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Fluorescence of 2-(Substituted anilino)benzoic Acids and Their Metal Chelate Compounds

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Abstract□ The substituent effects on the fluorescence of 2-(substituted anilino)benzoic acids and their aluminum chelate compounds were examined and satisfactory linear relationships between Hammett substituent constants, σ and the lowest excited singlet energy levels were obtained. But fluorescence intensity only made a qualitative relationship with σ values of substituent groups. Effects of solvents and metal ions on the native and metal chelate fluorescence of the above derivatives were also investigated.

Keywords□ 2-(Substituted anilino)benzoic acids, Hammett-type correlation, Fluorescence.

Derivatives of 2-anilinobenzoic acid, 2-(substituted anilino)benzoic acids, are well known as anti-inflammatory agents. Flufenamic acid, mefenamic acid and meclofenamic acid are now used widely in clinical field and others have been reported for having anti-inflammatory action on animal test.

These anti-inflammatory drugs have been determined by a variety of analytical techniques. The most commonly used are ultraviolet spectrometry[1] and gas chromagtography.[2,3]

Other methods include thin-layer chromatography,[4] acid-base titration[5] and high performance liquid chromatography.[6] There have been several studies of the fluorescence properties of 2-(substituted anilino)benzoic acids.

Dell *et al.*[7,8] have described a fluorometric assay after conversion to heterocyclic compounds and the solvent effect on the fluorescence. Metha and Schulman[9] found analytically useful native fluorescence and Strojny and de Silva[10] published a paper on the luminescence determination in plasma following seperation by thin-layer chromatography.

And recently Miller *et al.*[11] reported the fluorescence characteristics at low temperature.

The present paper describes the substituent effect on the fluorescence of 2-(substituted anilino)benzoic acids and their aluminum chelate compounds in terms of the correlation between Hammett substituent constant and the lowest excited singlet energy level. The effects of solvent on the fluorescence of both ligands and their aluminum chelate compounds and the fluorescence characteristics of compounds relating to 2-anilinobenzoic acid are also examined.

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EXPERIMENTAL

General

Fluorescence measurements were performed on a Shimatzu Model RF-501 Spectrofluorophotometer equipped with 15OW xenon lamp, R452 phototube and Varian 9176 Laboratory Strip Chart Recorder. Excitation and emission slits were set at 5 and 3 nm, respectively, and 10mm quartz cell was used.

The spectrofluorophotometer was standized with quinine bisulfate (1mcg/ml in 0.01N H_2SO_4, 50 relative fluorescence intensity units at excitation and emission wavelengths of 350 and 445 nm, respectively).

IR spectra were recorded on a Hitach-Perkin Elmer 264 and UV-Visible spectra were measured on a Pye-Unicam SP 8-100.

All the NMR spectra were taken on a Varian EM-360 with tetramethylsilane(TMS) as an internal standard. Chemical shifts are reported in parts per milion downfield from TMS.

Melting point determinations were made with a Thomas Unimelt type apparatus.

Elemental analysis were obtained on a Carlo Erba Strumentazione Model 1106 Elemental Analyzer.

Materials

All the solvents were fractionally distilled and only center cuts were collected, but chlorform and ethanol were adopted the HPLC grade (Merck).

Mefenamic acid (Asia Pharm.) was recrystallized from 80% ethanol. Quinine bisulfate (Sigma), benzoic acid(Tokyo Kasei), anthranilic acid(Metheson Coleman & Bell), and diphenylamine(Tokyo Kasei) were of reagent grade.

Flufenamic Acid [2-(3-trifluoromethyl anilino) benzoic Acid]

Dissolved 0.5g of aluminum flufenamate (Taisho Pharm.) into 3 ml of ethanol and stirred on the boiling water bath for 1 hour. Product then filtered and washed with d stilled water and recrystallized from 80% ethanol. Yellow crystalline powder, mp. 132°C, UV(Dioxane): λ_{max}=286($E_{1\%}$=575), 346(317), IR(KBr): 3320(-NH), 1652(COOH), 1330, 1252, 1215, H-NMR(DMSO-d_6): σ= 9.73(COOH), 8.1-6.6(H in pyridine/benzene), $C_{14}H_{10}F_3NO_2$ (281.2): Calculated: C:59.74, H:3.58, N:4.98, Found: C:59.01, H:3.35, N:4.71

2-(4-Methoxyanilino)benzoic Acid[12]

2.6g of 2-chlorobenzoic acid (Tokyo Kasei), 2.5g of *p*-anisidine(Yoneyama), 35ml of isoamyalcohol, 2.3g of potassium carbonate anhydrous and 0.03 g of copper dust were intimately mixed and refluxed at 130-140°C for 2 hours.

The product was then washed with isoamylalcohol and distilled water and recrystallized from 80% ethanol.

White crystalline powder, mp. 182°C. UV(Dioxane): λ_{max}=281($E_{1\%}$=565), 355 (320), IR(KBr): 3326(-NH), 1665(COOH), 1510, 1296, 1240, H-NMR(DMSO-d_6); σ= 9.15(COOH) 8.2-6.6(H in pyridine/benzene), 3.83(-OCH_3), $C_{14}H_{13}NO_3$(242.1): Calculated: 69.14, H:5.35, N:5.76, Found: C:70.61, H:5. 22,N:5.88

Aluminum Chelate Compounds of 2-(substituted anilino) benzoic Acids

15ml of 0.1M flufenamic acid in 1N sodium hydroxide was added into the 40 ml of aqueous 0.1M aluminum chloride solution while stirring continuously.

The pH of the solution was adjusted to 7.0–8.0 and then standing for one hour. Then, the product was filtered and dried in vacuum at 80°C for 5 hours.

For the same procedure aluminum chelate compounds of 2-anilinobenzoic acid, mefenamic acid and 2-(4-methoxyanilino) benzoic acid were prepared.

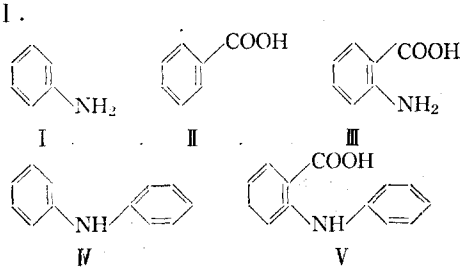
Metal Chelate Compounds of Flufenamic Acid

For the same procedure of the aluminum chelate compounds, four kinds of chelate compounds were prepared, with 10 ml of of 0.5M flufenamic acid in 1N sodium hydroxide and 40 ml of aqueous 0.5M metal salts solutions(zinc chloride, cadmium acetate, cobalt nitrate and copper acetate).

RESULTS AND DISCUSSION

Of the huge number of known organic compounds, only small fraction exhibits intense luminescence.

To figure out the possible fluorescence moiety, fluorescence characteristics of compounds relating to 2-anilinobenzoic acid in Scheme I were examined and shown in Table I.



Scheme I: The compounds relating to 2-anilinobenzoic acid.

Table I: Excitation and emission properties of compounds relating to 2-anilinobenzoic acid in dioxane.

Compounds	Ex-max. nm	Em-max. nm	Relative fluorescedce intensity
Benzoic acid	—	—	—
Aniline	296	331	7
2-Aminobenzoic acid	341	394	170
Diphenylamine	297	345	192
2-Anilinobenzoic acid	347	427	23

As shown in Table I, aniline[I] exhibited fluorescence but benzoic acid [II] did not.

Diphenylamine[IV] remained on the relatively same wavelengths of excitation and emission spectra but exhibited more intensive fluorescence than aniline[I] did.

On the contrary, 2-aminobenzoic acid[III] showing strong fluorescence produced shifts in both excitation and emission spectra to lower energy, on longer wavelength.

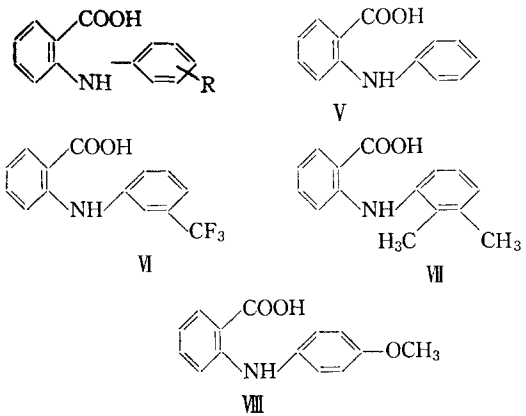
But in case of 2-anilinobenzoic acid[V], longer red shift was observed with decreasing fluorescence intensity.

No similiarity on fluorescence spectra and intensities was found among the compounds studied.

These might mean that no particular fluorescence moietiy is present on 2-(substituted anilino)benzoic acids, which probably have the lowest excited singlet of combined π, π^* configuration in measuring condition. It is also assumed that the carboxylic and amino groups are critical for having fluorescence.

2-(Substituted anilino)benzoic acids described in Scheme II were selected to

examine the substituent effects on the fluorescence phenomenon originated from 2-anilinobenzoic acid.



Scheme II: 2-(Substituted anilino)benzoic acids.

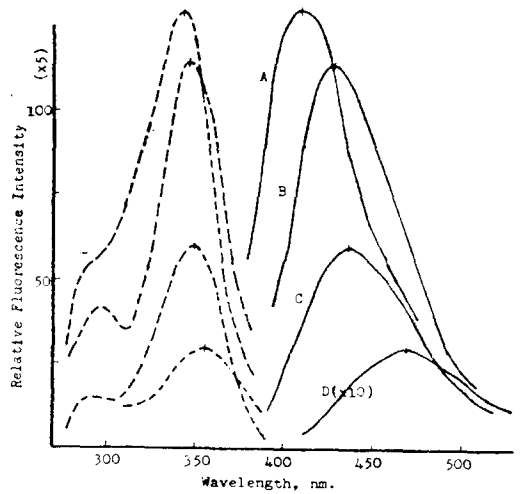


Fig. 1: Excitation (– –) and emission(—) spectra of 2-(substituted anilino)benzoic acids in dioxane.
Concentration; 10 mcg/ml.
A: Flufenamic acid, B: 2-Anilinobenzoic acid,
C: Mefenamic acid, D: 2-(4-Methoxyanilino) benzoic acid.

As shown in Fig. 1, each substituent groups made great influence on both excitation and emission spectra and the fluorescence intensities. The maximum excitation and emission wavelengths of 2-anilinobenzoic acid, flufenamic acid, mefenamic acid and 2-(4-methoxyanilino) benzoic acid were 347/427, 343/409, 348/434 and 356/468nm and their relative fluorescence intensities were 23, 26, 12 and 0.6, respectively. For the excitation spectra, the bathochromic shift was occured as decreasing the Hammett substituent constant of the respective substituent group of compounds examined, but no quantitative correlationship between them was found.

On the other hand, linear plots were obtained for maximum emission wavelength vs the Hammett substituent constant and shown in Fig. 2. Hammett substituent constant, for *m*-CF_3, H and *p*-OCH_3 were reported as 0.43, 0 and –0.268, respectively. But in case of mefenamic acid, the sum of σ of *o*-CH and *m*-CH , –0.21 was taken.[13-15]

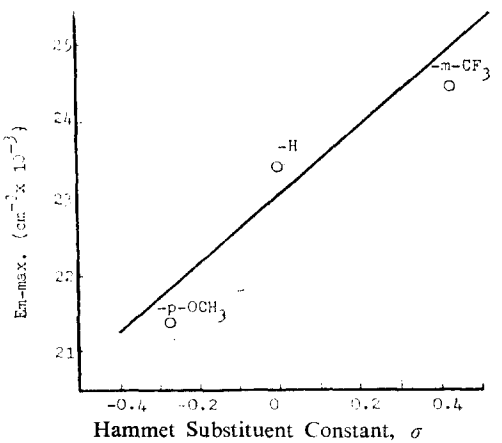


Fig. 2: Hammett plot for fluorescence emission maxima of 2-(substituted anilino) benzoic acids. Em-max($cm^{-1} \times 10^{-3}$)=4.21σ+22.85, n=3, r=0.895, s=0.701

The fluorescence intensities were increased by electron-withdrawing substituents and decreased by electron-donating ones, but were not correlated with the Hammett substituent constant of substituent groups discussed.

This result was agreed with the observation of Hirauchi and Amano[16] for substituent effect of 4'-substituted 4-nitrodiphenylamines, but was reverse of the generalized substituent effect of fluorescence intensity, which *ortho-para*-directing substituents, such as -OH, $-NH_2$ and $-OCH_3$, often enhance fluorescence or electron-donating groups do the same.[17~19]

The complexation of aromatic ligands with metal ion usually produces electronic spectral shifts and decreases or increases the fluorescence intensities of fluorescence molecules.

Especially the co-ordination of aromatic ligands by non-transition metal ions has the great effect on increasing the fluorescence intensity of ligands itself.

Aluminum chelate compounds of 2-(substituted anilino)benzoic acids were measured at the concentration of 1 mcg/ml in dioxane as shown in Table II.

The influence of the substituent groups on the maximum emission wavelength and fluorescence intensities were great, but a little effect were detected on the maximum excitation wavelength. This might mean that the effects of substituent group on the electronic state of ligands are also expanding to the aluminum chelate compounds in the same manner.

Table II: Effect of substituted groups on excitation and emission properties of aluminum-[2-(subsituted anilino)benzoic acids] in dioxane at the concentration of 1 mcg/ml.

Ligand	Ex-max. nm	Em-max. nm	Relative fluorescence intensity
2-Anilinobenzoic acid	366	458	47.3
Flufenamic acid	359	441	49.3
Mefenamic acid	360	455	25.0
2-(4-Methoxyanilino) benzoic acid	364	478	q16.3

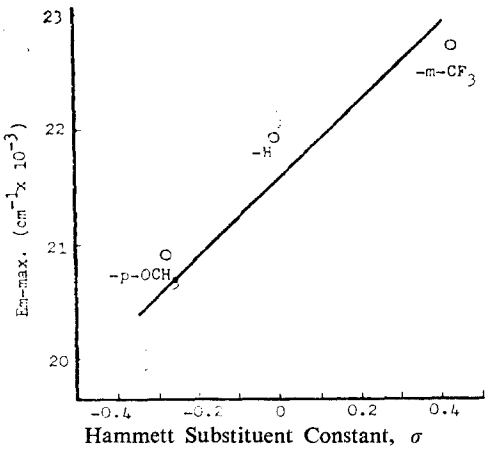


Fig. 3: Hammett plot for fluorescence emission maxima of aluminum-[2-(substituted anilino) benzoic acids] in dioxane.
Em-max($cm^1 \times 10^{-3}$)=2.47σ+21.67, n=3, r =0.972, s=0.190

As the ligands did, maximum emission wavelength of the aluminum chelate compounds studied made a linear relationship with the Hammett substituent constant of respective subitituent groups and shown in Fig. 3.

In the aluminum chelate fluorescence, the slope of the Hammett plot was lowered than that of ligands and could be explained as the decreasing of the effect of the substituent groups on the fluorescence spectra in chelate compounds.

As a results of Hammett type plots of both

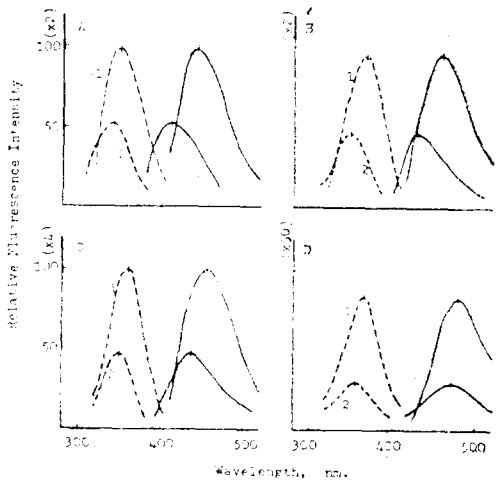


Fig. 4: Comparison of excitation (– –) and emission (—) spectra between native and aluminum chelate compounds of 2-(substituted anilino) benzoic acids in dioxane.
1; Aluminum chelate compounds, 1 mcg/ml, 2; Native compounds, 10 mcg/ml.
A; Flufenamic acid, B;2-Anilinobenzoic acid, C; Mefenamic acid, D; 2-(4-Methoxyanilino) benzoic acid.

ligands and their aluminum chelate compounds the exact significance of the Hammett-type correlations for excited state molecules cannot be speculated, but it should be emphasized that correlations between Hammett substituent constant values and excited state molecules seem to be possible.

Fig. 4 figures out the comparative data on the shift of both excitation and emission spectra and the fluorescence intensities between ligands and their aluminun chelate compounds.

The fluorescences of ligands and their aluminum chelate compounds were substantially different from one another.

With increasing Hammett the substituent constant of the substituent groups, the bathochromic shift of both excitation and emission spectra over the respective ligands increased.

But with decreasing the Hammett substituent constant, a little spectral shift was observed.

Moreover, for the fluorescence intensities of aluminum chelate compounds, stronger fluorescence intensities, increasing about over 20 times than that of ligands, were detected.

An explanation that why the aluminum chelates are more fluorescent than the ligands might be presented as follow; by forming chelates, the favored configuration on ceasing of rotation of which reduces an ideal situation for internal conversion could be achieved and the molecules might be the planar one in which stabilizing resonance can occur to fluoresce intensively.[20,21]

The influence of the metallic ion on the fluorescence characteristics of metal chelate compounds was observed and shown in Table Ⅲ.

Table III: Metallic ion effect on excitation and emission properties of metal chelate compounds of flufenamic acid in dioxane at the concentration of 1 mcg/ml.

Metal ion	Atomic number	Ex-max. nm	Em-max. nm	Relative fluorescence intensity
Al	13	359	441	49
Zn	30	343	409	3.8
Cd	48	343	408	3.1
Cu	29	343	408	2.7
Co	27	343	407	1.9

Among those metallic ions, except aluminum ion mentioned above, a change in the metallic ion has a little effect on the

Table IV: Solvent effect on excitation and emission properties on flufenamic acid at the concentration of 10 mcg/ml.

Solvent	Dielectric constant[a]	Ex-max. nm	Em-max. nm	Relative fluorescence intensity
Dioxane	2.21	343	409	26
Chloroform	4.81	348	424	25
Ethanol	24.55	348	436	1
Dimethyl-formamide	36.71	321	485	15

a) Data from Ref. 22.

positions of excitation and emission wavelengths but such change has a effect on the intensity of fluorescence because the metallic ions strongly effect the efficiency of the various pathways available for dissipating the excitation energy of the chelate.

Data shown in Table III also suggest that fluorescence intensity decreases with increasing atomic number of the non-transition metal ions(e.g. Al^{+3}, Zn^{+2}, Cd^{+2}) and non-transition metal chelates exhibit more intensive fluorescence than those of transition metal chelates (e.g. Cu^{+2}, Co^{+2}).[23,24]

Solvent effects on the fluorescence spectra and intensities are generally explained by the solvent-solute interaction; the general effect of the solvent in terms of polarity on the fluorescence spectra and intensity of flufenamic acid are shown in Table IV.

Emission spectra showed greater wavelenghs dependence on solvent polarity than did the excitation spectra, but solvent effects upon emission spectra were qualitatively similar to those upon excitation spectra.

The shift in both excitation and emission spectra to lower energy, or longer wavelength (red shift), was observed as the dielectric constant of the solvent increases.

Table V: Effect of solvents on exitation and emission properties of [aluminum-flufenamic acids] at the concentration of 1 mcg/ml.

Solvent	Dielectric constant[a]	Ex-max. nm	Em-max. nm	Relative fluorescence intensity
Dioxane	2.21	359	441	49
Chloroform	4.81	359	439	48
Ethanol	24.55	355	435	48
Dimethyl-formamide	36.71	355	438	33

a) Data from Ref. 22.

On the other hand, the effects of the solvent on the excitation and emission spectra of the aluminum-flufenamic acid chelate compound shown in Table V were different from that of ligand; relatively slight differencies on both spectra were shown.

The fluorescence intensity of flufenamic acid was also affected by solvent polarity. These effects were insignificant in nonpolar solvents but in polar solvents fluorescence intensities decreased in a large scale. On the fluorescence intensity of aluminum-flufenamic chelate, solvent polarity made a little effects against the ligand except dimethylformamide having the highest dielectric constant among the solvents studied.

Though the fluorescence solvent effects are less well known than the ultraviolet absorption solvent effects and the effects of pH on excitation and emission spectra, it is assumed that effects of solvent were originated from the interaction of solvent and -COOH/-NH groups of the compounds studied on both ground and excited state.

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