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Metal Chelates of Aromatic *o,o'*-Dihydroxyazo Compounds. I. The Fluorescence Properties of the Metal Chelates of *o,o'*-Dihydroxyazobenzene and Their Use in Fluorometry

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As fluorometric reagents for the determination of Al, Ga, In, *etc.*, 11 derivatives of *o,o'*-dihydroxyazobenzene were examined in an attempt to determine the most desirable structure of azo compounds. *o,p,o'*-Trihydroxyazobenzene type compounds were more sensitive than *o,o'*-dihydroxyazobenzene; CH_3O-group substitution at the *para* position was also efficient. The SO_3H substitution increased, whereas the Cl substitution decreased the fluorescence of metal complexes, and the CH_3-substituted compound did not form fluorescent metal complexes because of its low solubility in water and because of the steric hindrance. Superchrome Garnet Y and Lumogallion seemed to be excellent fluorometric reagents for Al and Ga; 2-(2,4-dihydroxyphenylazo)-1-hydroxybenzene and 3-(2-hydroxy-4-methoxyphenylazo)-4-hydroxybenzene sulfonic acid were also useful. Therefore, the several conditions for the fluorometric determination of Al and Ga with those compounds were also investigated.

It has been recognized that some azo compounds can be used as fluorometric reagents for aluminum, gallium, and other metal ions. Pontachrome Blue Black R[1] and Eriochrome Red B[2] are widely used for the determination of aluminum, while Lumogallion[3,4] is one of the most sensitive fluorometric reagents for aluminum and gallium and has been employed for the determination of aluminum in sea water.[5] However, few systematic investigations of the usefulness of azo compounds for the fluorometry of metal ions have yet been performed.

Freeman and White[6,7] studied the relationship between the structures of several *o,o'*-dihydroxyazo compounds and the fluorescent characteristics of the metal complexes. However, they did not observe any general regularity, because the fluorescence properties were obtained in an aqueous solution in some cases and in a dimethylformamide solution in other cases, and because the pH of the solution was not always the optimum for the fluorescence.

In this paper, the usefulness of *o,o'*-dihydroxyazo compounds in the fluorometric determination of aluminum and gallium will be discussed. *o,o'*-Dihydroxyazobenzene was chosen as the basic azo compound; ten substituted compounds, in which –OH, $-OCH_3$, $-SO_3H$, –Cl, and/or $-CH_3$ groups were substituted for hydrogen atoms at the *ortho*, *meta*, and *para* positions of azobenzen, were synthesized. Among these compounds, Superchrome Garnet Y,[8] Mordant Blue 31,[9] and Lumogalliion[3] had previously been reported on. The fluorescence measurements were done in an aqueous medium to avoid the complication of the operation and to make the comparison easy, and the usefulness of these azo compounds for the fluorometry was ex-

1) M. Ishibashi, T. Shigematsu, and Y. Nishikawa, *Bunseki Kagaku*, **6**, 568 (1957).
2) Y. Nishikawa, K. Hiraki, and K. Morishige, *J. Facul. Sci. Technol., Kinki Univ.*, **2**, 15 (1967).
3) Y. Nishikawa, K. Hiraki, K. Morishige, and T. Shigematsu, *Bunseki Kagaku*, **16**, 692 (1967).
4) Y. Nishikawa, K. Hiraki, K. Morishige, A. Tsuchiyama, and T. Shigematsu, *ibid.*, **17**, 1092 (1968).
5) T. Shigematsu, Y. Nishikawa, K. Hiraki, and N. Nagano, *ibid.*, **19**, 551 (1970).
6) C. Donald, J. Freeman, and C. E. White, *J. Amer. Chem. Soc.*, **78**, 2678 (1956).
7) C. E. White and G. G. Guilbault, eds., "Fluorescence," Marcel Dekker, New York (1967), p. 275.
8) K. Hiraki, This Bulletin, **45**, 1395 (1972).
9) K. Hiraki, *ibid.*, **45**, 789 (1972).

amined under the optimum analytical conditions.

Reagent and Apparatus

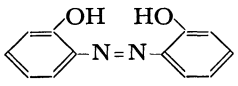
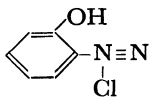
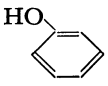
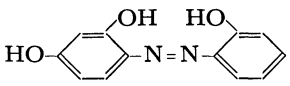
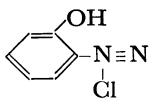
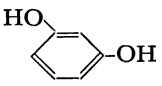
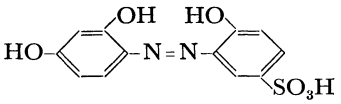
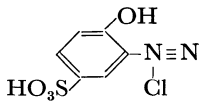
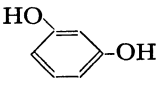
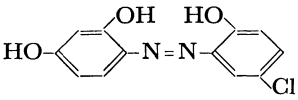
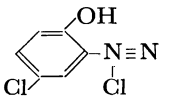
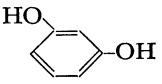
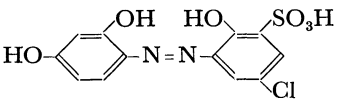
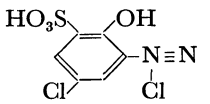
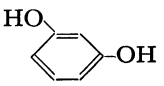
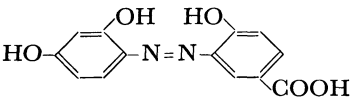
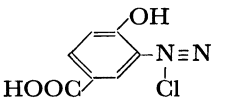
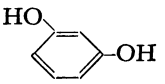
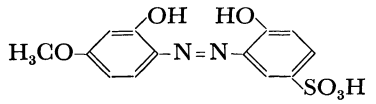
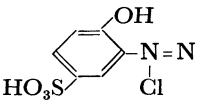
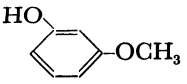
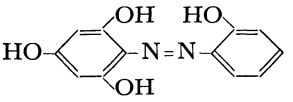
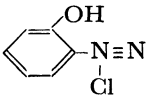
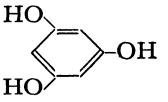
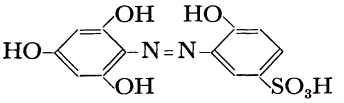
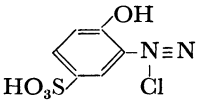
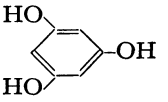
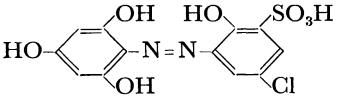
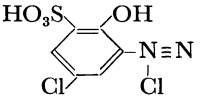
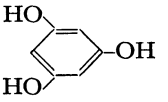
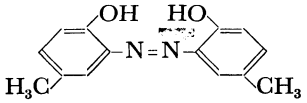
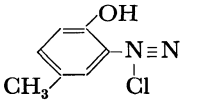
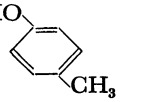
Synthesis of Azo Compounds. The eleven azo compounds used in this investigation are shown in Table 1. These azo compounds were synthesized by the usual diazotization and coupling, and were precipitated from an aqueous solution by adding concentrated hydrochloric acid. The products thus obtained were purified by repeated recrystallizations from 6M hydrochloric acid or an ethanol solution, and were then dried at room temperature on silica gel in a desiccator.

Azo Compound Solutions. A definite weight of each of the azo compounds was dissolved in redistilled water or purified ethanol, and then the solution was diluted to give a 0.1—0.01% solution.

Standard Solutions of Aluminum, Gallium, Scandium, and Indium. The stock solution of aluminum was prepared by dissolving 1.6803 g of aluminum in water containing 5 ml of concentrated hydrochloric acid, and by then diluting the solution to 100 ml. The stock solution was diluted with water to make a standard solution containing 1.00 μg aluminum per ml. The stock solutions of gallium, scandium, and indium were prepared by dissolving 99.99, 99.9, and 99.99% pure oxides respectively with 6M hydrochloric acid; the standard solutions were obtained by the subsequent suitable dilution of the stock solutions.

Rhodamine B Solution. 0.1 g of Rhodamine B was dissolved in water, and then the solution was diluted to 100 ml. The stock solution was diluted with water to make a solution containing 0.168 μg Rhodamine B

Table 1. Chemical formulae of o,o'-dihydroxyazo compounds

Compd. No.	o,o'-Dihydroxyazo compound		Diazonium component	Coupling component
I		2-(2-hydroxyphenylazo)-1-hydroxybenzene		
II		2-(2,4-dihydroxyphenylazo)-1-hydroxybenzene		
III		3-(2,4-dihydroxyphenylazo)-4-hydroxybenzenesulfonic acid		
IV		2-(2,4-dihydroxyphenylazo)-1-hydroxy-4-chlorobenzene		
V		3 (2,4-dihydroxyphenylazo)-2-hydroxy-5-chlorobenzenesulfonic acid		
VI		3-(2,4-dihydroxyphenylazo)-4-hydroxybenzenecarboxylic acid		
VII		3-(2-hydroxy-4-methoxyphenylazo)-4-hydroxybenzenesulfonic acid		
VIII		2-(2,4,6-trihydroxyphenylazo)-1-hydroxybenzene		
IX		3-(2,4,6-trihydroxyphenylazo)-4-hydroxybenzenesulfonic acid		
X		3-(2,4,6-trihydroxyphenylazo)-2-hydroxy-5-chlorobenzenesulfonic acid		
XI		3-(2-hydroxy-5-methylphenylazo)-4-hydroxy-1-methylbenzene		

per ml; this solution was employed as the reference fluorescence standard in adjusting the sensitivity of the instrument.

All the other chemicals used were the analytical grade. Ethanol, hydrochloric acid, and ammonia were distilled until they did not show fluorescence.

Apparatus. The spectrofluorometric measurements were made with a Hitachi spectrophotometer, Model EPU-2A, equipped with a Hitachi fluorometric attachment, Model G-3 (exciting source : 150 W Xenon lamp). A Hitachi-Horiba glass electrode pH meter, Model M-5, was used for the pH measurements.

Experimental

The general procedure was as follows. To 15—20 ml of a sample solution containing appropriate amounts of metal ions such as aluminum, gallium, scandium, *etc.*, a certain quantity of a 0.1—0.01% azo compound solution and 2 ml of a 20% ammonium acetate solution or a 20% ammonium chloride solution are added. The pH value is adjusted to a desired one with a dilute hydrochloric acid solution or an ammonia solution, after with the solution is diluted to 25 ml with water. If the reaction is slow, the solution is kept at 50—80 °C for 10 min before the final dilution. The fluorescence intensity is measured at the optimum-excitation and maximum-emission wavelengths, using a Rhodamine B solution as the reference standard.[10]

Results and Discussion

Fluorescence Properties of Metal-o,o'-dihydroxyazo Compounds. Table II summarized the fluorescence behavior of several metal complexes with the *o,o'*-dihydroxyazo compounds synthesized in the present work. As may be seen in the table, metal complexes with Compounds II, III, IV, V, and VII show intense fluorescence, but the complexes with Compounds VIII, IX, X, and XI are weakly fluorescent or non-fluorescent. Compound VI, which is a carboxyl-substituted product, can not be practically used in the fluorometry, because the reagent itself has a strong fluorescence.

The fluorescence intensities of the aluminum complexes obtained under the optimum conditions increase in this order: Compound IV<II=VII<V<III. The weaker fluorescence of the Compound II complex may result from its low solubility in water. The sulfo-substituted compound III forms a strong fluorescent complex, but this reagent fluorescences remarkably. On the other hand, chloro substitution decreases the fluorescence intensity of the complex because of the electron-withdrawing property of chlorine atom. The chloro- and sulfo-substituted compound V, also called Lumogallion, is a very useful and accurate fluorometric reagent for trace amounts of aluminum. This may be the result of the fact that the fluorescence of the aluminum complex decreases a little, while the blank fluorescence decreases markedly, in comparison with Compound III.

Freeman and White pointed out that the fluorescence intensity increased in the presence of an additional hydroxyl group or a methoxyl group on the position *para* to the azo group in *o,o'*-dihydroxyazobenzene. In the order of sensitivity presented above, Compound VII was less sensitive than Compound V. However,

Table 2. Optimum fluorescence conditions of various metal-*o,o'*-dihydroxyazo compound complexes

Compound No.	Ion	$\lambda_{Excitation, max.}$ (nm)	$\lambda_{Emission, max.}$ (nm)	pH	Standing time	Metal to ligand ratio	Fluorescence intensity[a]
I	—	—	—	—	—	—	—
II	Al	365, 480	568	5.0	5 min, room temp	1 : 1	83.5
	Ga	365, 485	575	3.0	5 min, room temp	1 : 1	11.0
	Sc	480	580	6.5	5 min, room temp		1.3
	Mg	480	560	12.0	5 min, room temp		0.5
III	Al	365, 485	562	5.5	10 min, 80 °C	1 : 1	195.0
	Ga	365, 485	570	3.0	5 min, room temp	1 : 1	25.5
	Sc	482	580	6.0	5 min, room temp		0.8
	In	480	570	5.0	5 min, room temp		0.3
IV	Al	365, 485	576	6.3	10 min, 50 °C	1 : 1	15.3
	Ga	365, 485	580	3.5	5 min, room temp		1.5
V	Al	365, 485	576	5.0	10 min, 80 °C	1 : 1	135.0
	Ga	365, 485	580	3.0	10 min, 80 °C	1 : 1	24.3
VI	—	—	—	—	—	—	—
VII	Al	495	570	4.0	5 min, room temp		81.6
	Ga	500	570	3.5	5 min, room temp		2.7
VIII	—	—	—	—	—	—	—
IX	—	—	—	—	—	—	—
X	—	—	—	—	—	—	—
XI	—	—	—	—	—	—	—

a) Fluorescence intensity was obtained on 1 μg of metal ions. Fluorometer was set at 60 div. with 0.168 μg Rhodamine B/ml standard solution. Longer excitation wavelength was selected.

10) The fluorescence spectra were not corrected, since the present research was done mainly for analytical purposes.

this comparison was made principally for analytical purposes. The sensitivity of the fluorometer was set in such a manner that the fluorescence intensity of the Rhodamine B reference standard gave a definite reading (60 division) under several optimum conditions for every complex. The optimum excitation wavelength and the maximum emission wavelength of the aluminum-Compound VII complex (λ_{ex} : 495 nm, λ_{em} : 570 nm) are somewhat different from those of the Compound-V complex (λ_{ex} : 485 nm, λ_{em} : 576 nm) and of the other complexes (λ_{ex} : 480—485 nm, λ_{em} : 560—580 nm). The Rhodamine B standard shows a higher fluorescence intensity under the measuring condition for the Compound VII complex; accordingly, the sensitivity of the instrument is reduced. When the sensitivity of the instrument is kept constant, the stronger fluorescence of the aluminum complex occurs in the order of V<VII<III.

On the gallium complexes, the effect of the substituent group is similar, but the fluorescence intensity is much weaker than that of the aluminum complexes.

Compounds II and III also form fluorescent complexes with scandium, indium, and magnesium, but their fluorescence is too weak to use for the fluorometry of scandium, indium, and magnesium.

Compound I, VIII, IX, X, and XI do not form fluorescent complexes. Compound I is hardly soluble in water, although the reagent forms fluorescent aluminum and gallium complexes in an alcoholic medium. The water-solubility of Compound XI is also very low, so the fluorescence intensity is reduced. In the case of Compounds VIII, IX, and X, the reasons for the non-fluorescence are not clear.

The excitation and the emission spectra of these complexes are shown in Figs. 1, 2, 3, and 4. The excitation spectra are very similar in shape except in the cases of Compound VII complexes. On the other hand, the emission spectra of the complexes with the same compound are deformed in a certain manner by

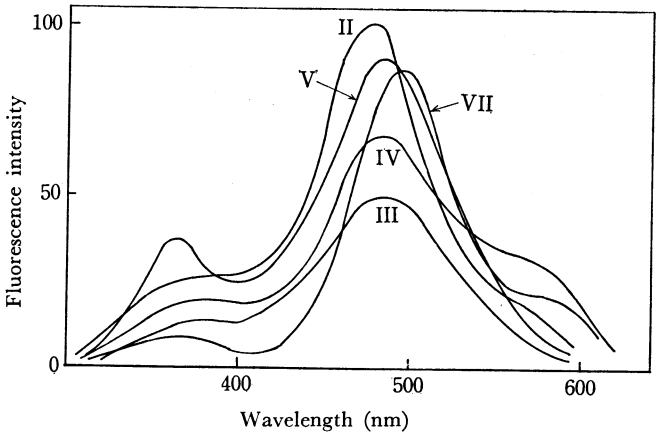


Fig. 1. Excitation spectra of aluminum complexes with *o,o'*-dihydroxyazo compounds.
II: Compound II (*cf.* Table 1, first column), emission monochromator set at 568 nm; III: Compound III, emission monochromator set at 562 nm; IV: Compound IV, emission monochromator set at 576 nm; V: Compound V, emission monochromator set at 576 nm; VII: Compound VII, emission monochromator set at 570 nm

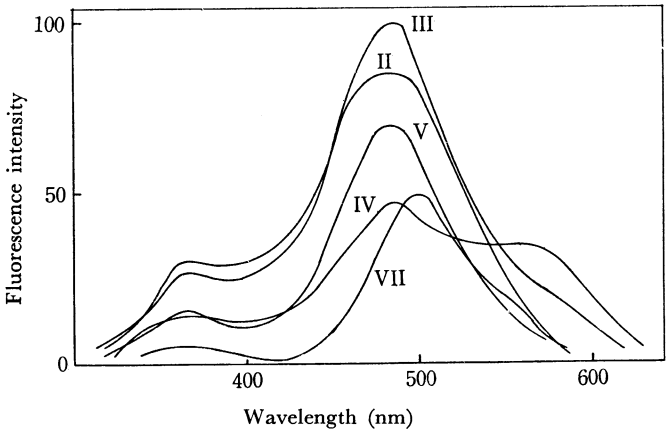


Fig. 2. Excitation spectra of gallium complexes with *o,o'*-dihydroxyazo compounds.
II: Compound II (*cf.* Table I, first column), emission monochromator set at 575 nm; III: Compound III, emission monochromator set at 570 nm; IV: Compound IV, emission monochromator set at 580 nm; V: Compound V, emission monochromator set at 580 nm; VII: Compound VII, emission monochromator set at 570 nm

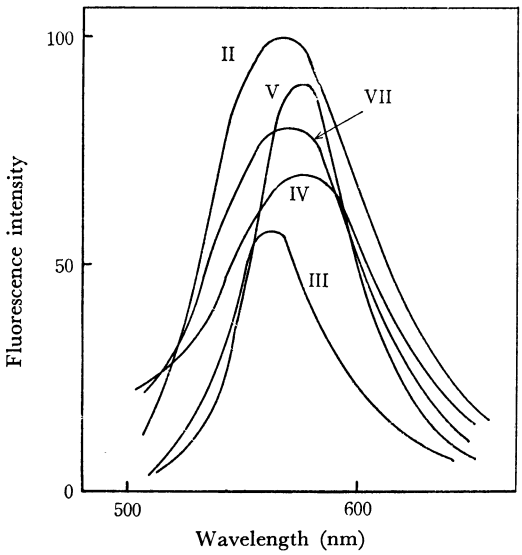


Fig. 3. Emission spectra of aluminum complexes with *o,o'*-dihydroxyazo compounds.
II: Compound II (*cf.* Table I, first column), excitation monochromator set at 480 nm; III: Compound III, excitation monochromator set at 485 nm; IV: Compound IV, excitation monochromator set at 485 nm; V: Compound V, excitation monochromator set at 485 nm; VII: Compound VII, excitation monochromator set at 495 nm

a change in the central metal ions. For example, the emission maximum of the gallium complex shifts to a slightly longer wavelength than that of the aluminum complex.

Fluorometric Determination of Aluminum and Gallium with 2-(2,4-Dihydroxyphenylazo)-1-hydroxybenzene (Compound II) and 3-(2-hydroxy-4-methoxyphenylazo)-4-hydroxybenzene Sulfonic Acid (Compound VII). As has been considered above, Compound III (Superchrome Garnet Y) and Compound V (Lumogallion) are excellent reagents; Compound II and Compound VII

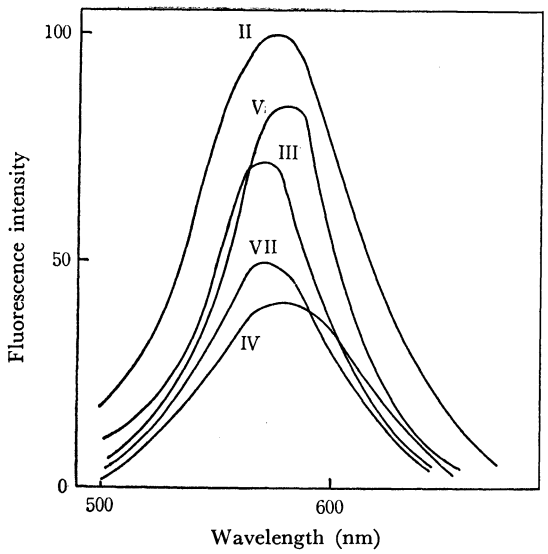


Fig. 4. Emission spectra of gallium complexes with *o,o'*-dihydroxyazo compounds.
II: Compound II (*cf.* Table I, first colum), excitation monochromator set at 485 nm; III: Compound III, excitation monochromator set at 485 nm; IV: Compound IV, excitation monochromator set at 485 nm; V: Compound V, excitation monochromator set at 485 nm; VII: Compound VII, excitation monochromator set at 500 nm

also seem to be useful for the fluorometric determination of aluminum and gallium. Analytical methods using Superchrome Garnet Y and Lumogallion were previously reported; in the present research, the procedures with the latter two reagents were studied.

(a) Effect of Standing Time: The fluorescence of these complexes developed fully within a few minutes after the preparation of the solutions, and it is stable for 2 hr at least.

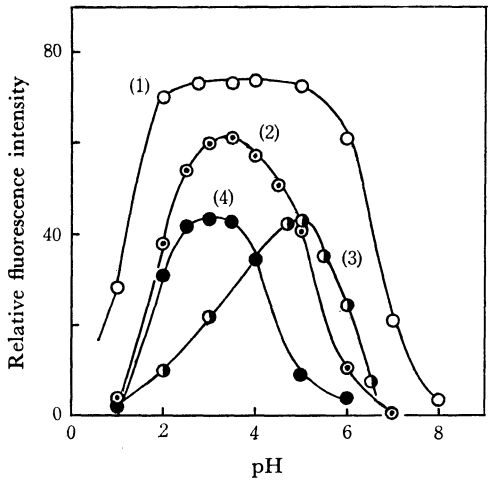


Fig. 5. Effect of pH of solution on fluorescence intensity.
(1) Al complex with compound VII, Al 1.0 μg, 495 nm/570 nm, 50 div. *vs.* 0.168 μg Rhodamine B/ml standard solution; (2) Ga complex with compound VII, Ga 10 μg, 500 nm/570 nm, 100 div. *vs.* 0.168 μg Rhodamine B/ml standard solution; (3) Al complex with compound II, Al 1.0 μg, 480 nm/568 nm, 30 div. *vs.* 0.168 μg Rhodamine B/ml standard solution; (4) Ga complex with compound II, Ga 5.0 μg, 485 nm/575 nm, 40 div. *vs.* 0.168 μg Rhodamine B/ml standard solution

(b) Effect of pH: The effect of the pH on the fluorescence intensities of metal complexes is shown in Fig. 5. The aluminum-Compound VII complex shows its maximum fluorescence in the pH range from 2.7 to 5; the gallium complex, at pH 3.5, the aluminum-Compound II complex, at pH 5, and the gallium complex, near pH 3.

(c) Effect of the Reagent Concentration: Various amounts of the reagent were added to solutions containing an adequate amount of aluminum (2 μg for Compound II and 1 μg for Compound VII) or 10 μg of gallium, after which the fluorescence intensity was measured at the optimum pH. The results, shown in Fig. 6, indicate that 1 ml of a 0.1% Compound VII solution is required for up to 1 μg of aluminum in 25 ml, and 3 ml, for 10 μg of gallium, while 0.5 ml and 0.8 ml of a 0.01% Compound II solution are desirable for 0.05—2 μg of aluminum and 0.5—12 μg of gallium respectively.

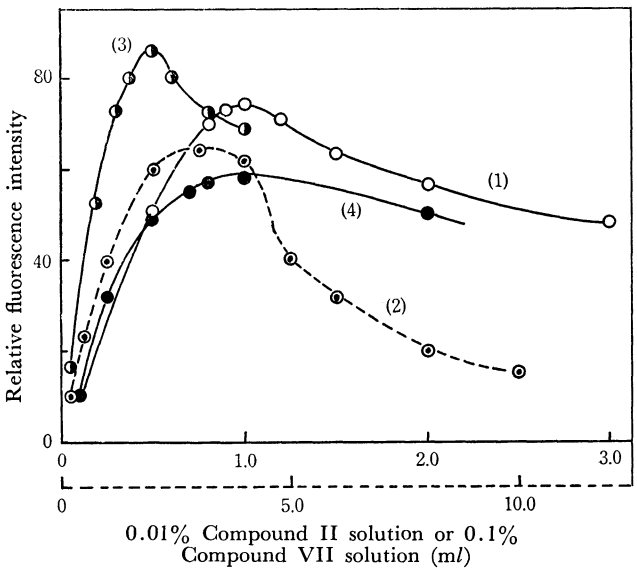


Fig. 6. Effect of reagent concentration.
(1) Al Complex with Compound VII, Al 1.0 μg, 495 nm/570 nm, 50 div. *vs.* 0.168 μg Rhodamine B/ml standard solution; (2) Ga complex with Compound VII, Ga 10 μg, 500 nm/570 nm, 100 div. *vs.* 0.168 μg Rhodamine B/ml standard solution; (3) Al complex with Compound II, Al 2.0 μg, 480 nm/568 nm, 30 div. *vs.* 0.168 μg Rhodamine B/ml standard solution; (4) Ga complex with Compound II, Ga 10 μg, 485 nm/575 nm, 40 div. *vs.* 0.168 μg Rhodamine B/ml standard solution

(d) Calibration Curves: Figure 7 presents the calibration curves for the fluorometric determinations of aluminum and gallium with Compound II and Compound VII; these curves were obtained under the above conditions. The sensitivity of the fluorometer was regulated by setting the fluorescence of the standard Rhodamine B solution (0.168 μg/ml) at a certain reading (40—100 division).

Linear relationships are observed between the fluorescence intensity and the metal-ion concentration; the determinable ranges, within an average error of 3 per cent, are 0.05—1 μg Al/25 ml and 0.5—12 μg Ga/25 ml with Compound II, and 0.05—2 μg Al/25 ml and 2—20 μg Ga/25 ml with Compound VII.

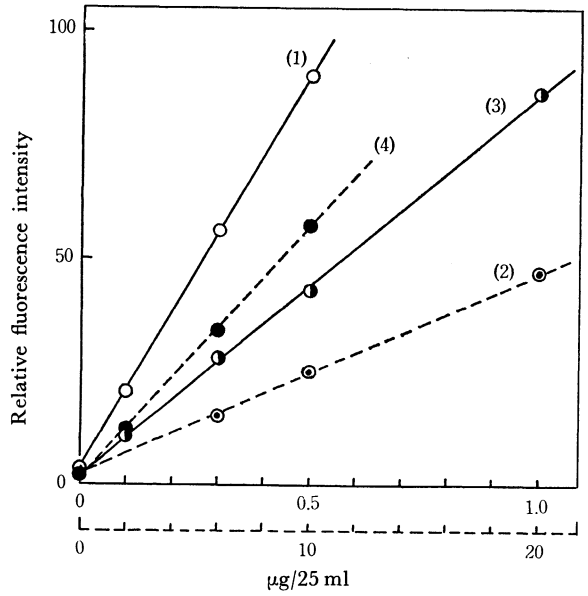


Fig. 7. Calibration curves for aluminum and gallium.
(1): Al-Compound VII system, 495 nm/570 nm, 100 div. *vs.* 0.168 μg Rhodamine B/ml standard solution; (2): Ga-Compound VII system, 500 nm/570 nm, 50 div. *vs.* 0.168 μg Rhodamine B/ml standard solution; (3): Al-Compound II system, 480 nm/568 nm, 60 div. *vs.* 0.168 μg Rhodamine B/ml standard solution; (4): Ga-Compound II system, 485 nm/575 nm, 40 div. *vs.* 0.168 μg Rhodamine B/ml standard solution.

(e) Effects of Diverse Ions: The interference of foreign ions was studied. The following metal ions diminish the fluorescence and give a negative error; in the aluminum-Compound II system, tenfold amounts of cobalt(II), copper(II), iron(III), gallium(III), zirconium(IV), vanadium(V), and chromium(VI); in the aluminum-Compound VII system, hundredfold amounts of cobalt(II), copper(II), nickel(II), tin(II), iron(III), gallium(III), thallium(III), zirconium(IV), titanium(III), vanadium(V), and chromium(VI); for the gallium-Compound II complex, sixfold amounts of copper(II), iron(III), vanadium(V), and molybdenum(VI); and for the gallium-Compound VII complex, twofold amounts of cobalt(II), copper(II), iron (III), and vanadium(V), and fivefold amounts of tin-(II), nickel(III), thallium(III), and chromium(VI). The aluminum ion gives a positive error in the determination of gallium.

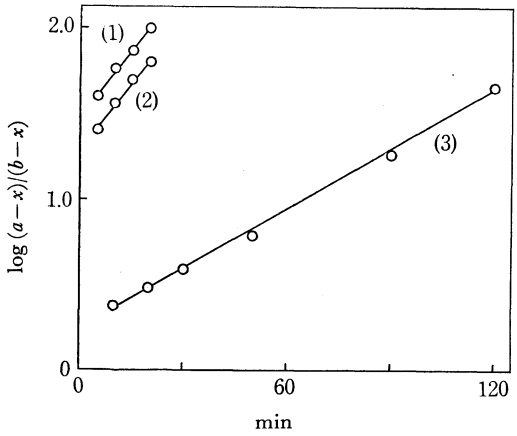


Fig. 8. Kinetics of chelate formation as indicated by fluorescence.
a is initial concentration of aluminum, *b* is initial concentration of compound II, and *x* is concentration of chelate as indicated by fluorescence (at 15 °C).
(1) *a*: 50 μmol, *b*: 5 μmol; (2) *a*: 5 μmol, *b*: 50 μmol; (3) *a*: 5 μmol, *b*: 5 μmol

(f) Determination of the Metal-to-Ligand Ratio: Although the existence of the 1 : 1 complex was expected, it could not be confirmed fluorometrically either by the mole-ratio method or by the continuous-variation method. Therefore, the kinetic method was used (reaction temperature—15 °C). Figure 8 shows that the formation of the aluminum-Compound II complex follows the second-order kinetics; this may support the above expected composition. Similar results were obtained in the aluminum-Compound VII, gallium-Compound II, and gallium-Compound VII systems; therefore, the complexes may be said to have the same composition as those of the complexes with Compounds III and V.

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