Structural Features for Fluorescing Present in Methoxycoumarin Derivatives

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Fluorometric analysis is one of the most sensitive methods for detecting organic and/or inorganic compounds, and therefore it has been widely used in many scientific fields with improving analytical instruments such as high-performance liquid chromatography (HPLC), capillary electrophoresis (CE), and the like. However, as most compounds in nature are not fluorescent, it is necessary to give them fluorescence by reacting them chemically with fluorescent or fluorogenic molecules. For this purpose, a great deal of effort has gone into the development of fluorescence derivatization reagents using various fluorophores. At the stage of molecular design, having sufficient information on the relationship between the chemical structures and fluorescence characteristics of fluorophores should facilitate the development of the reagents.

Recently, Imai and co-workers established a method of predicting the fluorescent behaviour of benzo furazane compounds, some of which are commonly purchased as fluorescence derivatization reagents, from a standpoint of the Hammett’s substituent effects1) and by semi-empirical molecular orbital calculations.2)

In a previous paper,3) we have also reported the fluorescence characteristics of methoxycoumarins, some of which have been utilized as the fluorophores of the analytical reagents,4) as well as benzofurazanes. Next, the structural features of strongly fluorescing methoxycoumarins were discussed in connection with their spectroscopic properties based on intramolecular charge-transfer (ICT) from the substituents to a coumarin ring. Furthermore, the presence of a lactone ring itself, including a carbonyl group, cyclic ether oxygen and ethylene bond as partial ring structures, was found to be essential for fluorescing in methoxycoumarins according to the fluorescent behaviors of chemically deformed model compounds.

Key words methoxycoumarin; fluorophore; fluorescence emission mechanism; intramolecular charge-transfer

Structural features of fluorescent methoxycoumarins were examined from the viewpoint of substituent effect and ring structure in connection with intramolecular charge-transfer (ICT). The fluorescence of methoxycoumarins depended primarily upon the ICT from a C2-electron-donating group to the substituents at the C2-position of the coumarin ring. Additionally, the presence of a lactone ring itself, including a carbonyl group, cyclic ether oxygen and ethylene bond as partial ring structures, was found to be essential for fluorescing in methoxycoumarins according to the fluorescent behaviors of chemically deformed model compounds.

Experimental

Materials All chemicals were of reagent grade, unless noted otherwise. The solvents (Luminalos) used for the fluorescence measurement were purchased from Dojindo Laboratories (Kumamoto, Japan). Compounds 1a—k were prepared by means of Knoevenagel condensation of 4,5-dimethoxysalicylaldehyde with the corresponding active methylene compounds, according to the methods described in the literature.5)—(10)

Typical synthetic procedures, as well as physical and spectral data of unknown compounds, were as follows:

1f: Yield 45%, mp 216—217 °C. 1H-NMR (CDCl3) δ: 3.93, 3.96 (3H, s, C7-OCH3), 6.88 (1H, s, C7-H), 6.98 (1H, s, C8-H), 8.37 (1H, s, C4-H), 10.22 (1H, s, C8-CHO). MS m/z: 234 (M+).

1b: Yield 26%, mp 167—168 °C. 1H-NMR (CDCl3) δ: 3.94, 3.98 (3H, s, C6-, C7-OCH3), 6.89 (1H, s, C7-H), 6.90 (1H, s, C8-H), 7.68 (1H, s, C4-H). MS m/z: 354 (M+).

1j: Yield 69%, mp 293—294 °C. 1H-NMR (CDCl3) δ: 3.97, 4.01 (3H, s, C6-, C7-OCH3), 6.87 (1H, s, C7-H), 6.89 (1H, s, C8-H), 8.15 (1H, s, C4-H). MS m/z: 231 (M+).

2a: A mixture of 2,5-dihydroxy-4-methoxybenzaldehyde11) (10 mmol) and ethyl acetooacetate (10 mmol) in absolute ethanol was refluxed in the presence of a few drops of piperidine for 10 min. After cooling, the resulting precipitates were recrystallized from ethanol to give 3-acetyl-6-hydroxy-7-(15-crown-5)-methyleneoxy benzaldehyde, which was prepared in the same manner as that of 1a, other than employing 3-acetyl-7-hydroxy-6-methoxycoumarin [mp 237—238 °C. 1H-NMR (CDCl3) δ: 2.71 (3H, s, C3-COCH3), 2.71 (3H, s, C7-OCOCH3), 2.73 (3H, s, C3-COCH3), 2.73 (3H, s, C7-OCOCH3), 3.90 (3H, s, C3-COCH3), 6.42 (1H, s, C7-H), 6.94 (1H, s, C1-H), 6.97 (1H, s, C8-H), 7.31 (1H, s, C4-H), 8.45 (1H, s, C1-H), 8.45 (1H, s, C8-H). MS m/z: 276 (M+).

Compound 2b was obtained in the same manner as that of 2a, other than employing 3-acetyl-7-hydroxy-6-methoxycoumarin [mp 237—238 °C. 1H-NMR (CDCl3) δ: 2.71 (3H, s, C3-COCH3), 2.71 (3H, s, C7-OCOCH3), 2.73 (3H, s, C3-COCH3), 2.73 (3H, s, C7-OCOCH3), 3.90 (3H, s, C3-COCH3), 6.91 (1H, s, C7-H), 7.31 (1H, s, C1-H), 8.45 (1H, s, C4-H). MS m/z: 276 (M+).

2c: A mixture of 2,5-dihydroxy-4-methoxybenzaldehyde (5 mmol) and 2-(tosloxyethyl)-15-crown-5-ether12) (5 mmol) in anhydrous acetone (50 ml) was refluxed in the presence of K2CO3 (5 mmol) for 72 h, filtered and evaporated to dryness under reduced pressure. The residue was extracted with dichloromethane and then washed successively with water and saturated sodium chloride solution. Evaporation of the solvent left an oil, 2-(tosloxyethyl)-15-crown-5-ether2) (15-crown-5)-methyleneoxy benzaldehyde, which was refluxed with ethyl acetooacetate (5 mmol) and a catalytic amount of piperidine in absolute ethanol (20 ml) for 10 min without purification. The resulting precipitates were recrystallized from ethanol to give yellow-green needles of 2e. Yield 35%, mp 148—150 °C. 1H-NMR (CDCl3) δ: 2.71 (3H, s, C3-COCH3), 2.34—4.20 (21H, m, C3-COCH3), 2.39 (3H, s, C7-OCOCH3), 3.89 (3H, s, C7-OCOCH3), 6.91 (1H, s, C7-H), 7.06 (1H, s, C1-H), 8.46 (1H, s, C4-H). MS m/z: 466 (M+).

Compound 2d was prepared in the same manner as that of 2e, other than employing 2,4-dihydroxy-5-methoxybenzaldehyde. Yield 26%, mp 138—140 °C. 1H-NMR (CDCl3) δ: 2.71 (3H, s, C3-COCH3), 6.34—4.20 (21H, m, C3-COCH3), 3.89 (3H, s, C7-OCOCH3), 6.91 (1H, s, C7-H), 6.94 (1H, s, C8-H), 8.48 (1H, s, C8-H). MS m/z: 466 (M+).

Compound 3 was obtained by cyclic condensation of 4,5-dimethoxysalicylaldehyde with methylvinylketone in the presence of K2CO3 in dioxane, according to the methods of Rene and Vincenzo.14) Yield 15%, mp 115—116 °C. 1H-NMR (CDCl3) δ: 2.38 (3H, s, C3-COCH3), 3.86, 3.88 (3H, s, C6-, C7-OCH3), 4.97 (2H, s, C7-H), 6.47 (1H, s, C8-H), 6.68 (1H, s, C1-H), 6.84

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(1H, s, C₆-H). MS m/z: 234 (M⁻).

Compound 4 was prepared by acetylation of 1,2-dihydro-6,7-dimethoxy-
napthalene[1] with acetic anhydride in the presence of aluminum chloride.
Yield 56%, mp 131—133 °C. ¹H-NMR (CDCl₃): δ: 2.43 (3H, s, COCH₃),
2.57 (2H, t, C₆-CH₂), 2.78 (2H, t, C₇-CH₂), 3.90 (3H, s, OCH₃), 3.92 (3H, s,
OCH₃), 6.73 (1H, s, C₅-H), 6.78 (1H, s, C₆-H). MS m/z: 232 (M⁻).

Compound 5 was prepared by the reaction of commercially available 3,4-
dimethoxybenzaldehyde with acetone according to the text.[16] Yield 65%,
mp 71—74 °C. ¹H-NMR (CDCl₃): δ: 2.37 (3H, s, COCH₃), 3.94 (6H, s,
OCH₃), 6.56 (1H, d, olefinic-H), 6.88 (1H, d, C₅-H), 7.08 (1H, s, C₆-H), 7.11
(1H, d, C₇-H), 7.46 (1H, d, olefinic-H). MS m/z: 206 (M⁻).

Apparatus and Measurements The melting points were measured on a
YNagamoto micro-melting point apparatus, and are uncorrected. ¹H-NMR
were obtained with a JEOL JNM-GSX 500FT-NMR spectrometer using
tetramethylsilane as an internal standard. The following abbreviations are
used: s, singlet; d, doublet; t, triplet; and m, multiplet. The mass spectra
were taken with a JEOL JMS-DX303 spectrometer, using the electron
impact ionization (EI) mode at 70 eV. The fluorescence spectra were taken
with a Hitachi F-4000 fluorescence spectrophotometer. Fluorescence quan-
tum yields were determined according to the method of Parker and Rees,[17]
and the value (0.55) for quinine sulfate in 0.5 M H₂SO₄ was used as the stan-
dard.

Fluorescence Quenching Efficiencies (FQE): These values were calculated
by means of the following equation: FQE (%) = (I₀ — Iₐ)/I₀ × 100, where I₀
and Iₐ are the fluorescence intensities (Ex 387 nm, Em 477 nm) of 2c or 2d
(5.0 × 10⁻⁶ M) in the absence and the presence of sodium acetate (5.0 × 10⁻⁴ M),
respectively, in methanol solution.

Stability Constants: Measurements for the stability constants (Kₛ) were
made on a methanol solution of 2c or 2d (5.0 × 10⁻⁵ M) and sodium acetate
(5.0 × 10⁻⁶ — 5.0 × 10⁻⁴ M). The Kₛ were estimated by the usual treatment of
Benesi–Hildebrand plots obtained from the changes in fluorescence intensi-
(Ex 387 nm, Em 477 nm).

Results and Discussion

Effects of Substituents at the C₃-Position In a recent publica-
tion,[18] we described that i) the arrangements of an electron-donating group at the C₆-position and an electron-
withdrawing group at the C₇-position on the coumarin ring contribute predominantly to the fluorescence enhancement of methoxycoumarins, ii) this enhancement can be appreciated by approximating the relationship of two substituents at the
C₆- and C₇-positions to the para-position in the disubstituted benzene model, and also iii) additional structural features of coumarins required for intense fluorescence include diether bonds at both the C₆- and C₇-positions, and an electron-
withdrawing group at the C₆-position, as shown in 3-acetyl-6,7-dimethoxycoumarin with a quantum yield of 0.52. On the
basis of these structural requirements, 3-substituted-6,7-dimethoxycoumarins, 1a—k, with two fixed methoxy groups
at the C₆- and C₇-positions were prepared to examine the effects of the substituents at C₃-position in this study. In
order to understand the contribution of these substituent groups to the fluorescence characteristics, a suitable, easily
available parameter was searched for. As is distinct from the
condensed-ring compounds, such as pyrene and anthracene, fluorescence characteristics of the heterocyclic compounds
such as coumarin and benzo[5,6]furan were attributed to the
electronic effects of substituents in the molecule. The Ham-
matt substituent constants (σᵣ, or σᵣₑ) are practical parameters
used to estimate the electronic effect in the chemical re-
actions. Although these constants represent gross values, in-
cluding the polar effect, the resonance effect, and the solvent
effect, they seemed suitable for representing total electronic effects. Thus, we tried to understand the fluorescence characteristics of 3-substituted-6,7-dimethoxycoumarins using Hammet σᵣₑ-values as suitable parameters. Fluorescence
spectral data of 1a—k, together with Hammett σᵣₑ-values[19]
are summarized in Table 1, where the relative fluorescence intensities (RFI) are relative values against the fluorescence intensity (100) of the standard compound, 1d. As shown in
Table 1, an increased fluorescence intensity was observed for 1e—j, substituted with electron-withdrawing groups
(σᵣₑ=0.31—0.66), compared with that of 1d. However, non-
fluorescence was observed for 1k, with the stronger electron-
withdrawing nitro group (σᵣₑ=0.78). On the other hand, comp-
ounds 1a and b, which were introduced electron-donating
groups such as hydroxy- and methoxy groups at the C₃-position,
showed a tendency to decrease in fluorescence intensity.
The fluorescence wavelengths of these compounds were also
shifted to longer wavelength regions, with increases in the
electron-withdrawing ability of substituents, except for 1a. It was suggested from these results that the fluorescence of
such coumarins are subject to the ICT effect through a
push–pull system between C₆- and C₇-electron-donating groups and electron-withdrawing groups on the lactone ring. As can be seen in Hammett’s plots (Fig. 1) obtained from the
data in Table 1, the fluorescence intensities in general in-
creased with an increase in the electron-withdrawing ability of
substituents, but a nonlinear relationship was observed.
The fluorescence intensity of 1k was particularly low. This
high fall-off in fluorescence intensity may be ascribed to
the conversion of the planar ICT state to a conformer dis-
playing full charge separation, a twisted ICT state,[20] which is
non-emissive in a polar solvent such as methanol, and by the
acceleration of ICT between the electron-donating methoxy
groups and the stronger electron-withdrawing C₃-nitro group.
This is also supported by the fact that the fluorescence of 1k
is restored in less polar solvents such as benzene and chloro-
form. Furthermore, ¹H-NMR spectroscopic study[21] gave add-
tional information on the substituent effect from the stand-
point of the polarization of molecules in the ground state.
The plot of ¹H-NMR chemical shifts of C₆-H on the
coumarin ring in deuterated chloroform, and σᵣₑ-values, gave
a positive correlation similar to that in Fig. 1, namely, the δ
values of C₆-H increased with an increase in the electron-
withdrawing ability of substituents at the C₃-position (Fig. 2).
Such downfield shifts apparently suggest an electron deficiency at the C$_4$-position caused by electron-withdrawing substituents at the adjoined C$_3$-position in the ground state. From these results, the fluorescence of such coumarins was found to be strongly dependent upon ICT between the electron-donating methoxy groups and the C$_3$-substituents. However, the virtual fluorescence mechanism is apparently not so simple.

Effects of Substituents at C$_6$- or C$_7$-position

To specify the contribution of the position of the C$_6$- or C$_7$-electron-donating groups to the fluorescence, 3-acetylcoumarin derivatives, $2a$--$d$, were prepared. Figure 3 shows the fluorescence spectra of $2a$, $b$, together with $1i$ for comparison. The fluorescence of $2a$, a derivative of $1i$ whose C$_6$-methoxy group was replaced with an electron-withdrawing acetoxy group, was quenched, and its blue-shifted spectrum resembled that of 7-methoxy-3-acetylcoumarin ($F_{\text{max}}$ 428 nm). In contrast with $2a$, a 7-acetoxy-compound, $2b$, still held about 20% of the fluorescence of $1i$. This fact suggests that the strong fluorescence of $1i$ is predominantly attributable to the ICT from the C$_6$-methoxy group to the C$_3$-acetyl group on the lactone ring. This estimate was examined in further detail by employing the newly prepared crowned-coumarins, $2c$, $d$. The 15-crown-5 ether moieties introduced into C$_6$- or C$_7$-positions of $2c$, $d$ have been well known to form complexes with Na$^+$ or K$^+$ by the electrostatic interaction of their electron-donating oxygens with metal cations. Therefore, this complexation event may prove the contribution of both electron-donating groups at the C$_6$- and C$_7$-positions in the fluorescence of $1i$, the same as the above substituent effect. In a recent study concerning fluorescent sensors for metal ions, photoinduced electron transfer (PET) has attracted attention as a novel operating principle. A number of excellent fluorescence signaling systems based on the PET mechanism have been proposed. Among them, de Silva’s group has reported a unique PET sensory system in which the benzocrown unit can act not only as a receptor for metal ions but also as an efficient fluorescence quencher for fluorophores such as anthracene. More recently, Nishizawa et al. reported a similar fluorescent PET sensor for metal ions using pyrene as a fluorophore and benzo-15-crown-5 as a receptor for metal ions and an electron donor (a fluorescence quencher of pyrene monomer) for exciplex formation at the same time. However, aliphatic crown ether, the 15-crown-5 ether moieties introduced into the C$_6$- or C$_7$-positions of $2c$, $d$ scarcely quenched their fluorescence, suggesting no PET interaction between the 15-crown-5 ether moieties and the coumarin fluorophore, as shown in Table 2. Thus, the effect of metal ions on the fluorescence spectra of $2c$, $d$ was examined in methanol. The fluorescence intensities of $2c$, $d$ in methanol were, in fact, decreased with the addition of sodium or potassium acetates. On the other hand, a corresponding derivative $1i$, without a 15-crown-5 ether moiety, showed no change in the fluorescence spectrum by adding these metal ions. This indicates the formation of complexes of $2c$ and $2d$ with metal ions. The fluorescence quenching of $2c$, $d$ is considered to lower the electron-donating ability of C$_6$- or C$_7$-substituents because of the electrostatic interaction of electron-donating oxygens on the crown ether with metal ions. FQE and stability constants ($\log K_s$) for $2c$, $d$ are shown in Table 2. It was noteworthy from the results that the stability constants of $2c$, $d$ were almost comparable; nevertheless, a remarkable difference in FQEs of $2c$ (16.9%) and $2d$ (10.3%) was observed. This supports the foregoing estimate, that is, the fluorescence in methoxycoumarins depends predominantly on ICT from the C$_6$-electron-donating group to C$_3$-electron-withdrawing substituents on the lactone ring.

Effects of Lactone Ring Structure

In addition to the above substituents effects, the contribution of ring structures to the fluorescence of this type of coumarin was examined.
Table 3. Fluorescence Spectral Properties of 1i, 2c, and 2d in Methanol at 25 °C in the Absence and Presence of Na+

<table>
<thead>
<tr>
<th>Compound</th>
<th>Structural Fmax/nm (Ex λ/nm)</th>
<th>Φ (rel)</th>
<th>FQE (%)</th>
<th>log Ks (Na+)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1i</td>
<td>479 (383)</td>
<td>100</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>2c</td>
<td>480 (383)</td>
<td>99</td>
<td>16.9</td>
<td>3.03</td>
</tr>
<tr>
<td>2d</td>
<td>479 (385)</td>
<td>103</td>
<td>10.3</td>
<td>3.11</td>
</tr>
</tbody>
</table>

Table 3. Fluorescence Spectral Data of 1i, 3, 4, and 5 in Methanol

<table>
<thead>
<tr>
<th>Compound</th>
<th>Structural formula</th>
<th>Fmax/nm (Ex λ/nm)</th>
<th>Φ (rel)</th>
<th>FQE (%)</th>
<th>log Ks (Na+)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1i</td>
<td><img src="image" alt="Structure of 1i" /></td>
<td>482 (385)</td>
<td>0.52</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3</td>
<td><img src="image" alt="Structure of 3" /></td>
<td>543 (375)</td>
<td>0.02</td>
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<tr>
<td>4</td>
<td><img src="image" alt="Structure of 4" /></td>
<td>491 (369)</td>
<td>0.01</td>
<td></td>
<td></td>
</tr>
<tr>
<td>5</td>
<td><img src="image" alt="Structure of 5" /></td>
<td>n.d.</td>
<td>n.d.</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

n.d. not detected.

from the viewpoint of the necessity of a carbonyl group and ether oxygen on the lactone ring, and also on the ring itself. For this purpose, compounds 3 (chromene type), 4 (dihydonaphthalene type) and 5 (styrene type) were prepared. Their fluorescence spectral data are listed in Table 3.

First, the contribution of a lactone carbonyl group to the strong fluorescence of 1i was estimated from the fluorescence behaviors of 3 lacking a carbonyl group. As shown in Table 3, the fluorescence quantum yield of 3 (Φ3 = 0.02) was remarkably low in comparison with that of 1i (Φ1i = 0.52). However, the Fmax (543 nm) of 3 shifted to a much longer wavelength region than that (482 nm) of 1i, and also, the emission color in the solution was distinct yellow in contrast to the whitish blue of 1i. Thus, the presence of a lactone carbonyl group on 1i from these results is suggested to contribute primarily to the fluorescence intensity. The remarkable red shift in Fmax of 3 may be attributed to the enhanced ICT effect from the C6-carbonyl group to the C5-acetyl group, due to the lack of a carbonyl group. This is also supported by the difference in Fmax of 6-methoxy-3-acetyl-coumarin (506 nm) and 7-methoxy-3-acetyl-coumarin (428 nm). The strong fluorescence of 1i, therefore, is presumed to result from two different ICT routes, from the C5- and C7-electron-donating groups to either a lactone carbonyl or a C7-electron-withdrawing group. The former route may contribute to the fluorescence intensity and the later to the fluorescence wavelength. Further investigation, however, is required to elucidate these mechanisms.

Subsequently, compound 4, which converted a lactone moiety into a cyclic ethylene structure, showed a lowered quantum yield and slight red shift in Fmax compared with 1i, and an additional lowering in quantum yield together with a blue shift compared with the cases of 3. The fluorescence behaviors of 4 are considered to be due to the effect of a slightly distorted cyclohexene ring. The fluorescence of 5 in the absence of a lactone ring structure was no longer detectable under the same conditions as the other compounds. These results indicate the requirement of at least a ring structure, as can be seen in 3, as a part of the coumarin ring for fluorescing. This chromene-type compound, 3, may also be promising for use as a novel fluorophore for fluorescent imaging because of emitting a distinct yellow.

Thus, such an approach to determining fluorescence characteristics by chemical tools was found to be practically effective for establishing reagent design structural requirements for various purposes.

References


