Chem. Pharm. Bull. 24(12)3045—3057(1976)

UDC 547.654.1'462.3.04.08 : 543.426.06.08

Screening Search for Fluorescent Thiol Reagents: Syntheses of N-Naphthylmaleimide Derivatives with a Dimethylamino or a Methoxy Group as an Auxochrome and Their Electronic Spectra[1]

Minoru Machida,[2a] Mitsue Bando, Yoshihiro Migita, Mikiko I. Machida, and Yuichi Kanaoka[2b]

Faculty of Pharmaceutical Sciences, Higashi Nippon Gakuen University[2a] *and Faculty of Pharmaceutical Sciences, Hokkaido University*[2b]

(Received April 1, 1976)

In order to search useful fluorescent thiol reagents, a naphthalene ring with a dimethylamino or methoxy group was selected as a fundamental fluorogenic group. On the basis of the design to combine such a fluorogenic and a maleimide group, **11** and **15** were synthesized as the candidate thiol reagents. Analogous model compounds (**13** and **17**) were also prepared. Their absorption and fluorescence spectra were measured, and the structural requirements for the candidate fluorogenic groups were discussed. Some of dimethylaminonaphthylmaleimides were found to be potential fluorescent thiol reagents.

Introduction

Fluorescence spectroscopy is one of the most sensitive and versatile of many physicochemical techniques for protein chemistry. The properties of the intrinsic fluorescence of proteins can be used to study changes on their conformation but it is difficult to exactly analyze the intrinsic fluorescence involving a number of parameters. On the other hand, extrinsic fluorescence properties of organic molecules covalently introduced to proteins are also becoming available for developing the new technique of the protein chemistry.[3] In this series of work, it has been found that certain N-substituted nonfluorescent maleimides react with thiol compounds to give addition products, which are strongly fluorescent.[4,5] For example, N-(*p*-(2-benzimidazoly)phenyl)maleimide (BIPM) is fairly useful as this sort of reagent due to its good reactivity with thiol compounds and the high fluorescence intensity of its derivatives.[5] However, there are still some important fluorescent properties to be improved in order to provide a variety of thiol reagents which can be conveniently utilized in various fields of biological research. For example, the emission maximum of BIPM (360 nm in H_2O) is, although long enough in the wavelength to be discriminated from the intrinsic fluorescence of usual biopolymers, still too short for the use in the histochemical problems in which emission in a visible region is definitely preferred. In addition, since the fluorescent spectra of BIPM are dependent to solvents only to very limited extent,[1] the reagent is not

1) Fluorescent Thiol Reagents. VII. Part VI; Y. Kanaoka, M. Machida, M.I. Machida, and T. Sekine, *Biochim. Biophys. Acta*, **317**, 563 (1973).
2) Location: *a*) *Tobetsu, Ishikari, Hokkaido 061-02 Japan*; *b*) *Kita-12, Nishi-6, Kita-ku, Sapporo 060 Japan*.
3) For example, see: *a*) G.M. Edelman and W.O. McClure, *Accounts Chem. Res.*, **1**, 65 (1968); *b*) L. Brand and J.R. Gohlke, *Ann. Rev. Biochem.*, **41**, 843 (1972) and papers cited therein.
4) Y. Kanaoka, M. Machida, H. Kokubun, and T. Sekine, *Chem. Pharm. Bull.* (Tokyo), **16**, 1747 (1968) and papers cited therein.
5) *a*) Y. Kanaoka, M. Machida, K. Ando, and T. Sekine, *Biochim. Biophys. Acta*, **207**, 269 (1970); *b*) K. Kimura, A. Watanabe, M. Machida, and Y. Kanaoka, *Biochem. Biophys. Res. Commun.*, **43**, 882 (1971); *c*) T. Sekine, K. Ando, M. Machida, and Y. Kanaoka, *Anal. Biochem.*, **48**, 557 (1972); *d*) T. Sekine, T. Ohyashiki, M. Machida, and Y. Kanaoka, *Biochim. Biophys. Acta*, **351**, 205 (1974); *e*) T. Sekine, K.A. Kato, K. Takamori, M. Machida, and Y. Kanaoka, *ibid.*, **354**, 139 (1974); *f*) M. Machida, T. Sekine, and Y. Kanaoka, *Chem. Pharm. Bull.* (Tokyo), **22**, 2642 (1974).

well suitable as a reporter[6] or a probe[3,7] which can offer spectroscopic information about microenvironments of the thiol group concerned. Therefore, a systematic search was undertaken to explore candidate fluorogenic groups which will fulfil such requirements as mentioned above. We report here some of our initial efforts in the systematic screening for the useful fluorogenic groups with regard to the thiol reagents in the studies of biological systems.

Most fluorescent compounds usually have at least one aromatic moiety as the fluorogenic group, while some extent of solubility in water is desired for the purpose of chemical modification of biopolymers. In view of these two requirements which are apparently contradictory from each other, we have decided to start initially with a naphthalene ring as the fundamental fluorogenic unit which seems to be the smallest aromatic ring potentially capable of exhibiting the required fluorescence. Dimethylamino and methoxy groups were selected as a common and simple auxochromic group to be attached to the naphthalene ring. In the present report we describe the syntheses of N-dimethylaminonaphthyl- and N-methoxynaphthyl-maleimides and their derivatives as well as their absorption and emission spectra in an attempt to experimentally confirm the correlation between fluorescent properties and structures including the effects of the nature and the position of these substituents on the naphthalene ring, the fundamental unit possessing the essential functional group, maleimide or succinimide.

Syntheses

The key intermediates for the syntheses of the desired maleimides are the suitably substituted naphthylamines whose amino group is ultimately to be converted into the maleimide

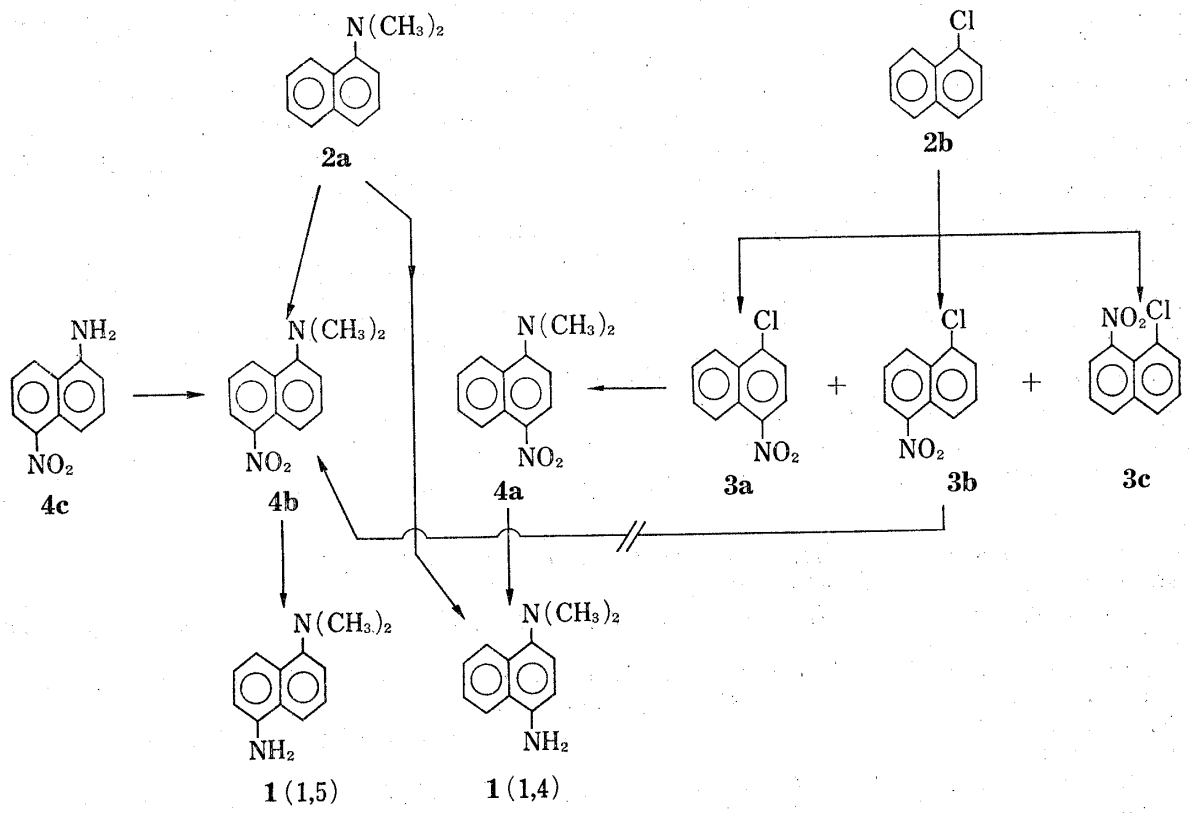


Chart 1

6) M. Burr and D.E. Koshland Jr., *Proc. Nat. Acad. Sci. U. S. A.*, **52**, 1017 (1964).
7) L. Stryer, *J. Mol. Biol.*, **13**, 482 (1965); *Science*, **162**, 526 (1968).

group. Since the isomers of dimethylaminonaphthylamine (**1**) are not commercially available except 1-dimethylamino-4-naphthylamine (**1**) (1,4), their syntheses were first examined. 1-Dimethylamino-4-naphthylamine (**1**) (1,4) was prepared by reduction of 4-*p*-sulfobenzeneazo-1-dimethylaminonaphthalene.[8a] Nitration of 1-chloronaphthalene (**2b**)[9] gave a mixture of mononitro compounds (**3**), from which the 4-nitro compound (**3a**) was isolated as a major product through alumina column chromatography, while 5-nitro and 8-nitro derivatives were obtained in poor yields. The former (**3a**) was converted to 1-dimethylamino-4-nitronaphthalene (**4a**)[8a] which on reduction afforded (**1**) (1,4). In a more practical synthetic route, 1-dimethylaminonaphthalene (**2a**) was nitrated in the presence of urea[10] to give 1-dimethylamino-5-nitronaphthalene (**4b**), whose structure was confirmed by independent synthesis from **4c**. Compound (**4b**) was catalytically reduced to 1-dimethylamino-5-naphthylamine (**1**) (1,5) (Chart 1).

Nitration of 2-dimethylaminonaphthalene (**2c**) under mild conditions gave a mixture of 5-nitro (**5a**) and 8-nitro compounds (**5b**). This mixture was reduced *in situ* followed by benzoylation ultimately to separate 2-dimethylamino-5-naphthylamine (**1**) (2,5) and 2-dimethylamino-8-naphthylamine (**1**) (2,8). Alternatively, nitration of N-acetyl-2-naphthylamine (**2e**) under controlled conditions afforded a mixture of mononitro-N-acetyl-naphthylamines

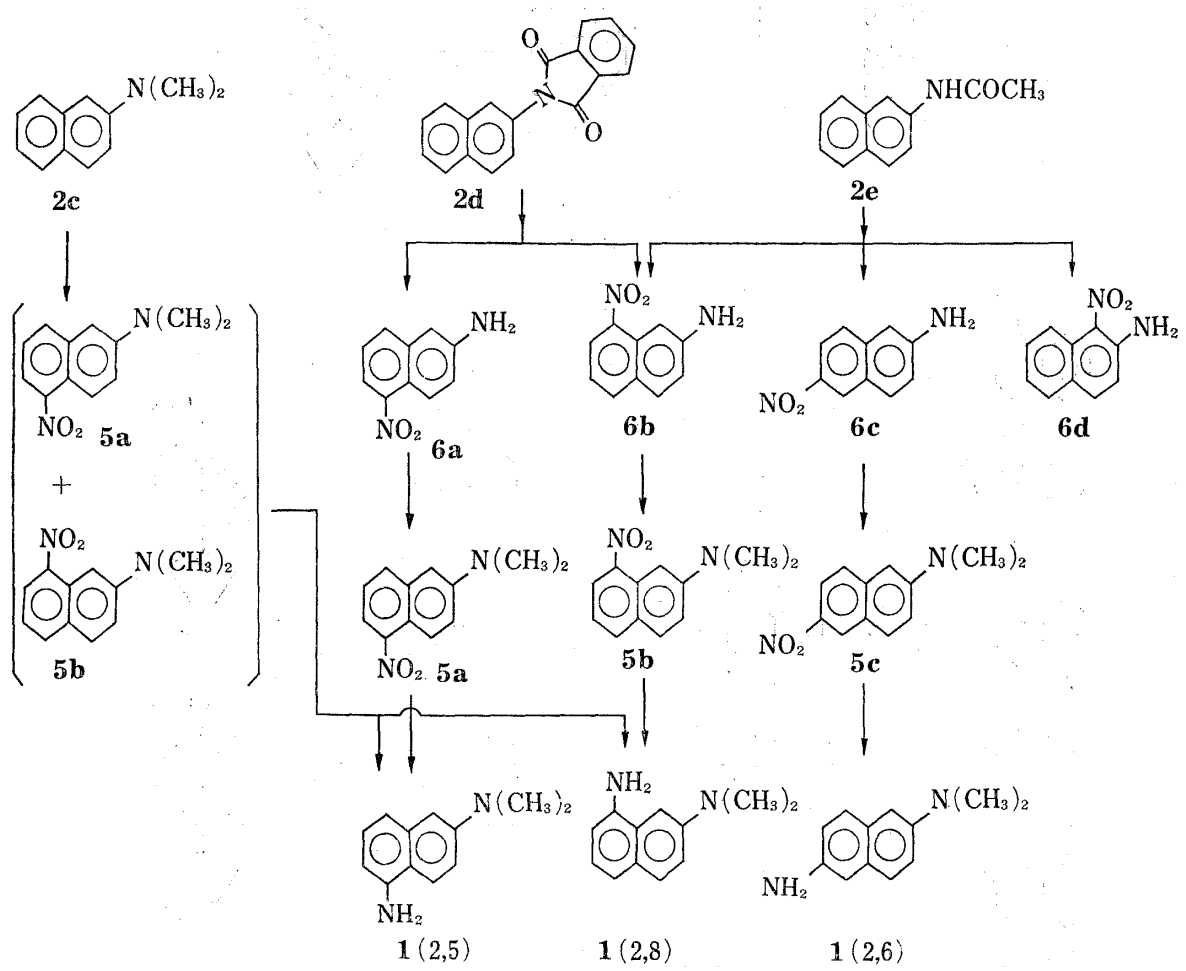


Chart 2

8) *a*) H.H. Hodgson and J.H. Crook, *J. Chem. Soc.*, **1936**, 1500; *b*) *Idem, ibid.*, **1936**, 1844.
9) P. Ferrero and C. Caflisch, *Helv. Chim. Acta*, **11**, 795 (1928).
10) H.H. Hodgson and W. Davey, *J. Chem. Soc.*, **1939**, 348.

which, on hydrolysis, gave 1-nitro- (**6d**), 6-nitro- (**6c**), and 8-nitro-2-naphthylamine(**6b**).[11] N-Phthaloyl-2-naphthylamine (**2d**) was also nitrated in a similar manner to give a mixture of 5-nitro and 8-nitro compounds. All the nitrated compounds (**6a**—**d**), separated and purified by silica gel column chromatography, were methylated with dimethyl sulfate to nitro-2-naphthylamines (**5**) which were hydrogenated to give the corresponding dimethylamino-naphthylamines (Chart 2).

The major synthetic routes to the N-substituted maleimides (**11**) and the succinimides (**13**) are shown in Chart 3 and 4. Upon mixing the amine (**1**) and maleic anhydride in an inert solvent, the maleamic acids (**10**) were obtained in nearly quantitative yields. The

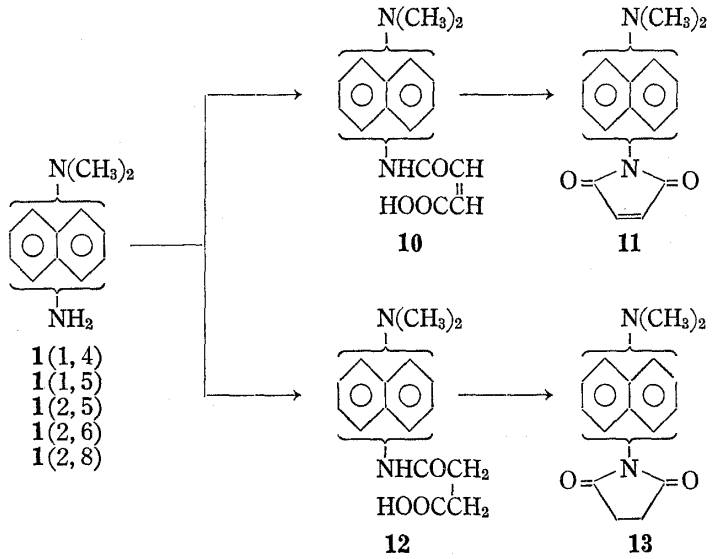


Chart 3

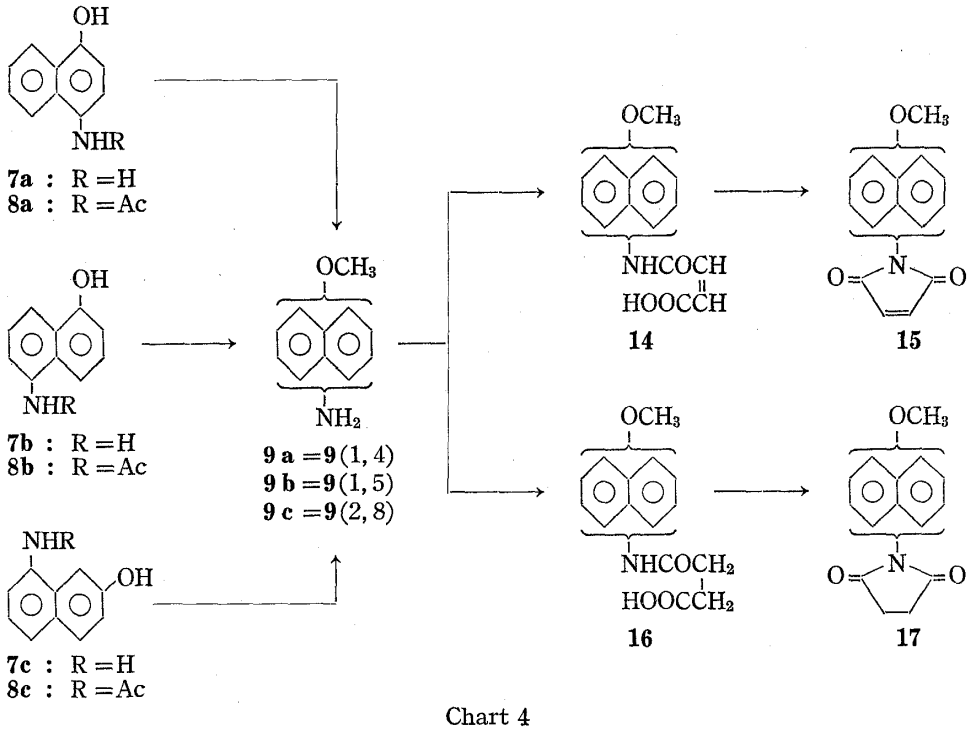


Chart 4

11) *a*) E.R. Ward and P.R. Wells, *J. Chem. Soc.*, **1961**, 4859; *b*) C.R. Saunders and C.S. Hamilton, *J. Am. Chem. Soc.*, **54**, 636 (1932); *c*) W.W. Hartman and L.A. Smith, "Organic Syntheses," Coll. Vol. II, John Wiley and Sons, Inc., New York N.Y., 1950, p. 438.

imide cyclization of these maleamic acids has been best carried out by heating the maleamic acids with the mixture of acetic anhydride (3 moles) and anhydrous sodium acetate (0.1 mole) to afford the desired maleimides (**11**). The corresponding N-substituted succinimides (**13**) are good model compounds for the adduct of a thiol group to a maleimide moiety,[4] and were prepared in a similar manner from the amines (**1**) as above using succinic anhydride in place of maleic anhydride. The N-(dimethylaminonaphthyl)maleimides (**11**) were purified by silica gel chromatography. Methoxy-naphthylamines (**9**) were obtained by methylation of N-acetylaminonaphthols (**8a**—**c**), which were prepared from acetylation of commercially available aminonaphthols (**7a**—**c**). These methoxynaphthylamines (**9**) were transformed into the

Table I. Preparation of the N-Substituted Maleimides

Maleamic acid mp (°C)	Maleimide	Yield (%)	mp (°C)	Recryst. solvent Appearance	Analysis[a] Found C	H	N	IR[b] (cm^{-1})
10(1, 4) 159.5—160.5	**11**(1, 4)	72	123—124.5	ether yellow prisms	72.12	5.41	10.58	1780(w) 1710(s)
10(1, 5) 115—118	**11**(1, 5)	64	120—122	ether–*n*-hexane yellow needles	72.02	5.23	10.45	1790(w) 1710(s)
10(2, 5) 185—186	**11**(2, 5)	10	163—165.5	benzene–*n*-hexane red prisms	71.94	5.12	10.26	1770(w) 1710(s)
10(2, 6) 212—213.5	**11**(2, 6)	17	180—182	AcOEt red needles	72.21	5.25	10.26	1770(w) 1710(s)
10(2, 8) 177—179	**11**(2, 8)	10	121—123	ether–*n*-hexane red prisms	72.12	5.24	10.63	1775(w) 1710(s)
14(1, 4) 192—194.5	**15**(1, 4)	56	177.5—178.5	EtOH yellow prisms	71.17	4.32	5.38	1795(w) 1720(s)
14(1, 5) 143.5—145	**15**(1, 5)	50	143.5—144.5	EtOH yellow columns	70.96	4.40	5.22	1775(w) 1720(s)
14(2, 8) 172—173	**15**(2, 8)	67	99.5—101.5	EtOH yellow plates	71.06	4.57	5.38	1782(w) 1715(s)

a) Calcd. for $C_{16}H_{14}O_2N_2$ (**11**): C, 72.16; H, 5.30; N, 10.52. Calcd. for $C_{15}H_{11}O_3N$ (**15**): C, 71.14; H, 4.38; N, 5.53.
b) imide carbonyl (w, weak; s, strong)

Table II. Preparation of the N-Substituted Succinimides

Succinamic acid mp (°C)	Succin-imide	Yield (%)	mp (°C)	Recryst. solvent Appearance	Analysis[a] Found C	H	N	IR[b] (cm^{-1})
12(1, 4) 134—136	**13**(1, 4)	61	148.5—150	ether colorless prisms	71.74	5.99	10.19	1785(w) 1710(s)
12(1, 5) 168—170.5	**13**(1, 5)	96	175—177	AcOEt–*n*-hexane colorless prisms	71.67	6.09	10.45	1780(w) 1720(s)
12(2, 5) 177—180	**13**(2, 5)	78	224—226	AcOEt colorless needles	71.38	6.21	10.43	1780(w) 1720(s)
12(2, 6) 183—185	**13**(2, 6)	12	249—251	AcOEt canary yellow needles	71.79	6.12	10.44	1775(w) 1710(s)
12(2, 8) 178—180	**13**(2, 8)	76	201.5—203	AcOEt–*n*-hexane colorless needles	71.47	6.08	10.38	1785(w) 1720(s)
16(1, 4) 209—212	**17**(1, 4)	90	216—217	EtOH colorless prisms	70.39	5.25	5.34	1780(w) 1710(s)
16(1, 5) 202—205	**17**(1, 5)	90	219.5—220.5	AcOEt colorless prisms	70.67	5.11	5.63	1782(w) 1710(s)
16(2, 8) 183—185	**17**(2, 8)	70	144—146	EtOH cclorless prisms	70.57	5.26	5.57	1775(w) 1715(s)

a) Calcd. for $C_{16}H_{16}O_2N_2$ (**13**): C, 71.62; H, 6.01; N, 10.44 Calcd. for $C_{15}H_{13}O_3N$ (**17**): C 70.58:H 5.13; N 5.49.
b) imide carbonyl (w, weak; s, strong)

Table III. NMR Data of the Imides[a)]

Compounds	$N(CH_3)_2$	OCH_3	Protons of the imide ring
11(1, 4)	2.91(6H, s)		6.86(2H, s)
13(1, 4)	2.88(6H, s)		2.92(4H, s)
13(1, 5)	2.88(6H, s)		2.95(4H, s)
15(1, 4)		3.99(3H, s)	6.87(2H, s)
15(1, 5)		3.97(3H, s)	6.86(2H, s)
15(2, 8)		3.83(3H, s)	6.92(2H, s)
17(1, 4)		3.96(3H, s)	2.92(4H, s)
17(1, 5)[b)]		3.99(3H, s)	2.94(4H, s)
17(2, 8)[b)]		3.95(3H, s)	2.93(4H, s)

a) δ (ppm); s, singlet *b*) in DMSO-d_6

corresponding maleimides (**15**) and succinimides (**17**) in a similar manner (Chart 4). Among the theoretically possible positional isomers, five N-(dimethylaminonaphthyl)maleimides (**11**), three N-(methoxynaphthyl)maleimides (**15**), and their corresponding succinimides were thus synthesized. The pertinent experimental data are listed in Table I—III.

Results

Ultraviolet Absorption Spectra of the Dimethylaminonaphthalene and the Methoxynaphthalene Derivatives

Fig. 1 shows the absorption spectra of some of the fundamental chromophores: *i.e.*, N-(naphthyl-1)succinimide, N-(naphthyl-2)succinimide and naphthalene. The succinimide group causes a faintly red shift with reference to the naphthalene. Absorption spectra of the parent fluorogenic compounds without an imide group are shown in Fig. 2. Both methoxy and dimethylamino groups cause a large red shift, and the bathochromic effect of a substituent at the β-position is greater than that of the same substituent at the α-position. Fig. 3 illustrates

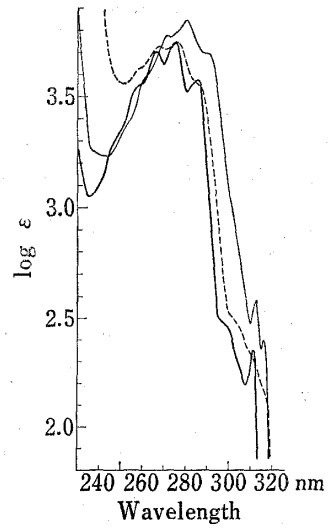


Fig. 1. Ultraviolet Absorption Spectra of Naphthalene and N-(Naphthyl) succinimides

———: naphthalene
———: N-(naphthyl-1)succinimide
———: N-(naphthyl-2)succinimide

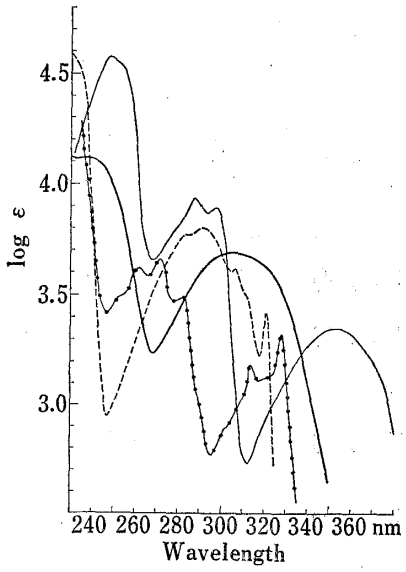


Fig. 2. Ultraviolet Absorption Spectra of Dimethylamino- and Methoxynaphthalenes

———: 1-dimethylaminonaphthalene
———: 2-dimethylaminonaphthalene
———: 1-methoxynaphthalene
—•—•—: 2-methoxynaphthalene

the absorption spectra of various methoxy-substituted naphthylimides (**15**, **17**). As shown in Fig. 4, N-(naphthyl-1)imides (**11**) (1,4) (1,5), (**13**) (1,4) (1,5) with a dimethylamino group at the α-position have their absorption maxima of the longest wavelength at about 320 nm. Absorption spectra of the analogous N-naphthylimides (**11**) (2,5) (2,6) (2,8), (**13**) (2,5) (2,6) (2,8) with a dimethylamino group at the β-position have the maxima at about 360 nm (Fig. 5—6).

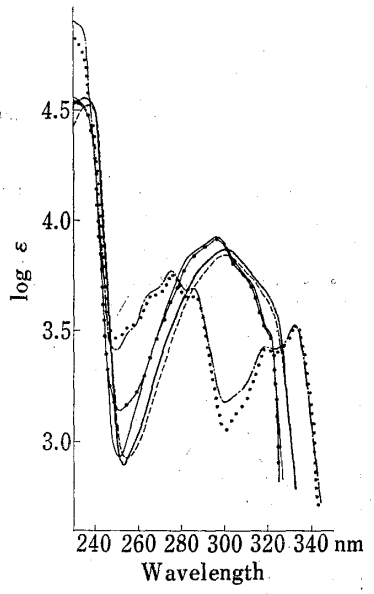


Fig. 3. Ultraviolet Absorption Spectra of Methoxynaphthalene Derivatives

——: **15**(1,4) —•—•—: **17**(1,4)
——: **15**(1,5) – – – – : **17**(1,5)
— — —: **15**(2,8) • • • • : **17**(2,8)

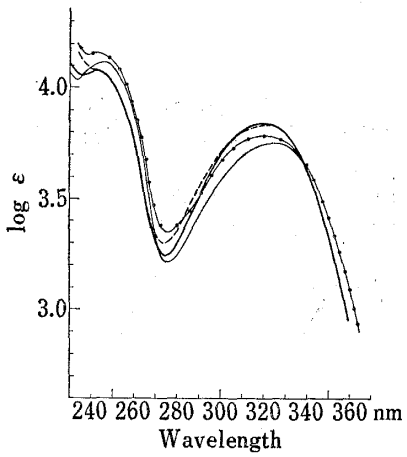


Fig. 4. Ultraviolet Absorption Spectra of Dimethylaminonaphthalene Derivatives

– – – – : **11**(1,4) ——: **13**(1,4)
—•—•—: **11**(1,5) ——: **13**(1,5)

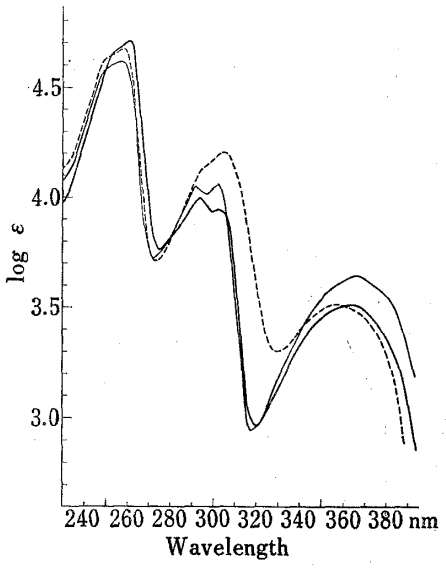


Fig. 5. Ultraviolet Absorption Spectra of N-(Dimethylaminonaphthyl)-succinimides

——: **13**(2,5)
– – – – : **13**(2,6)
——: **13**(2,8)

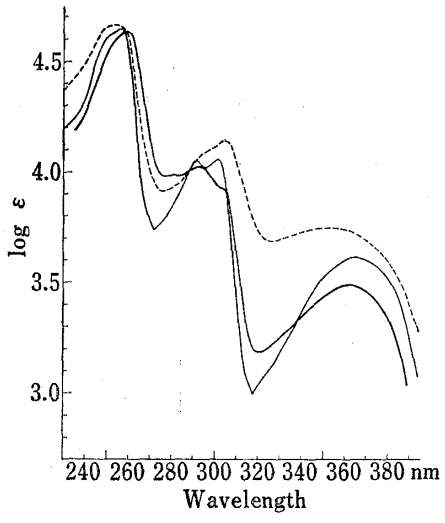


Fig. 6. Ultraviolet Absorption Spectra of N-(Dimethylaminonaphthyl)-maleimides

——: **11**(2,5)
– – – – : **11**(2,6)
——: **11**(2,8)

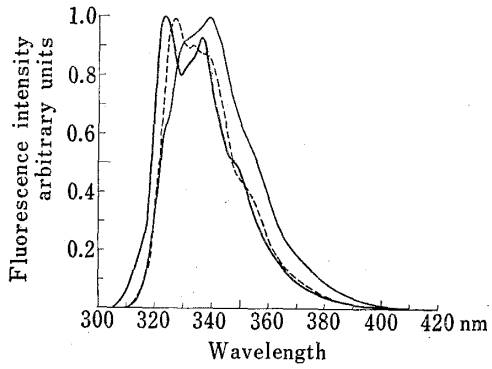


Fig. 7. Fluorescence Spectra of Naphthalene and N-(Naphthyl)succinimides

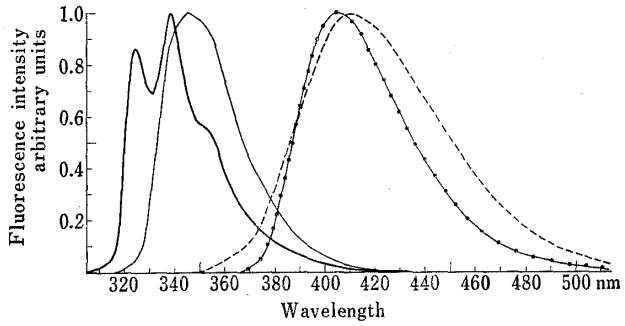


Fig. 8. Fluorescence Spectra of Dimethylamino- and Methoxynaphthalenes

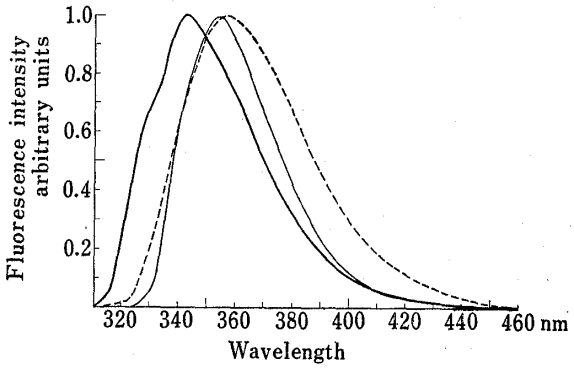


Fig. 9. Fluorescence Spectra of N-(Methoxynaphthyl)succinimides

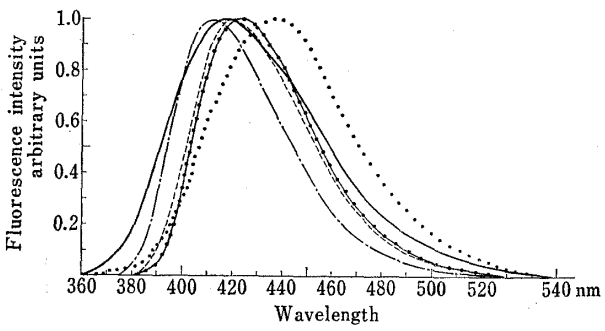


Fig. 10. Fluorescence Spectra of N-(Dimethylaminonaphthyl)succinimides

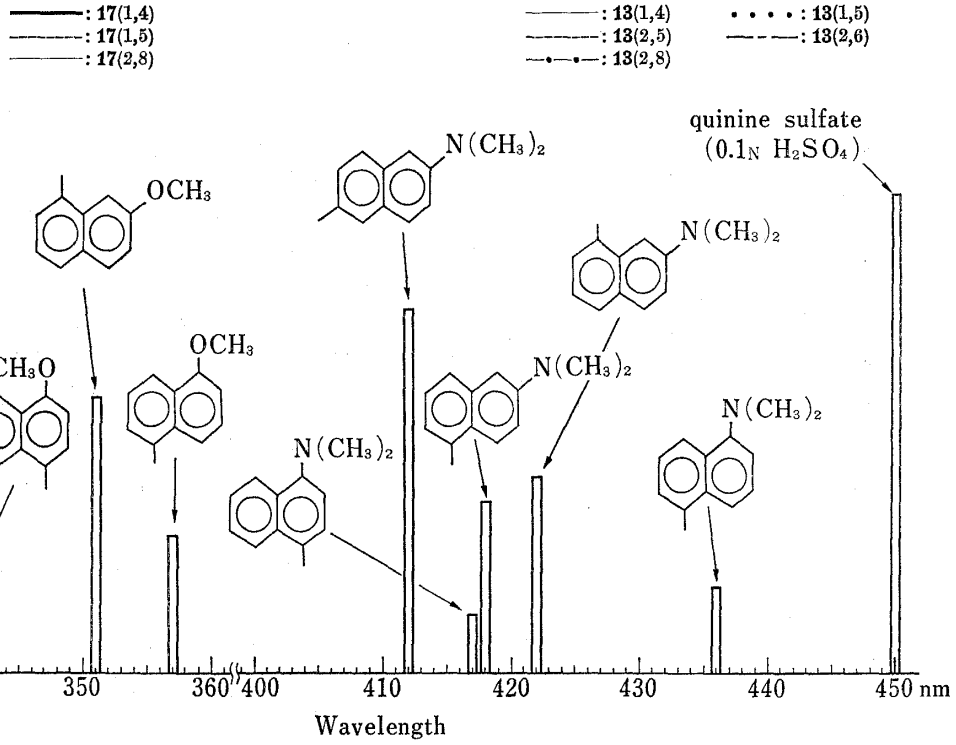


Fig. 11. Correlation of Quantum Yields and Emission Maxima of N-(Dimethylaminonaphthyl)succinimides and N-(Methoxynaphthyl)succinimides in Ethanol

Fluorescence Spectra of Dimethylamino- and Methoxynaphthalene Derivatives

Fluorescence compounds were excited with the light of the wavelength where their absorption spectra have their longest maxima. The fluorescence spectra of the succinimide derivatives were determined since they can be regarded as the model compounds of addition products of the maleimides with thiols.[5a] Fig. 7 gives the emission spectra of such fundamental fluorogenic compounds. The emission maxima of N-(naphthyl-2)succinimide caused a faintly red shift and the band at about 340 nm was intensified. The emission spectra of the parent compound containing a methoxy and a dimethylamino group as the auxochrome are given in Fig. 8. The fluorescence spectra of methoxynaphthylsuccinimides (**17**) (1,4), (**17**) (1,5) and (**17**) (2,8) show structureless peaks around 340—360 nm (Fig. 9), while the spectra of dimethylaminonaphthylsuccinimides compounds (**13**) have maxima in the 410—440 nm range (Fig. 10) with that of (**13**) (1,5) at the longest wavelength.

Quantum Yields, Excitation and Emission Maxima

Quantum yields of three methoxynaphthylsuccinimides (**17**) and five dimethylaminonaphthylsuccinimides (**13**) in ethanol solutions were obtained and compiled in Fig. 11. Quantum yields of N-(2-dimethylaminonaphthyl)succinimides are larger than that of N-(1-dimethylaminonaphthyl)succinimides in ethanol. Excitation spectra of the all succinimides are summarized in Fig. 12. Fluorescence spectra of the imides (**13**) were measured in 0.1M phosphate buffer at pH 7.0, since these reagents will be used in buffer

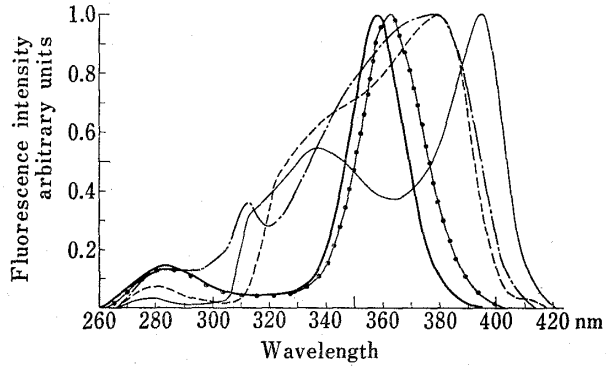


Fig. 12. Fluorescence Excitation Spectra of N-(Dimethylaminonaphthyl)succinimides

——: **13**(1,4) —•—•—: **13**(1,5)
— — —: **13**(2,5) ———: **13**(2,6)
———: **13**(2,8)

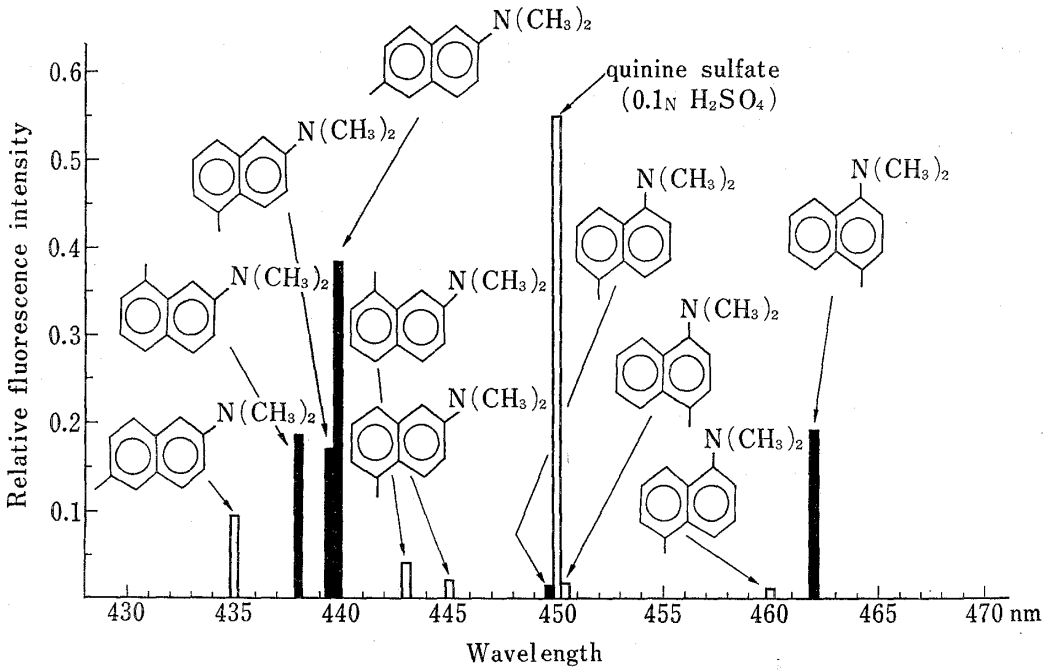


Fig. 13. Correlation of Relative Fluorescence Intensity and Emission Maxima of N-(Dimethylaminonaphthyl)succinimides and N-(Dimethylaminonaphthyl)-succinamic Acids in 0.1 M Phosphate Buffer at pH 7.0

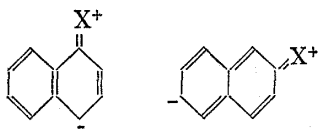
□: succinimide ■: succinamic acid

solutions in the procedures of their practical application. Because of very low quantum yields of these compounds in aqueous media, relative fluorescence intensities are shown in Fig. 13 for convenient comparison of them from each other. Comparison of their relative intensities was made by exciting at absorption maxima of the imides having the same optical density (OD) value with quinine sulfate as standard. The relative fluorescence intensities of the succinamic acid derivatives (**12**) were also included.

Discussion

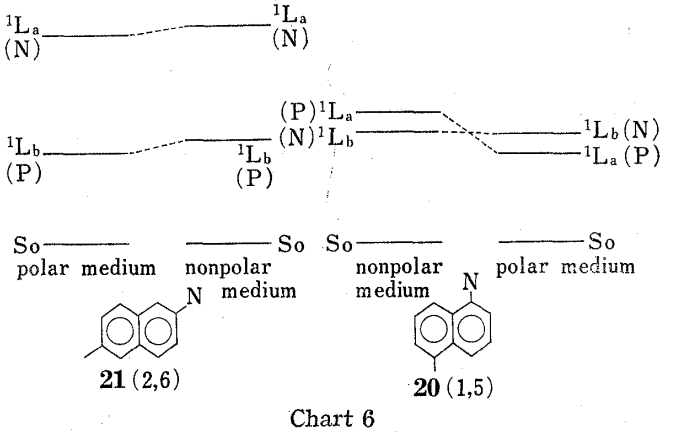
It is well known that naphthalene and its derivatives have three main bands in the ultraviolet region, and that these bands have been assigned to ^1L_b, ^1L_a, and ^1B_b, respectively[12] (Fig. 1). The ^1L_b and ^1B_b bands are associated with longitudinal polarization parallel to the long axis of the molecule, while the ^1L_a band is with transverse or perpendicular one to the long axis. The effects of the substituents on the absorption bands are generally explained by the direction in which the extending conjugation of the substituents effects. α-Substitution extends conjugation primarily in the short-axis direction (**18**), and hence should cause bathochromic and hyperchromic effects predominantly in the ^1L_a bands, while β-substitution extends it primarily to the long-axis direction (**19**) resulting in a red shift and intensifying in the ^1L_b band.[12] The ultraviolet absorption of the dimethylaminonaphthalenes and the methoxynaphthalenes, the fundamental units, agree properly with the above generalization, and those of the closely related compounds, naphthylamines and naphthols, analyzed by Baba and Suzuki.[13] Like the known spectra of naphthylamines,[13] in the spectrum of α-dimethylamino-naphthalene (Fig. 2) the ^1L_a and ^1L_b bands fuse (at around 310 nm) because of the large bathochromic displacement on the ^1L_a band due to the α-dimethylamino substituent. The β-dimethylamino group also causes the red-shift and intensification of the ^1L_b band (about 355 nm) but the ^1L_a band is hardly affected (Fig. 2). The spectra of α- and β-methoxynaphthalene show similar, but much smaller effects than with the dimethylamino substituent, in agreement with the behavior of naphthols.[13] From Fig. 3—6, it may be concluded that introduction of the maleimide and succinimide groups has no significant effect on the absorption spectra of the parent chromophores.

Since substitution in the aromatic ring shifts the wavelengths of fluorescence in accord with the effect of the same substituent on the absorption spectrum, the fluorescent spectra of the dimethylamino- and methoxy-substituted naphthalenes are in keeping with their absorption and the assignment of $^1L_b \rightarrow {}^1A$ transition. Correspondence of the fluorescent



18 **19**

Chart 5



21 (2,6) **20** (1,5)

Chart 6

12) H.H. Jaffe and M. Orchin, "Theory and Applications of Ultraviolet Spectroscopy," John Wiley and Sons, Inc., New York, 1962, p. 305.

13) *a*) H. Baba and S. Suzuki, *Bull. Chem. Soc. Japan*, **34**, 82 (1961); *b*) S. Suzuki, T. Fujii, and H. Baba, *J. Mol. Spectr.*, **47**, 243 (1973); *c*) S. Suzuki, *Bull. Res. Inst. App. Electr.*, **16**, 104 (1964).

excitation spectra and the absorption spectra is fairly satisfactory (Fig. 10 and 12). The methoxynaphthylsuccinimides(**17**)(in ethanol) had absorption in the 300—330 nm region with relatively smaller Stokes shift (20—60 nm), showing disadvantage to overlap with spectra of biopolymers. As a whole, on the other hand, the spectra of the dimethylaminonaphthylsuccinimides **13** showed larger substituent effect as well as Stokes shift (50—100 nm) giving rise to the fluorescence in the 410—440 nm region.

Regardless of detailed correlation of the energy and the position of substitution, the above spectral region of **13** (and **12**) is clear of the background emission of biopolymers. In Fig. 13, variation of the fluorescence maxima with change of the substituent from a succinimide group to a succinamic acid group seems rather complicated. It is known that the low energy excited states of aminonaphthalenes have considerable charge-transfer character[14] caused by mixing of the ^1L_a and ^1L_b states of naphthalene and the transition involving the lone pair electrons on the nitrogen. Although it is well known that fluorescent spectra of some anilinonaphthalene derivatives are highly solvent sensitive,[1,3,14] Hercules, *et al.* have very recently reported that in some dimethylaminonaphthalenesulfonates the emitting state is in fact solvent dependent.[15] Chart 6 summarizes solvent effects on the ^1L_a and ^1L_b states for 1-amino- and 2-amino-naphthalene derivatives. In 1-aminonaphthalenes (**20**) (1,5) the ^1L_a state is polar (P), and ^1L_b state is nonpolar (N) while, in 2-aminonaphthalenes (**21**) (2,6), the ^1L_b is polar and the ^1L_a is nonpolar. In nonpolar solvents fluorescence arises from the ^1L_b state of **20** (1,5) and, in polar solvents, from the ^1L_a state. In contrast, the emitting state for **21** (2,6) is ^1L_b in both solvents (Chart 6).[15] Such an inversion of the excited states may well occur in some of **12** and **13** to complicate interpretations of their spectral results.

Among the dimethylaminonaphthylimides (**11**) screened in the present work, **11** (1,4) **11** (1,5) and **11** (2,6) attracted our attention as the candidate reagents in view of the fluorescent properties of their models: *i.e.*, the red shift, **13** (1,4), **13** (1,5) and the intensity, **13** (2,6) (Fig. 11). Whereas synthesis of **11** (2,6) is difficult, **11** (1,4) is prepared very easily and, in addition, the hydrolyzed product (**12**) (1,4) has intense and red-shifted fluorescence (Fig. 13). Since addition products of thiols with a maleimide easily undergo hydrolytic ring opening.[16] **11** (1,4) may be used as a fluorescent thiol reagent.

In summary, systematic search for fluorogenic groups with simplest naphthalene derivatives showed that some of dimethylaminonaphthylmaleimides are candidates for the fluorescent thiol reagents. However an important direction, which has been previously unrecongnized, is suggested by this work. In order to develop a very useful reagent of this maleimide-type having excellent fluorescent properties as well as good solubility, it is desirable to have fluorogenic systems which, with a molecular size not larger than naphthalene, has more effective chromophore than at least a dimethylamino group. The conclusion obtained is applicable not only to the thiol reagents but also to generally designing fluorescent probes in biological studies. Studies for such relatively small-sized but efficient fluorogenic groups including coumarin derivatives[17] are currently in progress.

Experimental

Methods——All melting points were determined on a MP-1 melting point apparatus Yamato and uncorrected. Infrared (IR) and ultraviolet (UV) spectra were measured with a JASCO IR-S spectrophotometer, a Shimadzu double beam UV 200 spectrometer, and a Hitachi Recording spectrophotometer EPS 3T, respectively. Nuclear magnetic resonance (NMR) spectra were taken in $CDCl_3$ solution at 60 M Hz on a Hitachi H 60 spectrometer, with TMS as internal standard. The imides and the succinamic acids used for

14) C.J. Seliskar and L. Brand, *J. Am. Chem. Soc.*, **93**, 5405 (1971).
15) Y.-H. Li, L.-M. Chan, L. Tyer, R.T. Moody, C.M. Himel, and D.M. Hercules, *J. Am. Chem. Soc.*, **97**, 3118 (1975).
16) *a*) M.I. Machida, Thesis, Hokkaido University, 1973; *b*) M. Machida, M.I. Machida and Y. Kanaoka, in preparation.
17) M. Machida, N. Ushijima, M.I. Machida, and Y. Kanaoka, *Chem. Pharm. Bull.* (Tokyo), **23**, 1385 (1975).

spectroscopic measurement were analytically pure. All absorption and fluorescence spectra were measured in ethanol unless otherwise stated. Fluorescence spectra were measured with a Hitachi MPF-2A fluorescence spectrophotometer and uncorrected. The samples used for fluorescence studies have absorbances below 0.2 at the exciting wavelength. Quantum yields of the fluorescence spectra were obtained by the method described in a previous paper.[4]

Materials——N-(Naphthyl-1)succinimide, mp 155—157.5° (lit.,[18a] mp 152°), and N-(naphthyl-2)-succinimide, mp 186—188° (lit.,[18b] mp 183°), were prepared as described in literature. Dimethylaminonaphthalenes were obtained by methylation of naphthylamines: 1-dimethylaminonaphthalene, bp_{5-6} 120.5—121° (lit.,[19] bp_{12} 139—140°); 2-dimethylaminonaphthalene, bp_{16} 150—154° (lit.,[19] bp_{12} 160—161°). Naphthalene and methoxynaphthalenes were commercial products (Tokyo Kasei Co., Ltd.).

4-Dimethylamino-1-naphthylamine (1) (1,4) Dihydrochloride——(a) Sulfanilic acid was diazotised, coupled with **2a** in glacial acetic acid at 0° and resulting 4-*p*-sulfobenzeneazo-1-dimethylaminonaphthalene was reduced with stannous chloride in HCl[8a] to the diamine (**1**) (1,4). Recrystallization from EtOH–AcOEt gave pale yellow needles of mp 210—212°, yield 60%. **1** (1,4): *Anal.* Calcd. for $C_{12}H_{14}N_2 \cdot 2HCl$: C, 55.61; H, 6.22; N, 10.80. Found: C, 55.67; H, 6.34; N, 10.73. The N-acetyl derivative of **1** (1.4): mp 194.5—196.5° (lit.,[8a] mp 195°).

(b) **2b** was nitrated and the isolated **3a** (830 mg, 4 mmoles) was refluxed with dimethylamine hydrochloride (588 mg, 7.2 mmoles) and powdered $NaHCO_3$ (1 g, 12 mmoles) in pyridine for 3.5 hr. Recrystallization from EtOH afforded **4a** as yellow needles of mp 59—61° (lit.,[20] mp 64°). **1** (1,4) was obtained by catalytic hydrogenation of **4a** with Pt_2O in EtOH. After filtration of the catalyst dry HCl was bubbled into the solution, and the dihydrochloride of **1** (1,4) was recrystallized from EtOH–AcOEt, mp 211—213°.

1-Dimethylamino-5-naphthylamine (1)(1,5) Dihydrochloride——Urea (2.34 g, 34 mmoles) and **2a** (5.14 g, 30 mmoles) were dissolved in conc. H_2SO_4 (34 ml) and the ice-cooled solution was treated gradually with finely powdered KNO_3 (3.44 g) for 45 min and the mixture was further stirred for 1.5 hr at room temp. The mixture was poured on ice, neutralized with a NaOH solution and extracted with benzene, the organic phase was washed with water, dried over Na_2SO_4 and distilled *in vacuo*. **4b**, bp_3 160—165°, yield 64% (lit.,[20] bp_{14} 192—195°); picrate, mp 160—164° (lit.,[20] mp 164—166°). **4b** (4.32 g, 20 mmoles) in 20 ml of EtOH was hydrogenated in the presence of PtO_2 for 4 hr. After filtration of the catalyst the amine was converted to the dihydrochloride, which was recrystallized from MeOH–AcOEt to give **1** (1,5), mp 214—216° (decomp.), yield 70%. The hydrochloride of **1** (1,5): *Anal.* Calcd. for $C_{12}H_{14}N_2 \cdot 2HCl$: C, 55.61; H, 6.22; N, 10.80. Found: C, 55.67; H, 6.24; N, 10.99.

2-Dimethylamino-5-naphthylamine (1) (2,5) and 2-Dimethylamino-8-naphthylamine (1) (2,8) from 2-Dimethylaminonaphthalene (2c)——**2c** (6.84 g) was dissolved in 45 ml of conc. H_2SO_4. To this solution was added 4.76 g of finely powdered KNO_3 in a period of 1.5 hr with stirring and cooling. The reactant was poured on ice, neutralized with dil. NaOH and extracted with benzene, the organic layer was washed with water, dried over Na_2SO_4 and removed *in vacuo*. The reddish oily residue was purified twice by alumina column chromatography eluting with benzene–*n*-hexane (1: 3). The mixture of **5a** and **5b** thus obtained was recrystallized from EtOH to afford reddish needles, mp 45—47, yield 53%. This mixture was hydrogenated in the presence of Pt_2O for 15 hr as described above. The dihydrochlorides of the diamine (**1** (2,5) and **1** (2,8)), mp 210—220°; yield, 75%. To an aqueous solution of the dihydrochlorides (791 mg, 3 mmoles) were added CH_2Cl_2 (10 ml). K_2CO_3 (1.49 g, 10.8 mmoles) and benzoyl chloride (508 mg, 3.6 mmoles) with stirring. The pale greenish product immediately precipitated was collected, washed with aq. $NaHCO_3$ solution, water and dried. Recrystallization from acetone gave green needles, mp 233—235°; yield, 42%. The filtrate was further treated with 25 mg of benzoyl chloride for 4 hr. The white precipitate was collected and recrystallized from MeOH to give colorless needles, mp 238—240°; yield, 17%. The N-benzoyl derivative of mp 233—235° (290 mg, 1 mmole) was refluxed with 3 ml of 18% HCl in an oil bath for 2 hr. After cooling and filtration, the acid solution was evaporated to dryness *in vacuo*, and the solid mass was recrystallized from MeOH–AcOEt to give the dihydrochloride of **1** (2,8) as colorless needles of mp 222—224° (decomp); yield, 89%. *Anal.* Calcd. for $C_{12}H_{14}N_2 \cdot 2HCl$: C, 55.61; H, 6.22; N, 10.80. Found: C, 55.35; H, 6.26; N, 10.79. The N-acetyl derivative of **1** (2,8) recrystallized from benzene–*n*-hexane formed colorless needles of mp 160—162°. *Anal.* Calcd. For $C_{14}H_{16}ON_2$: C, 73.65; H, 7.06; N, 12.27. Found: C, 73.50; H, 6.97; N, 12.58. The N-benzoyl derivative of mp 238—240° was hydrolyzed with 18% HCl for 5 hr as above. Recrystallization from MeOH–AcOEt gave the dihydrochloride of **1** (2.5) as colorless needles of mp 219—222° (decomp), 77%. *Anal.* Calcd. for $C_{12}H_{14}N_2 \cdot 2HCl$: C, 55.61; H, 6.22; N, 10.80. Found: C, 55.45; H, 6.23; N, 10.75. The N-acetyl derivative of **1** (2,5) was recrystallized from AcOEt–*n*-hexane. *Anal.* Calcd. for $C_{14}H_{16}ON_2$: C, 73.65; H, 7.06; N, 12.27. Found: C, 73.43; H, 7.02; N, 12.31.

Nitration of N-Phthaloyl-2-naphthylamine (2d)——**2d** was added portionwise to nitric acid maintained below 15°.[8b] The mixture of nitro-N-phthaloyl-2-naphthylamines thus obtained were treated with hydrazine

18) *a*) R. Meyer, *Ann.*, **347**, 29 (1906); *b*) G. Koller, *Chem. Ber.*, **37**, 1599 (1904).
19) S. Hüning, *Chem. Ber.*, **85**, 1056 (1952).
20) V. Veselý and A. Vojtěch, *Coll. Czech. Chem. Comm.*, **1**, 104 (1929).

hydrate, and the reaction mixture was passed through a column of silica gel. Elution with benzene–AcOEt (5: 1) gave first the fractions containing 8-nitro-2-naphthylamine (**6b**), which forms reddish needles of mp 102—104° (lit.,[11b] mp 103.5°) from benzene (22%), and the fractions which afforded 5-nitro-2-naphthylamine **6a**, reddish orange needles of mp 128—134° (lit.,[10,8b] mp 143—144°) from benzene (8%).

Nitration of N-Acetyl-2-naphthylamine (2e)——The procedure given in "Organic Syntheses"[11c] was employed for the preparation of nitro-2-acetylaminonaphthalenes. After separation of 1-nitro-2-acetylaminonaphthalene, the residue was refluxed with 10% HCl and EtOH for 1.5 hr, neutralized with Na_2CO_3 and extracted with ether. The extract was dried over K_2CO_3 and evaporated *in vacuo*. The 6-nitro- and 8-nitronaphthylamines were purified through a silica gel column by elution with benzene–AcOEt (3: 2) to give **6c** as gold plates of mp 205—208° (lit.,[11b] mp 203°) and **6b** as dark reddish needles of mp 101—104° (lit.,[11b] mp 103.5°). 8-Nitro-2-naphthylamine (**6b**) was identified with the compound of mp 102—104° obtained by the hydrazinolysis from the nitro-N-phthaloyl derivatives by comparison of their IR spectra.

Methylation of Nitro-2-naphthylamines (6):——(a) **6a** was methylated with dimethyl sulfate at 160°.[20] Heating was carried out very gently until the oil bath temperature reached to 160°. The reaction mixture was then poured into water, neutralized with $NaHCO_3$ and extracted with benzene. The crude product obtained after removing the solvent *in vacuo* was submitted to column chromatography using silica gel. Recrystallization from EtOH–*n*-hexane gave 2-dimethylamino-5-nitronaphthalene (**5a**) as reddish needles of mp 68—70.5° (lit.,[20] mp 74°). (b) 2-Dimethylamino-8-nitronaphthalene (**5b**) was prepared from **6b** in a similar manner. Recrystallization from hexane gave **5b** as dark reddish needles of mp 75—78° (lit.,[20] mp 77). (c) The methylation of **6c** was carried out similarly. Recrystallization from benzene gave **5c** as reddish prisms of mp 162—164° (lit.,[20] mp 164°).

Hydrogenation of the Nitro Compounds 5: (a) **2-Dimethylamino-5-naphthylamine (1 (2,5)**; (b) **2-Dimethylamino-8-naphthylamine 1 (2,8)**; (c) **2-Dimethylamino-6-naphthylamine 1 (2,6)**——(a) **5a** was hydrogenated in ethanol with PtO_2 as catalyst. **1** (2,5) thus obtained was purified as the dihydrochloride, IR spectrum of which was identical with that of the diamine dihydrochloride obtained by hydrolysis from the benzoyl derivative of mp 238—240°. (b) **5b** was hydrogenated to give **1** (2,8) which was purified as the dihydrochloride of mp 222—223° (decomp.) from EtOH–AcOEt, 72%. Its IR spectrum was identical with that of diamine dihydrochloride obtained by hydrolysis from the benzoyl derivative of mp 233—235°. (c) **5c** was hydrogenated to give **1** (2,6), which was purified as the dihydrochloride of mp 245.5—247.5° from MeOH. The N-acetyl derivative of **1** (2,6): mp 179—179.5°. *Anal.* Calcd. for $C_{14}H_{16}ON_2$: C, 73.65; H, 7.06; N, 12.27. Found: C, 73.55; H, 7.10; N, 12.16.

4-Methoxy-1-naphthylamine 9(1,4)——Prepared by adaptation of the described procedure[21] from **8a**. The hydrochloride of **9** (1,4) on recrystallization from EtOH gave colorless needles of mp 268—275° (decomp.) (lit.,[21b] mp 278—279° (decomp.)).

5-Methoxy-1-naphthylamine 9(1,5)——Prepared from **8b** as above. Colorless needles from benzene–*n*-hexane, mp 78—79.5° (lit.,[22] mp 80°).

7-Methoxy-1-naphthylamine 9(2,8)——Prepared[23] from 7-hydroxy-1-naphthylamine. Recrystallization from ligroin gave plates of mp 78—79° (lit.,[23] mp 79—80°).

Maleamic Acids (10 and 14) (Table I)——General Procedure: To an ice cooled solution of maleic anhydride in THF is added a solution of equimolar amount of an amine (**1** and **9**) in ether. After the reaction mixture was stood overnight, the precipitated maleamic acid was collected and washed with THF. The crude acid was used for cyclization without purification.

Succinamic Acids (12 and 16) (Table II)——General Procedure: The succinamic acids are prepared as in the case of the maleamic acids. The reaction mixture is stood overnight, the solvent is removed *in vacuo*, and the residual acid was used for the cyclization. The mp of these succinamic acids are listed in Table II.

Imide Cyclization: Synthesis of the Maleimides (11 and 15) and the Succinimides (13 and 17) (Table II)—General Procedure: The mixture of an acid (1 mmole), acetic anhydride (3 mmole) and anhydrous NaOAc (0.3 mmole) is heated on an oil bath at 90° for 15—30 min until a clear solution results. After cooling ice water is added and the reactant is neutralized with $NaHCO_3$ and extracted with AcOEt. The extract is washed with sat. aq. NaCl, dried over Na_2SO_4 and evaporated *in vacuo*. The maleimides are purified through a silica gel column, while the succinimides are purified by recrystallization.

NMR Data of the Imides (Table III)——NMR data of the maleimides and the succinimides ($N(CH_3)_2$, OCH_3, vinyl protons of the maleimide ring, methylene protons of the succinimides) are summarized in Table III.

Acknowledgements We are grateful to Professor Hiroaki Baba for kind advice. This work was supported in part by grants from Tanabe Amino Acid Research Foundation, the Matsunaga Science Foundation, and the Ministry of Education, Science and Culture.

21) *a*) J. Lockett and W.F. Short, *J. Chem. Soc.*, **1939**, 787; *b*) G.B. Bachman and J.W. Wetzel, *J. Org. Org. Chem.*, **11**, 454 (1946).
22) H.J. Teuber and H. Lindner, *Chem. Ber.*, **92**, 927 (1959).
23) H.G. Fierz-David, J. Blangey, and W. von Krannichfeldt, *Helv. Chim. Acta*, **30**, 816 (1947).