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Studies on Benzothiazole Derivatives as Chelating Agents. V.[1] Fluorescence of Benzothiazole Derivatives and Their Zinc Chelates

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The relationship between the structure and the fluorescence of the benzothiazole derivatives and their metal chelates were investigated along with characteristics necessary for the fluorescence. By the chelation with zinc, N-salicylidene-4-aminobenzothiazole is held in plane and its electronic state was scarcely influenced, which induce fluorescence. In the fluorescent zinc chelates, the π electron distribution inclines towards the phenol portion from the thiazole portion in the transition from the ground state to the first excited state and the electron densities of the coordinate atoms to the central zinc ion do not almost change between both states. The fluorescence of the zinc chelates is due to π^*-π transition. The effect of additional substituent groups on the fluorescence was also examined along with the correlation between the stability constant and the fluorescence intensity of the metal chelates.

Keywords——benzothiazole derivatives; chelating agent; fluorescence and structure; π electron distribution; zinc chelate

A number of 2-, 4- and 7-substituted benzothiazole derivatives, shown in Table I, were synthesized to inquire into the relationship between the fluorescence of metal chelates and their structures, and to investigate their applications as analytical reagents.[3] Their acid-base equilibria and reactions with metal ions were examined potentiometrically[4] and the structures of their metal chelates both in the solid state and in solutions were reported in the preceding paper.[1]

The purpose of this work is to determine some of the characteristics necessary for fluorescence in the metal chelates of the benzothiazole derivatives, and to clarify some of the relationship between the fluorescence and the structures of reagents and their metal chelates.

Experimental

Syntheses of Reagents——N-(2-Hydroxy-5-chlorobenzylidene)-4-aminobenzothiazole[NS4ABT-5Cl]: To an ethanol solution of 4-aminobenzothiazole (0.7 g), was added an ethanol solution of an equivalent amount of 2-hydroxy-5-chlorobenzaldehyde. The mixture was refluxed for 1 hour on a water bath, cooled and the crystals were filtered. Recrystallization from ethanol gave 0.85 g of NS4ABT-5Cl as yellow needles, mp 168—169°. *Anal.* Calcd. for $C_{14}H_9ClN_2OS$: C, 58.23; H, 2.98. Found: C, 58.49; H, 3.14.

N-(2,5-Dihydroxybenzylidene)-4-aminobenzothiazole[NS4ABT-5OH]: 4-Aminobenzothiazole (0.5 g) and 2,5-dihydroxybenzaldehyde (0.65 g) were treated in the same procedure as the preparation of NS4ABT-5Cl. Yellow powder. 0.6 g. mp 218—219°. *Anal.* Calcd. for $C_{14}H_{10}N_2O_2S$: C, 62.21; H, 3.73. Found: C, 62.49; H, 3.61.

N-(4-Chlorobenzylidene)-4-aminobenzothiazole[N(ClB)4ABT]: 4-Aminobenzothiazole (0.5 g) and *p*-chlorobenzaldehyde (0.47 g) were treated in the same procedure as the preparation of NS4ABT-5Cl. Yellowish white powder. 0.5 g. mp 122—124°. *Anal.* Calcd. for $C_{14}H_9N_2SCl$: C, 61.65; H, 3.33. Found: C, 61.36; H, 3.19.

The syntheses of the benzothiazole derivatives listed with the abbreviations in Table I were described in the previous paper.[3]

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[1] Part IV: N. Shimidzu and T. Uno, *Chem. Pharm. Bull.* (Tokyo), **26**, 127 (1978).

[2] Location: *Yoshidashimoadachi-cho, Sakyo-ku, Kyoto.*

[3] N. Shimidzu and T. Uno, *Chem. Pharm. Bull.* (Tokyo), **21**, 184 (1973).

[4] N. Shimidzu and T. Uno, *Chem. Pharm. Bull.* (Tokyo), **25**, 2942 (1977).

Apparatus——The absorption spectra were obtained using a Hitachi recording spectrophotometer model EPS-2U. Fluorescence measurements were carried out with a Shimadzu Fluorospectrophotometer type GF-16 equipped with a Xenon arc lamp (Ushio Co. UXL-500 W) using 10 mm square quartz cells. The dissociation constants of the ligands and the stability constants of their metal chelates were determined potentiometrically. The details of the experimental conditions and the method were described previously.[4]

Extended Hückel Method——The atomic coordinates of the crystal structures of 2-methylaminobenzothiazole[5] and 2-chloro-N-salicylideneaniline[6] determined by X-ray analysis were used in calculation as the atomic coordinates of the benzothiazole portion and the other portion, respectively. The *d* electron of sulfur of benzothiazole was ignored in calculation.

Modified Hückel LCAO-MO Method (ω-Technique)——Parameters of hetero atoms and substituent groups, summarized by Yonezawa *et al.*,[7] were used in calculation. The value, 4.42, of coulomb integral of zinc was estimated from the ionization potential and the value of exchange integrals for the metal-oxygen or metal-nitrogen bond was 0.2—0.3. The inductive parameter was set equal to 1/10 for adjacent atoms. The calculation was repeated until the self-consistent charge distribution was obtained by the method of Ettinger.[8]

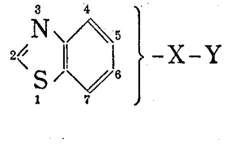
Results and Discussion

Although many factors affect the fluorescence of metal chelates, it is evident that rigid and planar structures are conductive to fluorescence production. The compounds shown in Table I have in common a single axis, X, about which two major portions of the molecule can rotate to some degree. The chelate formation of these compounds with metal ions may prohibit the internal rotation and induce rigidity into the overall molecule to promote fluorescence.

Table I. Benzothiazole Derivatives and Their Abbreviations

Compound	Abbreviation	X[a]	Y[a]
N-Salicylidene-2-aminobenzothiazole	NS2ABT	–N=CH–	C_6H_5OH
2-(*o*-Hydroxybenzyl)-aminobenzothiazole	2(HB)ABT	$-NHCH_2-$	C_6H_5OH
2-(2-Benzothiazolylazo)-4-methoxyphenol	2(2BTA)4MePh	–N=N–	$C_6H_4OHOCH_3$
2-(2-Benzothiazolylazo)-4-chlorophenol	2(2BTA)4ChPh	–N=N–	C_6H_4OHCl
1-(2-Benzothiazolylazo)-2-naphthol	1(2BTA)2NT	–N=N–	$C_{10}H_6OH$
2-(*o*-Hydroxyphenylimino)-methylbenzothiazole	2(HPI)MBT	–CH=N–	C_6H_5OH
2-(*o*-Hydroxyanilino)-methylbenzothiazole	2(HA)MBT	$-CH_2NH-$	C_6H_5OH
N-Salicylidene-4-aminobenzothiazole	NS4ABT	–N=CH–	C_6H_5OH
4-(*o*-Hydroxybenzyl)-aminobenzothiazole	4(HB)ABT	$-NHCH_2-$	C_6H_5OH
2-(4-Benzothiazolylazo)-4-methoxyphenol	2(4BTA)4MePh	–N=N–	$C_6H_4OHOCH_3$
1-(4-Benzothiazolylazo)-2-naphthol	1(4BTA)2NT	–N=N–	$C_{10}H_6OH$
2-(7-Benzothiazolylazo)-4-methoxyphenol	2(7BTA)4MePh	–N=N–	$C_6H_4OHOCH_3$
1-(7-Benzothiazolylazo)-2-naphthol	1(7BTA)2NT	–N=N–	$C_{10}H_6OH$
N-Salicylidene-7-aminobenzothiazole	NS7ABT	–N=CH–	C_6H_5OH

a)



Previous investigations clarified that the 7-substituted benzothiazole derivatives and the azomethine compounds derived from 2-aminobenzothiazole are poor in the reactivity with metal ions because of the steric hindrance.[3,4] However, many of the 2- and 4-substituted benzothiazole derivatives behave as planar terdentate chelating agents with divalent metal

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5) M. Fehlmann, *Acta Cryst.*, **1970**, B26, 1736.

6) J. Bregman, L. Leiserowitz, and K. Osaki, *J. Chem. Soc.*, **1964**, 2086.

7) T. Yonezawa, T. Nagata, H. Kato, A. Imamura, and K. Morokuma, "Introduction to Quantum Chemistry," Vol. 1, Kagakudojin Co., Kyoto, 1963.

8) R. Ettinger, *Tetrahedron*, **20**, 1579 (1964).

ions.[1] Table II shows the fluorescence of the benzothiazole derivatives and their metal chelates. The 2-substituted benzothiazole derivatives do not form fluorescent complexes with metal ions, while some of the 4-substituted form fluorescent complexes. For instance 2(HPI)MBT, an azomethine compound of the 2-substituted, exhibits fluorescence and its metal chelates do not, while NS4ABT, an azomethine compound of the 4-substituted, does not fluoresce and some of its metal chelates fluoresce. Especially, the fluorescence of the zinc chelate is very strong and NS4ABT is a high sensitive reagent for the fluorometric determination of zinc.[9] The structures of these compounds are shown in Fig. 1. Although the possibility of the rotation about the C(12)—C(13) bond and the N(15)—C(16) bond is present in NS4ABT, the infrared spectrum of NS4ABT suggested that the strong hydrogen bond exists between the N(15) and the phenol hydrogen.[1] Therefore, the rotation was considered to occur only about N(15)—C(16) axis. Accordingly, the total electron energies of NS4ABT were calculated by extended Hückel method in cases that NS4ABT is in plane as seen in Fig. 1 and that the plane of the benzothiazole portion rotates 90° and 180° about the N(15)—C(16) bond against the plane of the phenol portion. Similarly the total electron energies of 2(HPI)MBT were also calculated in cases that 2(HPI)MBT is in plane as seen in Fig. 1 and that the plane of the benzothiazole portion rotates 90° and 180° about the C(11)—C(14) bond against the plane of the phenol portion because of the hydrogen bond between the N(16) and the phenol hydrogen being suggested in the infrared spectrum. The results are as follows—2(HPI)MBT: plane, −1574.664581 eV; 90°, −1574.552155 eV; 180°, −1574.701782 eV and NS4ABT: plane, −1578.936462 eV; 90°, −1579.258636 eV; 180°, −1577.338501 eV. These results suggest that 2(HPI)MBT is the most stable in the plane structure as well as NS4ABT in 90°. The steric hindrance may exist between the H(14) and N(27) in the 180° structure of NS4ABT. Figures 2 and 3 show the absorption spectra of 2(HPI)MBT and NS4ABT along with those of their zinc chelates, respectively. The absorption spectrum of 2(HPI)MBT changes greatly in both ultraviolet and visible regions on chelating with zinc ion, while the absorption spectrum of NS4ABT no changes in the ultraviolet region on chelating with zinc ion. This spectral change of 2(HPI)MBT in the ultraviolet region suggests that the appreciable interaction between 2(HPI)MBT and zinc exists in its zinc chelate. On the basis of these results the following explanation may be given. In the absence of metal ions 2(HPI)MBT may be in plane and exhibits a fluorescence, while NS4ABT may be not in plane when it does not chelate

TABLE II. Fluorescence of Benzothiazole Derivatives and Their Metal Chelates

Compound	Ligand	Metal chelate
NS2ABT	Non-fluorescence	
2(HB)ABT	Non-fluorescence	
2(2BTA)4MePh	Non-fluorescence	Non-fluorescence
2(2BTA)4ChPh	Non-fluorescence	Non-fluorescence
1(2BTA)2NT	Non-fluorescence	Non-fluorescence
2(HPI)MBT	Fluorescence	Non-fluorescence
2(HA)MBT	Non-fluorescence	Non-fluorescence
NS4ABT	Very weak fluorescence	Zn very strong Cd very strong Mg strong
4(HB)ABT	Very weak fluorescence	
2(4BTA)4MePh	Non-fluorescence	Non-fluorescence
1(4BTA)2NT	Non-fluorescence	Al weak
2(7BTA)4MePh	Non-fluorescence	
1(7BTA)2NT	Non-fluorescence	
NS7ABT	Non-fluorescence	

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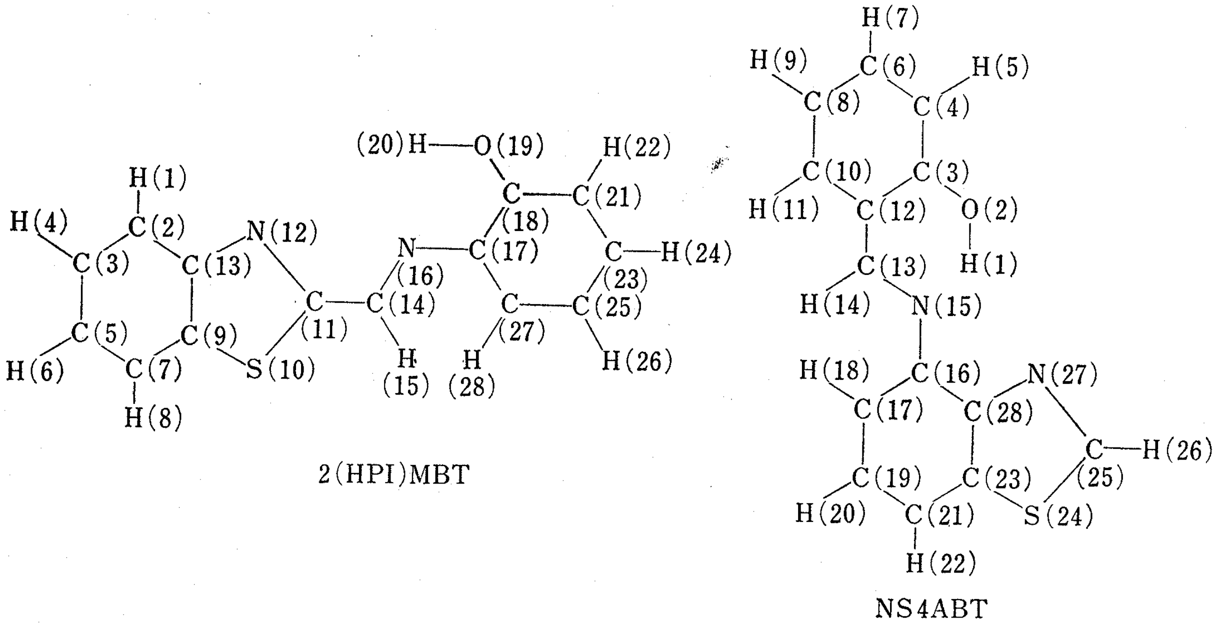
[9] N. Shimidzu and T. Uno, *Chem. Pharm. Bull.* (Tokyo), 21, 762 (1973).

with metal ions and scarcely fluoresce. Both 2(HPI)MBT and NS4ABT act as a terdentate ligand with zinc and their zinc chelates have an octahedron structure. In this structure the ligands may have a plane structure, which may be favorable for fluorescence. However, in the zinc chelate of 2(HPI)MBT the electronic state of the ligand may be influenced by the appreciable interaction between the ligand and zinc, and the zinc chelate does not exhibit a fluorescence. In contrast, the electronic state of NS4ABT may not be influenced by the chelation with zinc. In addition, NS4ABT is fixed in plane by the chelation with zinc. Therefore, the zinc chelate of NS4ABT may exhibit a strong fluorescence.



2(HPI)MBT

NS4ABT

Fig. 1. Structures of 2(HPI)MBT and NS4ABT

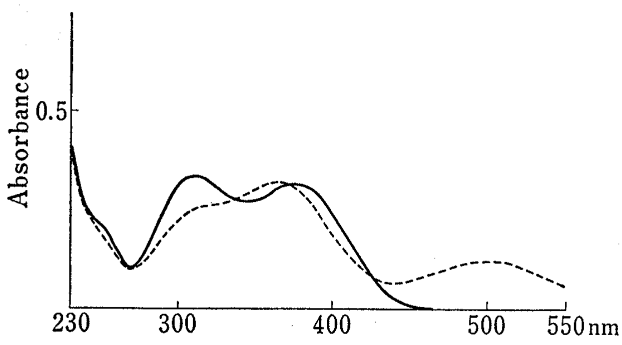


Fig. 2. Absorption Spectra of 2(HPI)MBT (Solid Line) and Its Zinc Chelate (Dotted Line) in 90% Ethanol

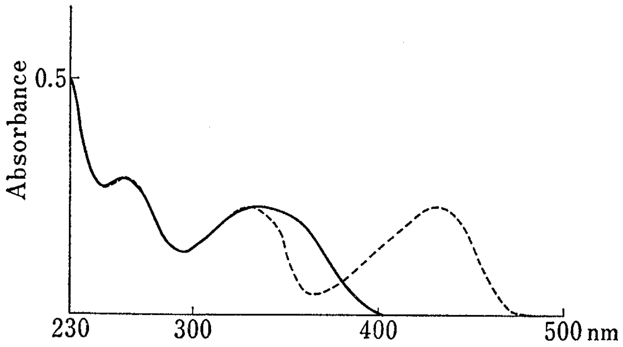


Fig. 3. Absorption Spectra of NS4ABT (Solid Line) and Its Zinc Chelate (Dotted Line) in 90% Ethanol

It is of importance from analytical point of view to inquire into the influence of the addition of substituent groups to the ligand on the fluorescence of its metal chelates. A few examinations of this subject have been reported,$^{10)}$ but any general regularity has not been observed because of the insufficiency of the data. Additional substituent groups were introduced into NS4ABT which exhibits the strongest fluorescence on chelating with metal ions and the effects on the fluorescence was examined; an electron-withdrawing hydroxyl

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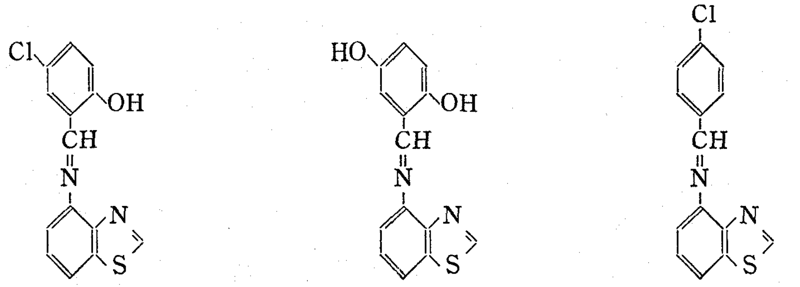
10) R.J. Argauer and C.E. White, *Anal. Chem.* **36**, 2141 (1964); K. Hiraki, *Bull. Chem. Soc. Japan*, **46**, 2438 (1973).

group or an electron-donating chloro group was introduced at the *para* position of the hydroxyl group of NS4ABT and N(ClB)4ABT, which has not the hydroxyl group, was synthesized in order to examine the contribution of the hydroxyl group in NS4ABT to the fluorescence of the metal chelates. The structures of the compounds synthesized are shown in Fig. 4. N(ClB)4ABT does not form fluorescent metal complexes and this result demonstrates the contribution of the hydroxyl group to the fluorescence. NS4ABT-5OH reacts with Zn^{2+} and Cd^{2+} to exhibit an orange fluorescence and NS4ABT-5Cl reacts with Zn^{2+}, Cd^{2+}, and Mg^{2+} to exhibit a green fluorescence. Table III shows the fluorescence behavior of these metal chelates. Compared with that of NS4ABT, the fluorescence intensity of NS4ABT-5Cl is approximately equal in the zinc chelate and somewhat weaker in the cadmium chelate. The emission maximal wavelength of the metal chelates of NS4ABT-5Cl is about the same as that of NS4ABT. In contrast, the fluorescence of the metal chelates of NS4ABT-5OH decreases and the emission maximum band is shifted to longer wavelength in comparison with that of NS4ABT. These results may be ultimately due to the effect of the substituent group upon the electronic state in the ligand.



NS4ABT-5Cl NS4ABT-5OH N(4ClB)4ABT

Fig. 4. Structures of NS4ABT Related Compounds

Table III. Excitation and Emission Band Maxima, and Fluorescence Intensity of NS4ABT and Its Related Compounds

Compound	Metal	λex. (nm)	λem. (nm)	Relative Intensity
NS4ABT		390	445	
	Zn^{2+}	428	510	75
	Cd^{2+}	428	510	25
	Mg^{2+}	380	510	12
NS4ABT-5Cl		420	520	
	Zn^{2+}	450	515	85
	Cd^{2+}	440	520	9.6
NS4ABT-5OH		430	570	
	Zn^{2+}	465	570	2.27
	Cd^{2+}	460	570	0.27

Z. Holzbecher pointed out a direct proportionality between the fluorescence intensities and the stability constants of the metal salts of the salicylaldehyde condensation products.[11] Table IV shows the stability constants obtained potentiometrically and the fluorescence intensities of the zinc and cadmium chelates of NS4ABT and NS4ABT-5Cl. The measurement of the stability constants of NS4ABT-5OH metal chelate is unsuccessful because of the slight solubility. Clear accounts can not be given for this problem because of the insufficiency of the data, but a proportionality may not exist exclusively between the fluorescence intensity

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11) Z. Holzbecher, *Collection Czech. Chem. Commun.*, **24**, 3915 (1954).

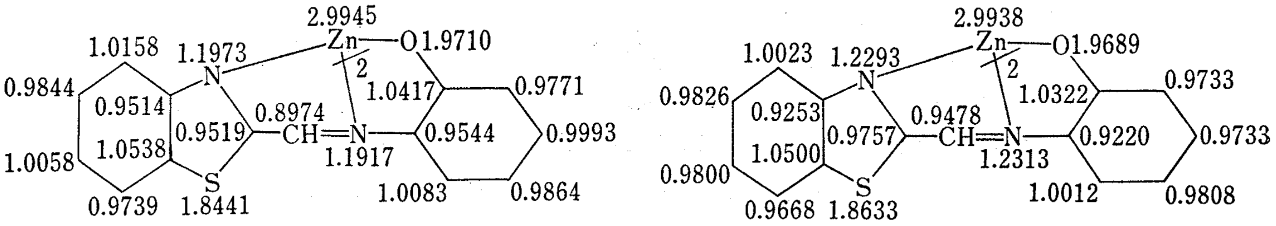
and the stability constant. The interaction between metals and ligands may be significant. In both cases that the electronic state of ligands does not almost influenced by the chelation with metal ions and that the influence is similar among the metal chelates, the proportionality may be expected.

Table IV. Fluorescence Intensity and Formation Constants of Metal Chelates

Compound	Zn^{2+}		F.I.[a]	Cd^{2+}		F.I.[a]
	$\log K_{ML}$	$\log K_{ML_2}$		$\log K_{ML}$	$\log K_{ML_2}$	
NS4ABT ($pK_a=12.2$)	7.7	6.7	75	6.4	5.0	25
NS4ABT-5Cl ($pK_a=10.62$)	6.1	6.8	85	5.4	5.3	9.6

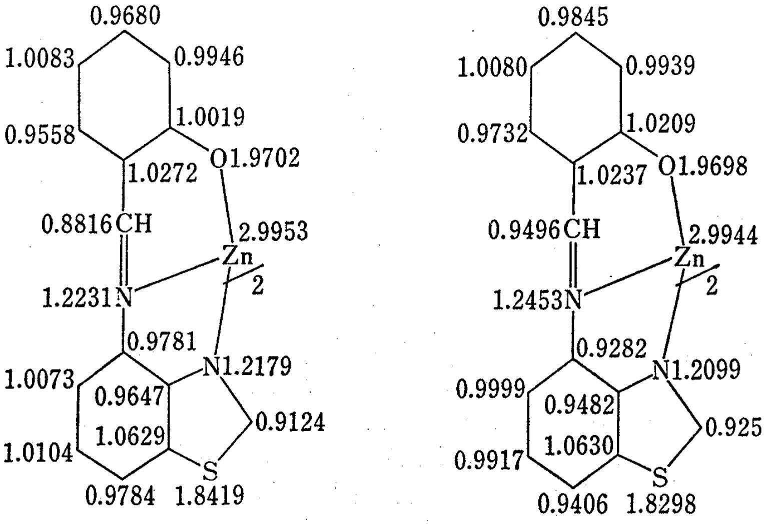
[a] Fluorescence intensity.

Fluorescence is emission process which commonly occurs between the first excited singlet and the ground state singlet. The π electron distributions of the zinc chelates of the benzothiazole derivatives were calculated at the ground and the first excited state by modified Hückel LCAO-MO method. All of the ligands examined act as terdentate ligand with zinc to form the 2: 1 ligand-to-metal chelates of octahedron structures in which the ligand may be in plane.



ground state first excited state

Non-fluorescent zinc chelate of 2(HPI)MBT



ground state first excited state

Fluorescent zinc chelate of NS4ABT

Fig. 5. The π-Electron Distribution in Non-fluorescent and Fluorescent Zinc Chelates

Therefore, the calculation was made for the zinc chelates which have the structures described above. As an example of the results Fig. 5 shows the total π electron densities of the non-fluorescent and the fluorescent zinc chelate at the ground and the first excited state. Table V shows the change of the electron density of each portion of the zinc chelate between the ground and the first excited state. The results shown in Table V may suggest the fact described below. In the fluorescent zinc chelates of NS4ABT and its related compounds, the sum of the total π electron densities of the benzothiazole portion tends to decrease and that of the phenol portion tends to increase slightly at the first excited state than at the ground state. In the non-fluorescent zinc chelates, the sum of the total π electron densities of the benzothiazole portion, in contrast, tends to increase and that of the phenol or naphthol portion tends to decrease at the first excited state than at the ground state. In other words, the π electron distribution in the fluorescent zinc chelates inclines towards the phenol portion from the benzothiazole portion in the transition from the ground state to the first excited state. On the other hand, the π electron distribution in the non-fluorescent zinc chelates towards the benzothiazole portion from the phenol or naphthol portion in the transition from the ground to the first excited state. The results shown in Table V may also suggest the following fact. The π electron densities of the coordinate atoms to the central zinc ion increase in all of the zinc chelates at the first excited state than at the ground state, but in the fluorescent zinc

TABLE V. The Change of π-Electron Density between the Ground State and First Excited State by Modified Hückel MO Method

Compound		Zn		Benzothiazole		–N=N– or –CH=N–		Phenol or naphthol		Total of coordinate atoms	
			ES−GS		ES−GS		ES−GS		ES−GS		ES−GS
$Zn[2(HPI)MBT]_2$	GS[a]	5.9889	−0.0014	19.9568	−0.0062	4.1782	+0.1800	15.8764	−0.1730	8.7200	+0.1390
	ES[b]	5.9875		19.9506		4.3582		15.7034		8.8590	
$Zn[2(2BTA)\text{-}4MePh]_2$	GS	5.9885	+0.0006	19.9420	+0.3356	4.3256	+0.4198	19.7438	−0.7564	8.5944	+0.3914
	ES	5.9891		20.2776		4.7454		18.9874		8.9858	
$Zn[1(2BTA)2NT]_2$	GS	5.9884	0	19.9746	−0.0168	4.3566	+0.2698	23.6802	−0.2432	8.6154	+0.2166
	ES	5.9884		19.9578		4.6264		23.4270		8.8320	
$Zn(NS4ABT)_2$	GS	5.9905	−0.0017	19.9480	−0.2746	4.2094	+0.1804	15.8520	+0.0960	8.8224	+0.0276
	ES	5.9888		19.6734		4.3898		15.9480		8.8500	
$Zn(NS4ABT\text{–}5OH)_2$	GS	5.9905	−0.0014	19.9658	−0.0188	4.1488	+0.1848	19.8954	−0.1650	8.4682	+0.0358
	ES	5.9891		19.9470		4.3336		19.7304		8.5040	
$Zn(NS4ABT\text{–}5Cl)_2$	GS	5.9905	−0.0016	19.9630	−0.1520	4.1432	+0.1278	19.9034	+0.0258	8.4660	+0.0168
	ES	5.9889		19.8110		4.2710		19.9292		8.4828	
$Zn[2(4BTA)\text{-}4MePh]_2$	GS	5.9899	−0.0001	19.7840	+0.3382	4.3544	+0.4058	19.8716	−0.7422	8.7118	+0.2022
	ES	5.9898		20.1222		4.7602		19.1294		8.9140	
$Zn[1(4BTA)2NT]_2$	GS	5.9900	+0.0003	19.8160	+0.1412	4.3866	+0.2573	23.8075	−0.3990	8.7185	+0.1367
	ES	5.9903		19.9572		4.6439		23.4085		8.8552	

a) Ground state. b) First excited state.

TABLE VI. Calculated and Observed Emission Maxima

Compound	ES[a)]–GS[b)]	λmax. (nm)	
		Calcd.	Observed
$Zn(NS4ABT)_2$	-0.8826β	498.06	505
$Zn(NS4ABT\text{–}5OH)_2$	-0.7523β	584.32	570
$Zn(NS4ABT\text{–}5Cl)_2$	-0.8732β	503.42	515

a) First excited state. b) Ground state.
Calculated as $\beta = -2.82$ eV.

chelate the increase is very slight. In other words, the electron densities of the coordinate atoms do not almost change in the fluorescent zinc chelates between the ground state and the first excited state.

Table VI shows the observed and the calculated emission maxima obtained from the energy difference between the first excited state and the ground state in the π-system of the zinc chelates. The calculated values agree with the observed values. This result suggests that the fluorescence of the zinc chelates of the benzothiazole derivatives is due to π^*-π transition.