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The Quenching Action of Benzoic Acid and Methyl Benzoate on the Fluorescene of Naphthalene Derivatives. II

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The quenching action of benzoic acid on the fluorescence of naphthalene derivatives, for instance, *N*, *N*-dimethyl-2-naphthylamine, is phenomenologically ascribed to its dimer. In order to examine its molecular mechanism, absorption and fluorescence spectra have been measured on solutions of *N*, *N*-dimethyl-2-naphthylamine with and without benzoic acid, using as a solvent cyclohexane to which various amounts of dioxane or ethyl ether has been added. The quenching action of benzoic acid has also been measured in similar solvents. A good parallelism has been found between the change in the absorption spectra of benzoic acid and the change in the quenching action of benzoic acid when both are examined as functions of the concentration of dioxane or ether. From this finding, and from the more precise analysis of the data described in a previous paper (This Bulletin, **38**, 529 (1965)), it has been concluded that the quenching action of benzoic acid may be ascribed to the direct interaction between the fluorescer and the benzoic acid dimer. Further, similar quenching actions have been observed in *o*-, *m*- and *p*-toluic acids, and *p*-methoxybonzoic acid.

In a previous paper,[1] the authors investigated the quenching action of benzoic acid and methyl benzoate on the fluorescence of naphthalene derivatives from the standpoint of the charge-transfer mechanism of quenching. It was shown that benzoic acid has a rather large quenching action on *N*, *N*-dimethyl-2-naphthylamine and 2-naphthyl methyl ether compared with that of methyl benzoate; this quenching action was phenomenologically attributed to the benzoic acid dimer. As for the molecular mechanism, the charge transfer interaction between the fluorescer and the benzoic acid dimer seemed plausible. However, it was not yet quite clear whether the benzoic acid ring dimer itself really has a quenching action or whethere the ring dimer dissociates partially (this produces the linear dimer) and gives rise to quenching by forming a hydrogen bond with an excited fluorescer molecule. The present paper deals with: i) the effect of the addition of ether-type compounds on the quenching action of benzoic acid and methyl benzoate on the fluorescence of *N*, *N*-dimethyl-2-naphthylamine, and ii) the quenching action of benzoic acid derivatives on the fluorescence of *N*, *N*-dimethyl-2-naphthylamine. The results lead to the conclusion that the dimer itself has the quenching action.

Experimental

p-Dioxane was purified by the method of Eigenberger.[2] Benzoic acid from the Natural Resources Institute (standard sample for calorimetry) was zone-refined ten times, and then recrystallized from benzene. *o*-Toluic acid of Wako Junyaku (G. R. grade) was recrystallized four times from a benzene - *n*-hexane mixture. *m*-Toluic acid of Wako Junyaku (G. R. grade) was recrystallized once from benzene, twice from *n*-hexane, and once from a water-methanol mixture. *p*-Toluic acid of Tokyo Kasei (G. R. grade) was recrystallized three times from benzene. *o*-Methoxy- and *p*-methoxybenzoic acid of Tokyo Kasei (G. R. grade) were recrystallized twice from benzene, and twice from a ligroin-benzene mixture. Naphthalene of Kanto Kagaku (G. R. grade) was recrystallized three times from ethanol, and then zone-refined ten times. Benzene and cyclohexane were purified in the manner described in the previous paper. The *N*, *N*-dimethyl-2-naphthylamine was the same sample as in the earlier work. The absorption spectra were measured with a Hitachi EPS spectrophotometer. The fluorescence and action spectra were measured with an Aminco-Bowman spectrofluorimeter. Measurements were made for both degassed and non-degassed solutions at room temperature.

Results and Discussion

I) The Effect of the Addition of Ether-type Compounds on the Quenching Action of Benzoic Acid.—This effect was studied in a cyclohexane solution of *N*, *N*-dimethyl-2-naphthylamine. Before investigating the quenching action, the hydrogen bonding equilibrium in the ground state was studied by analyzing the absorption spectra. In nonpolar solvents, benzoic acid exists mainly

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[1] T. Miwa and M. Koizumi, This Bulletin, **38**, 529 (1965).

[2] E. Eigenberger, "Technique of Organic Chemistry," Ed. by A. Weissberger et al., Vol. VII, Interscience Pub., New York (1955), p. 372.

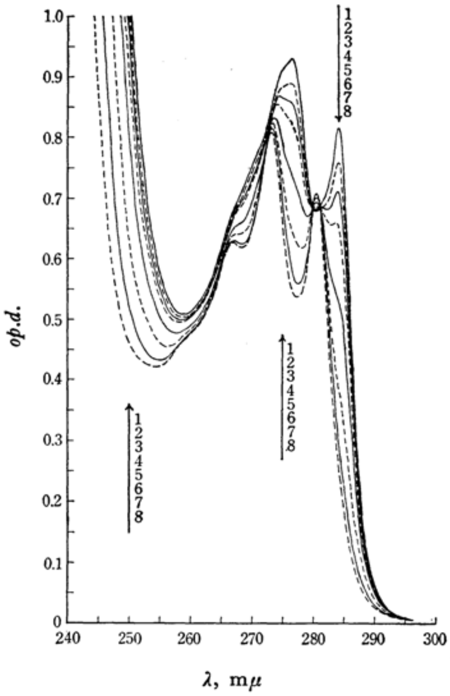

Fig. 1a. Absorption spectra of benzoic acid 1.50 $\times 10^{-2}$ M in cyclohexane plus dioxane (0—1.17 M). The molar concentrations of dioxane:
1) 0, 2) 3.50×10^{-2}, 3) 7.02×10^{-2}, 4) 1.17×10^{-1}, 5) 2.34×10^{-1}, 6) 4.68×10^{-1}, 7) 7.02×10^{-1}, 8) 1.17

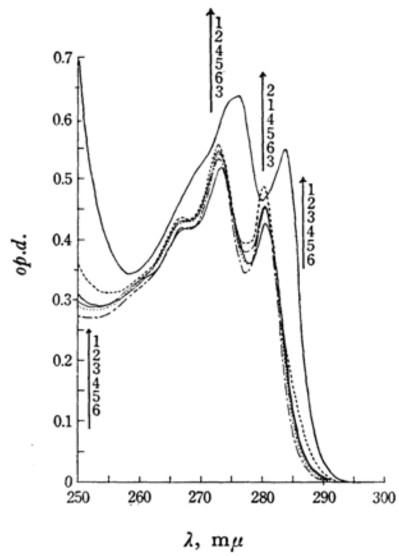

Fig. 1b. Absorptioon spectra of benzoic acid 1.00 $\times 10^{-2}$ M in dioxane-cyclohexane mixed solvent. The molar concentrations of dioxane:
1) 0, 2) 0.583, 3) 11.7 (dioxane only), 4) 8.74, 5) 5.83, 6) 2.91

as a ring dimer formed by intermolecular hydrogen bonding.* When dioxane or ethyl ether, either

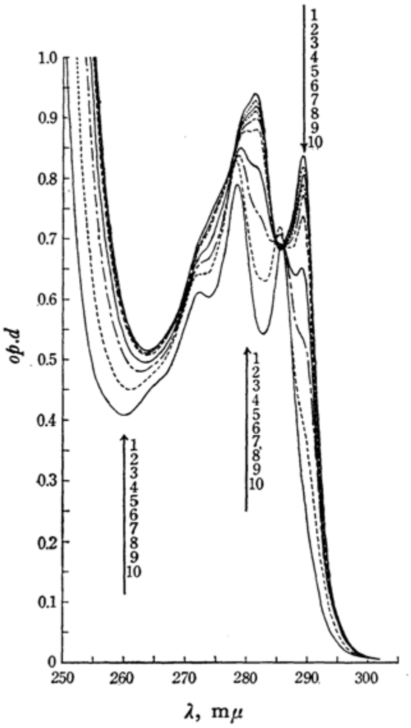

Fig. 2. Absorption spectra of benzoic acid 1.50×10^{-2} M in cyclohexane plus ethyl ether (0—1.55 M).
The concentrations (M) of ether:
1) 0, 2) 9.70×10^{-3}, 3) 1.94×10^{-2}, 4) 3.88×10^{-2}, 5) 5.82×10^{-2}, 6) 9.72×10^{-2}, 7) 1.94×10^{-1}, 8) 3.88×10^{-1}, 9) 7.76×10^{-1}, 10) 1.55

of which is capable of forming a hydrogen bond with benzoic acid, is added, a newly-established equlibrium of hydrogen bonding causes a considerable change in the absorption spectra as the concentration of the added substance is increased. The results are shown in Figs. 1a, 1b and 2.

The spectral behavior is very regular, and we can evaluate the equilibrium constant of hydrogen bond ing between benzoic acid and dioxane or ethyl ether in the following way. The optical density at a fixed wavelength is given by:

$$op.\,d. = (\varepsilon_{A_2}[A_2] + \varepsilon_A[A] + \varepsilon_{AB}[AB])d \qquad (1)$$

where A_2, A, AB, ε and d are, respectively, the benzoic acid dimer, its monomer, the hydrogen-bonded complex between benzoic acid and an added substance, the molar extinction coefficient, and the depth of the optical path. If one makes the plausible assumption that $\varepsilon_{AB}\approx\varepsilon_A$,3-5) Eq. 1

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* Hereafter, "the dimer" means the benzoic acid ring dimer and not the linear dimer.

3) H. Hosoya, J. Tanaka and S. Nagakura, *J. Mol. Spectry,* **8**, 257 (1962).

4) M. Ito, H. Tsukioka and S. Imanishi, *J. Am. Chem. Soc.,* **82**, 1559 (1960).

5) M. Ito, *J. Mol. Spectry,* **4**, 144 (1960).

Table I. Evaluation of hydrogen bond formation constant between benzoic acid and dioxane
Benzoic acid (1.50×10^{-2} M) - dioxane (0—1.17 M) in cyclohexane

[dioxane], M	*op.d.*	R	[A_2]	[A]	[B]	[AB]	K_2
0.00	0.840	0.616	7.03×10^{-3}	9.40×10^{-4}	0.00	0.00	—
5.83×10^{-3}	0.840	0.615	7.03	9.40	5.83×10^{-3}	0.00	0.00
1.17×10^{-2}	0.817	0.592	6.77	9.23	1.12×10^{-2}	0.543×10^{-3}	52.8
3.51×10^{-2}	0.779	0.554	6.33	8.93	3.37×10^{-2}	1.44	48.0
7.02×10^{-2}	0.730	0.505	5.77	8.52	6.76×10^{-2}	2.60	45.2
1.17×10^{-1}	0.675	0.450	5.14	8.04	1.13×10^{-1}	3.91	43.0
2.34×10^{-1}	0.556	0.331	3.78	6.90	2.27×10^{-1}	6.74	43.0
4.68×10^{-1}	0.414	0.189	2.16	5.21	4.58×10^{-1}	10.2	42.6
7.02×10^{-1}	0.294	0.069	0.789	3.15	6.89×10^{-1}	13.1	60.5
1.17	0.249	0.024	0.274	1.86	1.16	14.3	66.6

$R=op.d.-\varepsilon_A[A]_0d=op.d.-0.225$, *op.d.* : *op.d.* at 284.1 mμ.

Table II. Evaluation of hydrogen bond formation constant between benzoic acid and ethyl ether
Benzoic acid (1.50×10^{-2} M) - ethyl ether (0—1.55 M) in cyclohexane

[Et_2O], M	*op.d.*	R	[A_2]	[A]	[B]	[AB]	K_2
0.00	0.840	0.615	7.03×10^{-3}	9.40×10^{-4}	0.00	0.00	—
0.970×10^{-2}	0.823	0.598	6.84	9.27	0.930×10^{-2}	0.401×10^{-3}	46.5
1.94×10^{-2}	0.809	0.584	6.68	9.16	1.87	0.732	42.8
3.88×10^{-2}	0.792	0.567	6.48	9.03	3.77	1.14	33.4
5.82×10^{-2}	0.775	0.550	6.29	8.89	5.67	1.54	30.5
0.970×10^{-1}	0.741	0.516	5.90	8.61	9.47	2.34	28.7
1.94×10^{-1}	0.653	0.428	4.89	7.85	19.0	4.43	29.8
3.88×10^{-1}	0.530	0.305	3.49	6.62	38.1	7.36	29.2
7.76×10^{-1}	0.400	0.175	2.00	5.02	76.6	10.5	27.3
1.55	0.290	0.065	0.743	3.06	154	13.2	28.1

od.d.: *op.d.* at 284.1 mμ, $R\equiv op.d.-\varepsilon_A[A]_0d$.

can be rewritten as:

$$op.\,d.=[A_2](\varepsilon_{A_2}-2\varepsilon_A)d+\varepsilon_A[A]_0d$$

where $[A]_0$ is the added concentration of benzoic acid. Hence:

$$[A_2]=(op.d.-\varepsilon_A[A]_0d)/(\varepsilon_{A_2}-2\varepsilon_A)d \qquad (2)$$

Since ε_{A_2} and ε_A can be estimated from the extreme cases, $[A_2]$ can be calculated with the above formula. In the actual calculation, the optical densities of the dimer peak at 284.1 mμ, and the value of $K_1=7950$ reported by Hosoya et al.[3] for the solution of benzoic acid in *n*-heptane at 30°C, were used. Then, from the following relations:

$$\left.\begin{aligned}[A] &= \sqrt{[A_2]/K_1}\\ [AB] &= P-[A]=P-\sqrt{[A_2]/K_1}\\ [B] &= [B]_0-[A]\\ P &= [A]_0-2[A_2]\end{aligned}\right\} \qquad (3)$$

[A], [AB] and [B] were evaluated, and the formation constant of the hydrogen bond between benzoic acid and the added compound, $K_2=[AB]/([A]\cdot[B])$, could be obtained. The results obtained are given in Tables I and II. The K_2 values between benzoic acid and dioxane or ether are about 40 and 30 respectively.

The next item was to investigate how the quenching effect of benzoic acid depends on the amount of added dioxane or ether. First, the effect of the addition of benzoic acid on the absorption spectra of *N*, *N*-dimethyl-2-naphthylamine in cyclohexane containing various amounts of dioxane or ethyl ether was examined; it was confirmed that there

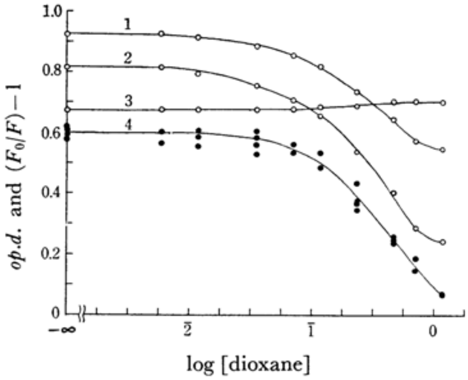

Fig. 3. Fluorescence intensity ratio $(F_0/F)^{-1}$ of *N*, *N*-dimethyl-2-naphthylamine (1.00×10^{-4} M) in the absence (F_0) and in the presence (F) of benzoic acid (1.50×10^{-2} M) and optical densities of benzoic acid (1.50×10^{-2} M) as functions of the added amount (M) of dioxane.
1) *op.d.* at 276.5 mμ, 2) *op.d.* at 284.1 mμ, 3) *op.d.* at 280.6 mμ, 4) $(F_0/F)-1$

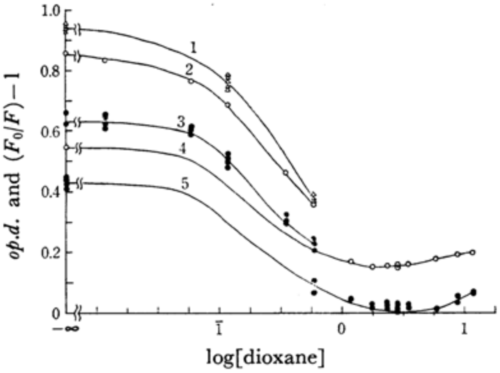

Fig. 4. Fluorescence intensity ratio $(F_0/F)-1$ of N, N-dimethyl-2-naphthylamine (1.00×10^{-4} M) in the presence (F) and in the absence (F_0) of benzoic acid (A) and optical densities of benzoic acid as functions of the added amount (M) of dioxane.

1) $(F_0/F)-1$, [A]: 1.50×10^{-2} M, degassed soln.
2) *op.d.*, [A]: 1.50×10^{-2} M, aerated soln.
3) $(F_0/F)-1$, [A]: 1.50×10^{-2} M, aerated soln.
4) *op.d.*, [A]: 1.00×10^{-2} M, aerated soln.
5) $(F_0/F)-1$, [A]: 1.00×10^{-2} M, aerated soln.

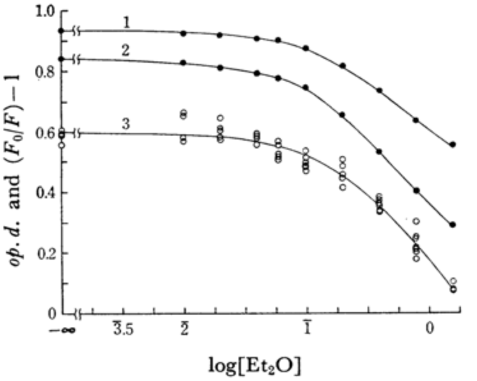

Fig. 5. Fluorescence intensity ratio $(F_0/F)-1$ of N, N-dimethyl-2-naphthylamine (1.00×10^{-4} M) in the presence (F) and in the absence (F_0) of benzoic acid (1.50×10^{-2} M) and optical densities of benzoic acid (1.50×10^{-2} M) as functions of the added amount (M) of ethyl ether.

1) *op. d.* at 277.0 mμ, 2) *op. d.* at 284.2 mμ,
3) $(F_0/F)-1$.

is not essential effect. This means that there is no appreciable interaction between the ground-state fluorescer and the benzoic acid dimer or monomer (hydrogen-bonded with ether or dioxane). Next, the fluorescence intensity, F_0, of N, N-dimethyl-2-naphthylamine 10^{-4} M in the above solvent system and that, F, of a similar solution containing 1.5×10^{-2} M or 1.0×10^{-2} M benzoic acid were measured in order to evaluate the quenching effect of benzoic acid. In Figs. 3, 4 and 5, the values of of $(F_0/F)-1$ and the optical densities of benzoic acid at several wavelengths are plotted against the concentration of dioxane or ether. In Fig. 4, the filled circles and triangles show the results for non-degassed and degassed solutions respectively. The quenching action of benzoic acid on the fluorescence is greater in the degassed solutions than in the non-degassed solutions, but no anomaly due to the dissolved oxygen was observed. Figures 3, 4 and 5 show a very good correlation between the spectral change in benzoic acid and the change in the quenching action of benzoic acid. Since the optical densities at 277 and 284 mμ, which are the absorption maxima of the dimer, give the measure for the dimer concentration, this correlation enables us to rule out a quenching mechanism in which the benzoic acid dimer dissociates partially or completely and forms a hydrogen bond with the excited fluorescer. If such were the case, the benzoic acid monomer hydrogen-bonded with dioxane or ether, the formation constant of which is much smaller than that of the dimer, would have a larger quenching action than the dimer. This expectation does not fit the results obtained; one can, therefore, safely conclude that if the dimer does act as a quencher, it really remains as the dimer throughout the quenching process.

II) The Effect of the Addition of an Ether-type Compound on the Quenching Action of Methyl Benzoate.—Similar experiments have been made using methyl benzoate as a quencher and N, N-dimethyl-2-naphthylamine as a fluorescer, in order to get a better understanding of the quenching action of benzoic acid. First, the absorption and fluorescence spectra of the solutions of methyl benzoate and N, N-dimethyl-2-naphthylamine were measured using dioxane-cyclohexane mixtures of various compositions as solvents. The absorption spectra of methyl benzoate in cyclohexane do not change when a small amount of dioxane is added. With the increase in dioxane concentration, the spectra of methyl benzoate gradually become broad as a result of the so-called solvent effect, but there is no marked effect. Next, the absorption and fluorescence spectra and the fluorescence intensity of N, N-dimethyl-2-naphthylamine were measured with and without 0.800 M methyl benzoate, using the above-mentioned solvents. The addition of methyl benzoate intensifies the absorption spectra of N, N-dimethyl-2-naphthylamine and causes a shift to longer wavelengths; the fluorescence spectra also shift to red and become broad. These shifts are more marked than the shifts observed in absorption spectra. On the further addition of methyl benzoate, a new emission band appears near 480 mμ.* The quenching action of methyl benzoate increases with the increase in the dioxane concentration. The fluorescence intensity (F) of the solution with the quencher and that of the solution without it (F_0) were determined at the maxima of the

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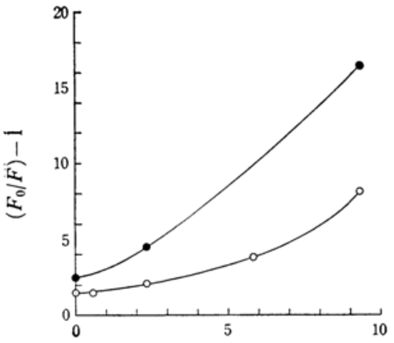
* The details will be described elsewhere.



[dioxane]: M

Fig. 6. The change of the quenching action of methyl benzoate due to the variation of the composition of solvent.
The concentration of methyl benzoate: 8.800 M, solvent: cyclohexane-dioxane mixture.
●: degassed soln., ○: non-degassed soln.

fluorescence spectra. No correction was made for the spectral sensitivity distribution of the fluorimeter. The calculated values are summarized in Fig. 6.

In this figure, the open circles show the values for the aerated solutions, while the filled circles show the values for the deaerated solutions. It is evident that there is no extraordinary effect of the dissolved oxygen. It is worthwhile to note here that a similar effect was also found in the case of benzoic acid systems. Thus, as may be seen from Fig. 4, the quenching action of benzoic acid at an extremely high concentration region of dioxane, begins to increase to some extent after having passed through a minimum, at which point the absorption spectra of the benzoic acid dimer must have completely turned to monomer-type ones (hydrogen-bonded with dioxane). At the same time, the absorption spectra of the hydrogen-bonded complex with dioxane begin to suffer the so-called solvent effect and the spectra become broad, as in the case of methyl benzoate (see Fig. 1b). These results strongly suggest the same quenching mechanism in both cases and favors the C. T. quenching mechanism which was assumed for the quenching action of the methyl benzoate in a previous paper.1)

III) The Quenching Action of the Benzoic Acid Derivatives on the Fluorescence of *N*, *N*-Dimethyl-2-naphthylamine.—In order to find other examples of quenching action similar to that of benzoic acid and to examine the relation between the quenching constants and the formation constants of the ring dimer, we have examined the quenching action of the benzoic acid derivatives on the fluorescence of *N*, *N*-dimethyl-2-naphthylamine. This was investigated only with aerated solutions, because it had already been confirmed that there is no extraordinary effect due to dissolved oxygen. The benzoic acid derivatives used as quenchers were *o*-, *m*-, *p*-toluic acid, and *o*-, *p*-methoxybenzoic acid.

Cyclohexane Solution.—The absorption spectra of these substituents of benzoic acid show a concentration effect analogous to that of benzoic acid. The dimerizations of benzoic acid and its derivatives have been extensively investigated on the basis of the concentration dependence of their absorption spectra. Especially, Forbes et al.6,7) measured the absorption spectra of benzoic acid and its derivatives systematically, and also examined the relationship between the molecular structure and the tendency toward dimerization. They expressed the ability to dimerize by "critical maximal concentration," which is the concentration above which the values of ε_{max} become approximately independent of the concentration, and below which the acid appreciably exists in the monomeric form. The values in Table III are quoted from their paper, thus facilitating an examination of the correlation between the ability to dimerize and the ability to quench. When benzoic acid derivatives are added to a solution of *N*, *N*-dimethyl-2-naphthylamine, the absorption and fluorescence spectra

TABLE III. THE QUENCHING ACTION OF BENZOIC ACID DERIVATIVES ON THE FLUORESCENCE OF *N*, *N*-DIMETHYL-2-NAPHTHYLAMINE

N, *N*-Dimethyl-2-naphthylamine: 1.00×10^{-4} M Solvent: cyclohexane

Quencher	The concn. range of quencher, M	Excitation wavelength mμ	Quenching constant	Critical max*,7,8) concn., M
Benzoic acid	$0-1.75\times10^{-2}$	350	36	8.0×10^{-4}
o-Toluic acid	$0-4.00\times10^{-2}$	355	37	$11\ \times10^{-4}$
m-Toluic acid	$0-4.00\times10^{-2}$	370	38	1.6×10^{-4}
p-Toluic acid	$<5.00\times10^{-3}$	can not be obtained		4.7×10^{-4}
o-Methoxybenzoic acid	$<1.00\times10^{-2}$	can not be obtained		—a)
p-Methoxybenzoic acid	$<5.00\times10^{-3}$	can not be obtained		0.34×10^{-4}

* Values for cyclohexane solutions or cyclohexane solutions containing small amount of ethyl ether, e. g. 5%.
a) Not within range

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6) W. F. Forbes and A. R. Knight, *Can. J. Chem.*, **37**, 334 (1959).

7) W. F. Forbes, A. R. Knight and D. L. Loffen, ibid., **38**, 728 (1959).

TABLE IV. THE QUENCHING ACTION OF BENZOIC ACID DERIVATIVES ON THE FLUORESCENCE OF *N*, *N*-DIMETHYL-2-NAPHTHYLAMINE

N,*N*-Dimethyl-2-naphthylamine 1.00×10^{-4} M Solvent: benzene

Quencher	The concn. range of quencher, M	Excitation wavelength mμ	Quenching constant	Critical max. concn.,* M
Benzoic acid	$0-4.00\times10^{-1}$	350	48	8.0×10^{-4}
o-Toluic acid	$0-4.00\times10^{-1}$	355	40	11×10^{-4}
m-Toluic acid	$0-3.00\times10^{-1}$	370	47	1.6×10^{-4}
p-Toluic acid	$0-4.00\times10^{-2}$	370	37	4.7×10^{-4}
o-Methoxybenzoic acid	$0-9.00\times10^{-2}$	370	71	—a)
p-Methoxybenzoic acid	0 and 5.00×10^{-3}	370	38	0.34×10^{-4}

* Same as in Table III.

of the fluorescer do not change, but the fluorescence intensity decreases considerably. The phenomena are quite similar to those observed with benzoic acid. The fluorescence intensities of the solutions of *N*,*N*-dimethyl-2-naphthylamine with the quenchers (F) and without them (F_0) were measured, and the quenching constants were determined from the slope of the $(F_0/F)-1$ vs. $[A]_0$ plot. This treatment is approximately correct, since, in the concentration range of the present experiments, the quenchers examined exist almost entirely in the dimeric form, as judged from the values of the critical maximal concentration and from the linearity of the $(F_0/F)-1$ vs. $[A]_0$ plot. The quenching constants obtained are summarized in Table III. By comparing the quenching constants with the critical maximal concentration, it may be observed that although the critical maximal concentrations are distinctly different from compound to compound, the quenching constants are almost the same, and there is no correlation between the two quantities.

Benzene Solutions.—Since benzene absorbs the light at wavelengths shorter than 280 mμ, one can not measure the absorption spectra of benzoic acid and its derivatives. It may be assumed, however, that the behavior of benzoic acid and its derivatives in benzene is essentially the same as that in cyclohexane. The absorption spectra and the fluorescence spectra of *N*,*N*-dimethyl-2-naphthylamine are scarcely affected, but its fluorescence intensity is considerably quenched by the addition of benzoic acid and its derivatives. The results are quite similar to those for cyclohexane solutions. In the lower concentration range of quenchers, the plots of $(F_0/F)-1$ against the quencher concentrations are nearly linear, but in the higher concentration range, the plots deviate upwards. The quenching constants obtained from the slope of the $(F_0/F)-1$ vs. $[A]_0$ plot at the low concentration range are summarized in Table IV.

The critical maximal concentration of those derivatives obtained with cyclohexane solutions or with a cyclohexane-ether mixture are also listed. From this table, it may be seen that there are appreciable differences among the quenching constants of benzoic acid, *o*- and *m*-toluic acid. This finding is different from that in the case of a cyclohexane solution.

It is remarkable that *o*-methoxybenzoic acid has a very large quenching constant. As has been pointed out by Forbes et al.,[6,7] this substance scarcely exists as a dimer, but it exists in the form of a monomer with an intramolecular hydrogen bond between methoxy and carbonyl groups. It is interesting that such an intramolecularly hydrogen-bonded monomer has a large quenching action. It should be noted, however, that the apparent quenching constants for the other compounds forming a ring dimer are refered to the monomer unit and that the quenching constant of the dimer is about twice the apparent one. Therefore, it can be said that the quenching power of the intramolecularly hydrogen-bonded monomer of *o*-methoxybenzoic acid is almost the same as that of the intermolecularly hydrogen-bonded dimer of other benzoic acid derivatives. Another notable result is that *p*-substituted compounds with a rather large tendency toward dimerization are less powerful quenchers than other compounds. In summary, there is no correlation between the dimerization tendency and the quenching efficiency.

IV) A More Rigorous Analysis of the Quenching Data.—Although all the results described in I), II) and III) favor the view that the dimer and not the monomer of benzoic acid or its derivatives acts as a quencher, still this is not conclusive. Hence, it is desirable to examine further whether only the dimer or only the free monomer, which exists in a small quantity, or both, act as quenchers; this examination is to be done by a more rigorous analysis of the quenching data. As has been stated in the previous paper, the $(F_0/F)-1$ vs. $[A]_0$ plot is linear, but this treatment is approximate in nature if only the dimer is a quencher. It is only when the dimer acts as twice as efficient a quencher as the monomer that this relation strictly holds. However, the mechanism for quenching by the monomer, if it exists, is considered to be quite different from that of the dimer, and this possibility seems improbable. Therefore, an analysis will be given below for the case when only the dimer or only the monomer acts as a quencher.

i) The Quenching Action of Benzoic Acid on N, N-Dimethyl-2-naphthylamine, 2-Naphthyl Methyl Ether or 2-Naphthol.—In these cases the addition of benzoic acid does not affect the absorption spectra nor the fluorescence spectra in spite of a large quenching effect.

a) If the dimer acts as a quencher and the monomer does not, the following scheme holds:

$$D + h\nu \rightarrow D^*$$

$$D^* \xrightarrow{k_d} D$$

$$D^* \xrightarrow{k_f} D + h\nu$$

$$D^* + A_2 \xrightarrow{k_q} D + A_2$$

$$A + A \underset{K_1}{\rightleftarrows} A_2$$

and the steady-state method leads to the following formula:

$$F_0/F = 1 + \{k_q/(k_f+k_d)\}[A_2] \qquad (4)$$

It should be noted that, in this case, the formation of a molecular complex between the fluorescer and a free monomer of benzoic acid need not be taken into account, because it scarcely affects the concentration of the dimer as long as the equilibrium constant of this reaction is not very large.

b) When only the monomer acts as a quencher, the scheme may be written as follows (in this case the possibility of the fluorescer being hydrogen-bonded with the quencher is taken into account because the monomer has a free carboxyl group):

$$\begin{tikzcd}
D^* + A \arrow[r, "k_q"] \arrow[d, shift right, "k_d+k_f"'] & D^*A \arrow[d, shift right] \\
D + A \arrow[u, shift right] \arrow[r, shift left] & DA \arrow[l, shift left, "K_3"] \arrow[u, shift right]
\end{tikzcd}$$

From this scheme the following relation is derived:

$$\frac{F_0}{F} = 1 + \left\{\frac{k_q}{k_d+k_f} + K_3\right\}[A] + K_3\frac{k_q}{k_d+k_f}[A]^2 \quad (5)$$

The concentrations of the benzoic acid monomer and the dimer are calculated from the analytical concentration of benzoic acid, using the value of $K_1=7950$.³⁾ When the solvent is cyclohexane, the results of analyses are as follows. The linear relationship is slightly better in the $(F_0/F)-1$ vs. $[A_2]$ plot than in the $(F_0/F)-1$ vs. $[A]_0$ plot. The slopes of these plots are given in Tables V and VI. The $(F_0/F)-1$ vs. [A] plot is concave upwards, while the $\{(F_0/F)-1\}/[A]$ vs. [A] plot is nearly linear. The values of the slopes and intercepts are also given in Tables V and VI. For benzene solutions, the results are quite similar to those for cyclohexane solutions as long as the quencher concentration is 0—5×10^{-2} M. At higher concentrations of the quencher, however, both the $(F_0/F)-1$ vs. $[A_2]$ plot and the $\{(F_0/F)-1\}/[A]$ vs. [A] plot deviate upward from linearity. Some examples of these analyses are shown in Figs. 7, 8, 9 and 10, and the results are summarized in Table VI.

From the above results one can rule out the mechanism in which only the free monomer acts as a quencher (case b). As may be seen in Tables

TABLE V. THE VALUES OF THE SLOPE OF $(F_0/F)-1$ VS. $[A]_0$ AND $(F_0/F)-1$ VS. $[A_2]$ PLOTS, AND THE VALUES OF THE SLOPE AND THE INTERCEPT OF $\{(F_0/F)-1\}/[A]$ VS. [A] PLOT

Solvent: cyclohexane, quencher: benzoic acid, A: the slope of $(F_0/F)-1$ vs. $[A]_0$ plot, B: the slope of $(F_0/F)-1$ vs. $[A_2]$ plot, C: the slope of $\{(F_0/F)-1\}/[A]$ vs. [A] plot, D: the intercept of $\{(F_0/F)-1\}/[A]$ vs. [A] plot

Fluorescer	Excitation wavelength mμ	A	B	C	D
N, N-Dimethyl-2-naphthylamine	350	36	79	6.8×10^5	−40
2-Naphthylamine	330	55	114	9.8×10^5	−50
2-Naphthyl methyl ether	320	17	36	3.1×10^5	−40
2-Naphthol	320	43	92	9.0×10^5	−130

TABLE VI. THE VALUES OF THE SLOPE OF $(F_0/F)-1$ VS. $[A]_0$ AND $(F_0/F)-1$ VS. $[A_2]$ PLOTS, AND THE VALUES OF THE SLOPE AND THE INTERCEPT OF $\{(F_0/F)-1\}/[A]$ VS. [A] PLOT

Solvent: benzene, quencher: benzoic acid. A: the slope of $(F_0/F)-1$ vs. $[A]_0$ plot, B: the slope of $(F_0/F)/1$ vs. $[A_2]$ plot, C: the slope of $\{(F_0/F)-1\}/[A]$ vs. [A] plot, D: the intercept of $\{(F_0/F)-1\}/[A]$ vs. [A] plot

Fluorescer	Excitation wavelength mμ	A	B	C	D
N, N-Dimethyl-2-naphthylamine	350	50	104	8.9×10^5	−100
2-Naphthylamine	330	48	101	9.1×10^5	−100
2-Naphthyl methyl ether	328	22	48	4.8×10^5	−50
2-Naphthol	330	35	71	5.7×10^5	∼ 0

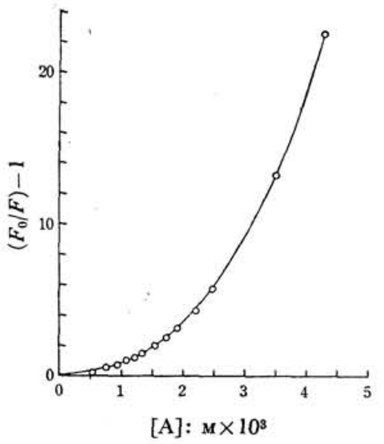

Fig. 9. $(F_0/F)-1$ vs. [A] plot in the system of *N, N*-dimethyl-2-naphthylamine (1.00×10^{-4} M)-benzoic acid (0—4.00×10^{-1} M) in benzene. Excitation wavelength: 350 mμ.

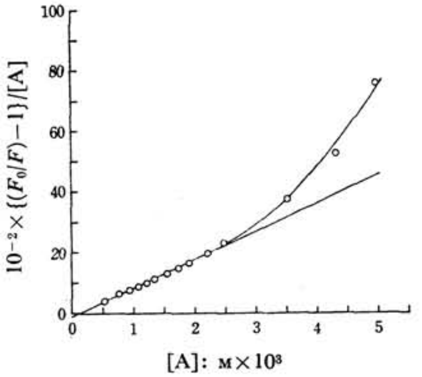

Fig. 10. $\{(F_0/F)-1\}/[A]$ vs. [A] plot in the system of *N, N*-dimethyl-2-naphthylamine (1.00×10^{-4} M) - benzoic acid (0—4.00×10^{-1} M) in benzene. Excitation wavelength: 350 mμ.

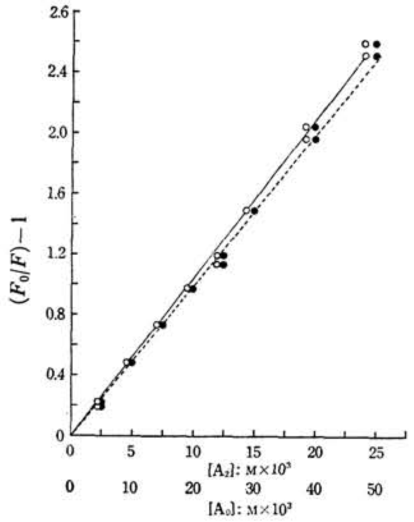

Fig. 7. $(F_0/F)-1$ vs. $[A]_0$ and $[A_2]$ plots in the system of *N, N*-dimethyl-2-naphthylamine (1.00×10^{-4} M)-benzoic acid (0—5.00×10^{-2} M) in benzene. Excitation wavelength: 350 mμ.
○; $(F_0/F)-1$ vs. $[A_2]$, ●; $(F_0/F)-1$ vs. $[A]_0$

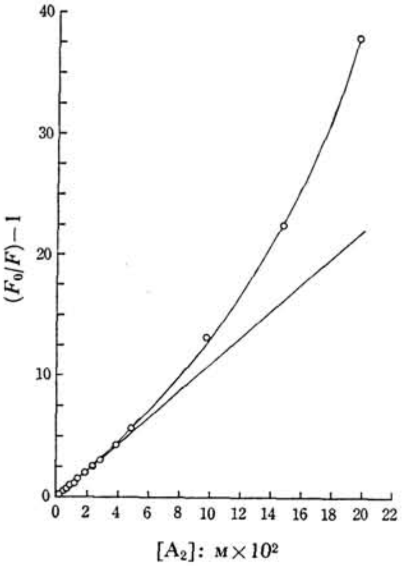

Fig. 8. $(F_0/F)-1$ vs. $[A_2]$ plot in the system of *N, N*-dimethyl-2-naphthylamine (1.00×10^{-4} M)-benzoic acid (0—4.00×10^{-1} M) in benzene. Excitation wavelength: 350 mμ.

V and VI, each $\{(F_0/F)-1/\}[A]$ vs. [A] plot has an intercept that is nearly zero or that has a small negative value. However, this is not conceivable, because the intercept which corresponds to $K_3+k_q/(k_d+k_f)$ can never be negative. Furthermore, all the values of the slopes, which are K_3 multiplied by the quenching constant, $k_q/(k_d+k_f)$, are larger than 3×10^5; these figures cannot be reconciled with the b) mechanism for the following reason. The value of K_3 is estimated to be 100* at most, and so the quenching constant is estimated to be larger than 3×10^3; this is far too large for a value expected from the diffusion controlled process.[8] Such a large value can only be interpreted on the basis of static quenching mechanism, which would contradict the present situation. Thus, it seems impossible that only the benzoic acid monomer is responsible for the quenching action.

It should be added that the above discussion is scarcely affected even when the values of K_1 are

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8) T. Miwa and M. Koizumi, This Bulletin, **36**, 1619 (1963).

* In the system consisting of 2-naphthylamine (fluorescer) and benzoic acid (quencher), which will be described later, the hydrogen bond is formed between the fluorescer and the quencher, to an extent observable by means of a study of the ultraviolet absorption spectra. From the spectral change the hydrogen bond formation constant is evaluated as 110.

changed over a wide range. The result that the $\{(F_0/F)-1\}/[A]$ vs. [A] plot deviates from linearity is also not affected by changing K_1 over a wide range. Thus, the above analysis leads to the conclusion that only the a) mechanism is reasonable. The deviation from linearity shown by the $(F_0/F)-1$ vs. $[A_2]$ plot in benzene solutions may be ascribed to static quenching by A_2; in this case the $(F_0/F)-1$ vs. $[A_2]$ plot is not essentially affected by the value of K_1, since K_1 is large enough for most of the benzoic acid molecules to exist in the dimeric form.

ii) The Quenching Action of Benzoic Acid on 2-Naphthylamine.—The solutions of 2-naphthylamine in cyclohexane show no spectral change with the addition of benzoic acid up to the limit of solubility. The solutions of 2-naphthylamine in benzene show a remarkable change in absorption spectra due to hydrogen bonding (Fig. 11), but they do not show any change in fluorescence spectra. Therefore, the hydrogen-bonded complex in this case is also nonfluorescent. In the previous paper, the equilibrium constant of hydrogen bonding was calculated from the spectral data, using an analytical concentration of benzoic acid. However, this procedure was not rigorous, and so first a more exact value was calculated, using 7950 as K_1[3]:

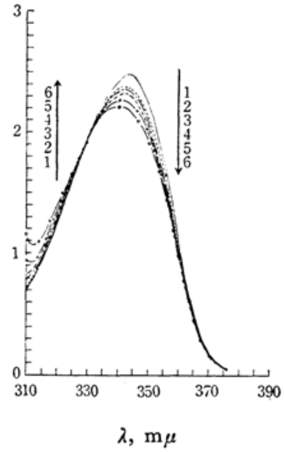

Fig. 11. Absorption spectra of the solutions containing 2-naphthylamine (1.00×10^{-4} M) and benzoic acid (0—4.00×10^{-1} M). Solvent: cyclohexane.
The concentrations (M) of benzoic acid are as follows.
1) 0, 2) 2.00×10^{-2}, 3) 6.00×10^{-2}, 4) 1.00×10^{-1}, 5) 2.00×10^{-1}, 6) 4.00×10^{-1}

$$A + D \rightleftarrows AD: K_3$$

$$K_3=\frac{[AD]}{([A]_0-2[A_2]-[AD])([D]_0-[AD])}$$

$$\approx\frac{[AD]}{([A]_0-2[A_2])([D]_0-[AD])} \qquad (6)$$

$[D]_0$: 1.00×10^{-4} M

At 5.00×10^{-4} M of benzoic acid, the lowest concentration used in this experiment, [A] is equal to 1.49×10^{-4} M when [D] is zero. Therefore, K_3 may be approximated as above by neglecting [AD], so long as the equilibrium constant, K_3, is of the order of a few hundred. By combining Eq. 6 and $op.\,d.=(\varepsilon_{AD}[AD]+\varepsilon_D[D])d$ at a suitable wavelength, one at which there is no absorption of benzoic acid or of its dimer, the following formula is obtained:

$$\frac{1}{P}K_3(\varepsilon_{AD}-\varepsilon_D)[D]_0\frac{1}{op.d.-\varepsilon_D[D]_0d}-K_3 \qquad (7)$$

$$P\equiv[A]_0-2[A_2]=[A]+[AD]\approx[A]$$

As Fig. 12 shows, the $1/P$ vs. $[D]_0/(op.d.-\varepsilon_D[D]_0d)$ plot is linear. From the intercept and the slope of this plot, $K_3=110$ and $\varepsilon_{AD}\doteqdot1400$ are obtained respectively. The fluorescence intensity is analyzed as in the case of *N, N*-dimethyl-2-methylamine under the assumption that the formation of the hydrogen-bonded complex, AD, causes scarcely any change in the concentration of A. The results obtained are very similar to those obtained for *N, N*-dimethyl-2-naphthylamine, etc. At higher concentrations of benzoic acid, the $\{(F_0/F)-1\}/[A]$ vs. [A] curve deviates from linearity, while the curve has a very large slope, 8.90×10^5, at lower concentrations of benzoic acid. When 110 is used as K_3, the quenching constant becomes 8.01×10^3, which is ridiculous as a dynamical quenching constant. Thus, in this case also, it can be concluded that the quenching of the fluorescence is not due to the direct action of the monomer.

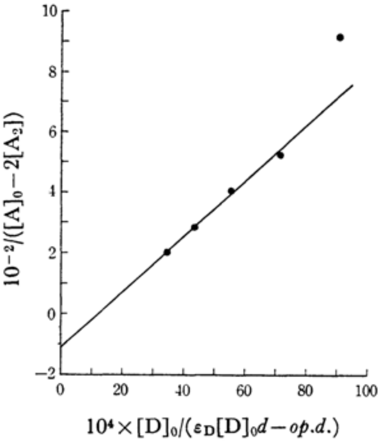

Fig. 12. The evaluation of the hydrogen-bond formation constant between 2-naphthylamine (1.00×10^{-4} M) and benzoic acid (0—4.00×10^{-1} M), using the formula (4) and the *op.d.* at 350 mμ. Solvent: benzene, temperature: 20°C.