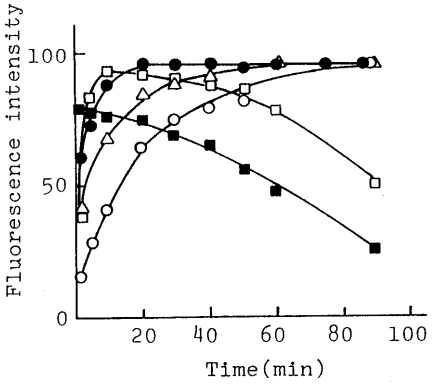


Fig. 1. Effects of Oxidation Time and Oxidation Temperature on Fluorescence Intensity of the Product

Amount of NP taken: 0.3 μg/ml. ○—○, at 0 °C; △—△, at 20 °C; ●—●, at 50 °C; □—□, at 80 °C; ■—■, on a boiling water bath.

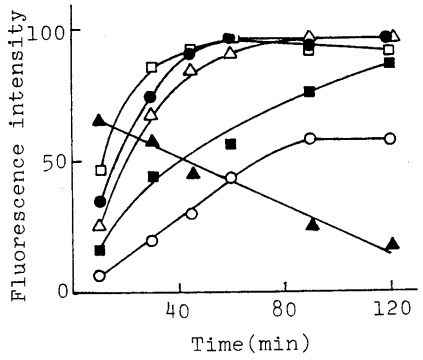


Fig. 2. Effects of Reaction Time and Reaction Temperature on Fluorescence Development

Amount of NP taken: 0.3 μg/ml. ○—○, at 20 °C; ■—■, at 40 °C; △—△, at 60 °C; ●—●, at 75 °C; □—□, at 80 °C; ▲—▲, on a boiling water bath.

The effects of oxidation temperature and time were examined by varying the reaction temperature and time, respectively. The maximum and constant fluorescence intensity was found in the temperature range from 30 to 60 °C and at oxidation times longer than 20 min (Fig. 1), so the oxidation of NP was carried out at 50 °C for 30 min.

**C. Reaction Conditions for Fluorescence Development** Constant fluorescence intensity was found in the concentration ranges of 1—10% (w/v) sulfuric acid solution and 0.15—0.25% AQT solution. Therefore, 5% (w/v) sulfuric acid solution and 0.2% AQT solution were selected for the standard procedure.

Figure 2 shows that the fluorescence intensity was constant in the range of 60—80 °C at 90 min; the optimum temperature was found to be 75 °C. At this temperature, the fluorescence intensity reached the maximum after 60 min and was constant for at least 120 min.

**D. Extraction of Fluorescent Product** The red fluorescent product formed in the standard procedure must be extracted with an immiscible solvent from the alkalized reaction mixture as described previously.[2] In this paper, 5 ml of 20% sodium hydroxide solution was added to the reaction mixture, followed by extraction with the same solvent ($CCl_4$) as described in the previous paper.[2]

**E. Calibration Curves and Precision** Sample solutions containing NP at various concentrations were assayed by the recommended procedure. The calibration curves were linear in the ranges from 0.01 to 0.3 μg/ml and from 0.1 to 1.2 μg/ml of NP. By repeated determinations ($n=10$) of sample solutions of 0.3 μg/ml or 1.0 μg/ml, the coefficient of variation was calculated to be 1.3% or 0.8%, respectively.

**F. Fluorescence Characteristics of Naphthols and Interfering Compounds** When 19 kinds of commercially available naphthols (Table II) were examined by the recommended procedure, only 3 kinds of naphthols showed red fluorescence resembling that of NP and their fluorescence

TABLE II. Fluorescence Characteristics of Naphthols

| Compound[a] | Ex.[b] (nm) | | Em.[c] (nm) | R.F.I.[d] |
|---|---|---|---|---|
| 1-Naphthol | 535 | 575 | 600 | 100 |
| 2-Naphthol | — | — | — | — |
| 1,3-Dihydroxynaphthalene | — | — | — | — |
| 1,4-Dihydroxynaphthalene | 536 | 575 | 601 | 116 |
| 1,5-Dihydroxynaphthalene | — | — | — | — |
| 1,6-Dihydroxynaphthalene | — | — | — | — |
| 1,7-Dihydroxynaphthalene | — | — | — | — |
| 2,3-Dihydroxynaphthalene | — | — | — | — |
| 2,6-Dihydroxynaphthalene | — | — | — | — |
| 2,7-Dihydroxynaphthalene | — | — | — | — |
| 1-Hydroxy-2-naphthoic acid | — | — | — | — |
| 2-Hydroxy-1-naphthoic acid | — | — | — | — |
| 3-Hydroxy-2-naphthoic acid | — | — | — | — |
| 2-Nitroso-1-naphthol | — | — | — | — |
| 1-Nitroso-2-naphthol | — | — | — | — |
| 4-Amino-1-naphthol hydrochloride | 537 | 575 | 600 | 96 |
| 4-Chloro-1-naphthol | 536 | 575 | 600 | 55 |
| Sodium 4-hydroxy-1-naphthalenesulfonate | — | — | — | — |
| Sodium 6-hydroxy-2-naphthalenesulfonate | — | — | — | — |

*a*) Amount taken: 0.3 μg/ml. *b*) Excitation maxima. *c*) Emission maximum. *d*) Relative fluorescence intensity.

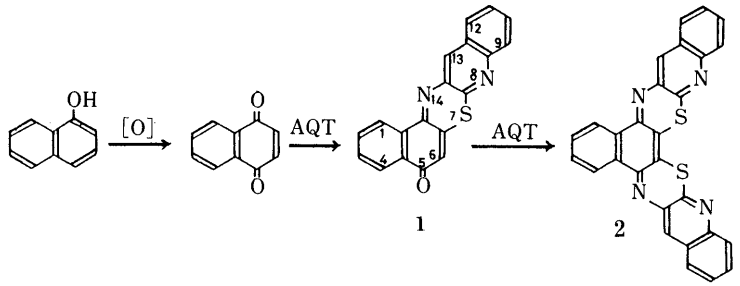


Chart 1

TABLE III. Determination of NP in the Presence of Various Compounds

| Compound | Added (μg) | Recovery[a] (%) |
|---|---|---|
| Sodium 4-hydroxy-1-naphthalenesulfonate | 150 | 102.9 |
| Sodium 6-hydroxy-2-naphthalenesulfonate | 150 | 100.4 |
| Naphthalene | 150 | 100.8 |
| 1-Chloronaphthalene | 150 | 100.0 |
| Glucose | 150 | 102.5 |
| Acetamide | 150 | 103.5 |
| *m*-Dinitrobenzene | 150 | 101.5 |
| Methylamine | 150 | 101.8 |
| 2-Naphthol | 75 | 102.6 |
| 2,3-Dihydroxynaphthalene | 75 | 104.6 |
| 2,6-Dihydroxynaphthalene | 75[b] | 98.3 |
| 2-Hydroxy-1-naphthoic acid | 75[b] | 99.6 |
| Benzamide | 75 | 98.2 |
| Picric acid | 75 | 98.9 |
| Formaldehyde | 75 | 97.8 |
| Acetaldehyde | 75 | 98.9 |
| Phenol | 75 | 100.0 |
| *m*-Cresol | 75 | 102.8 |
| 1,3-Dihydroxynaphthalene | 45[b] | 97.1 |
| 2,7-Dihydroxynaphthalene | 45[b] | 99.2 |
| 3-Hydroxy-2-naphthoic acid | 45[b] | 100.5 |
| 2-Nitroso-1-naphthol | 45[b] | 101.8 |
| 1-Nitroso-2-naphthol | 45 | 102.2 |
| *p*-Nitrophenol | 15 | 99.4 |
| Vanillin | 15 | 104.0 |
| 1,5-Dihydroxynaphthalene | 1.5 | 100.7 |
| 1,6-Dihydroxynaphthalene | 1.5 | 97.6 |
| 1,7-Dihydroxynaphthalene | 1.5 | 97.0 |

*a*) Amount of NP taken: 1.5 μg. *b*) 0.1% Fremy's salt was used.

spectra were closely similar to those of the fluorophore derived from NP.

No fluorescence was observed when various other compounds (Table III) was tested by the present method. No interference was caused by 2-naphthol in the presence of at least 50-fold excess concentration over NP. These results suggested that the identification and microdetermination of NP in the presence of 2-naphthol can be done without using a tedious reported method.[13]

**Determination of NAC** **A. Hydrolysis of NAC and Other Conditions** NAC did not give fluorescence when examined by the method described for NP, so conditions for hydrolysis of NAC were examined. NAC was completely hydrolyzed to NP with 0.5 M KOH solution (1 ml) over the range of time from 10 to 60 min at the ambient temperature. To adjust to pH 2—7, which is suitable for the oxidation of NP with PNS, about 1 ml of 0.5 M $H_3PO_4$ solution was added to the above solution. Other optimum conditions for the NAC determination were almost the same as those of the NP determination method.

**B. Calibration Curves and Precision** The calibration curve was linear in the range from 0.01 to 1.8 μg/ml of NAC. By repeated determination ($n=10$) of a sample solution containing 0.3 μg/ml of NAC, the coefficient of variation was calculated to be 1.5%.

**C. Fluorescent Product** To elucidate the reaction pathway and the structure of the fluorescent product, we carried out the reaction of NQ with AQT by the method described in the experimental section and obtained the intermediate compound as reddish-brown needles from the ethanolic extract. From the elemental analysis data, the fragment at *m/z* 286 [loss of CO (28) from the molecular ion (*m/z* 314)] in the MS, the strong absorption at 1627 $cm^{-1}$ (carbonyl group) in the IR spectrum, and the singlet signal at 7.08 ppm due to the C-6 proton in the $^1$H-NMR spectrum, the structure of intermediate **1** was concluded to be as shown in Chart 1.

From the residue insoluble in EtOH, a black-violet amorphous product (**2**) was obtained after recrystallization from DMF. The $CCl_4$ solution of **2** showed a red fluorescence and the spectra were identical with those of the fluorescent product obtained by the recommended procedure and that obtained by the reaction of **1** with AQT. Additionally, the absence of a carbonyl group absorption in the IR spectrum and the molecular ion at *m/z* 470 in the MS of **2** suggested that the fluorescent product was a condensation compound of NQ and AQT, which was similar to the compound obtained from the reaction of NQ with *o*-aminothiophenol by Akatsuka and Yoshinaga.[19] From these results, the fluorescence reaction was considered to proceed as shown in Chart 1.

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