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Structural development of canthin-5,6-dione moiety as a fluorescent dye and its application to novel fluorescent sensors

Hidetomo Yokoo,[a] Ayumi Ohsaki,[b] Hiroyuki Kagechika,[a] Tomoya Hirano[a,*]

[a]*Institute of Biomaterials and Bioengineering, Tokyo Medical and Dental University (TMDU), 2-3-10 Kanda-Surugadai, Chiyoda-ku, Tokyo 101-0062, Japan*

[b]*College of Humanities and Sciences, Nihon University, 3–25–40 Sakura–Josui, Setagaya-ku, Tokyo 156-8550, Japan*

*Corresponding author. Tel.: +81-3-5280-8128; fax: +81-3-5280-8127; e-mail: hira.chem@tmd.ac.jp

Abstract

Canthin-5,6-dione is a common structure of the fluorescent natural products amarastelline A and nigakinone. Here, we synthesized various derivatives, and studied their fluorescence properties. The effects of substituent groups at the N^3-, 4- and 10-positions were clarified. N^3-Substitution influenced the solvent-dependent fluorescent change by modulating tautomerization between canthin-5,6-dione and the 5-hydroxy-6-one form. 10-Substitution also influenced the fluorescence, especially in aqueous solution, in combination with N^3-substitution. Among the synthesized compounds, **5** showed an "OFF-ON-OFF"-type fluorescence change with increase of pH, and therefore served as a novel fluorescent sensor for a specific range of pH. Our findings suggest that canthin-5,6-dione should be useful as a fluorophore moiety for fluorescent labelling of biomolecules and for development of fluorescent probes and sensors.

1. Introduction

Fluorescent dyes have been widely employed for analytical and industrial purposes. They have also been utilized to develop probes and sensors whose fluorescence properties are changed by binding to or reacting with specific molecular species, including enzymes, or in response to changes of microenvironment,[1] such as pH[2] and viscosity.[3] In particular, small molecule-based fluorescent molecules are practically useful, and a number of well-established fluorophores have been reported.[4] However, for example, in the case of multi-color imaging applications, probes emitting in distinct wavelength regions are needed, and therefore it is important to expand the range of available fluorophores. For this purpose, our group has constructed a library of fluorescent compounds obtained by organic synthesis or by isolation of natural products.[5] Amarastelline A was isolated as a novel fluorescent natural product from the bark of *Quassia amara*.[6] It has strong fluorescence and could stain cytoplasm of living cells (Fig. 1). We also isolated and identified the known compound, named as Nigakinone (**1**),[7] from the same plant, and found the solvent-dependent fluorescence properties. We considered that these compounds might be useful as novel fluorophores. Therefore, in the present work, we synthesized various derivatives of the core canthin-5,6-dione structure to clarify the structure-fluorescence relationship. Among the compounds obtained, one showed pH-dependent fluorescence change, and could be utilized a fluorescent pH sensor, confirming the practical value of this fluorescent moiety.
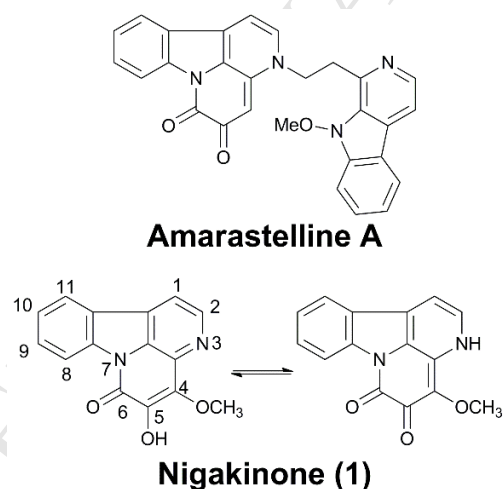

Amarastelline A

Nigakinone (1)

Fig. 1 Structures of fluorescent natural compounds, amarastelline A and nigakinone (**1**).

2. Results and Discussions

2.1. Design and synthesis of canthin-5,6-dione derivatives.

Amarastelline A and nigakinone contain a common canthin-5,6-dione structure, which is a tautomer of 5-hydroxy-canthin-6-one, as shown in Fig. 1 for the case of nigakinone. Tautomerization is expected to alter the fluorescence properties, and could be hindered by the introduction of functional groups at the N^3-position, as well as by the methoxy group at the 4-position adjacent to the diketone (or α-hydroxyketone) moiety of 1. Functional groups on the benzene moiety linked to the tautomerizable bicyclic 1,5-naphthyridin-2-one moiety might also influence the fluorescence properties. Thus, we designed compounds 1 – 10, having a variety of groups at the N^3-, 4- and 10-positions (Fig. 2).

1: R_1 = H R_2 = OCH$_3$ **6**: R_1 = H R_2 = OCH$_3$
2: R_1 = H R_2 = H   **7**: R_1 = H R_2 = H
3: R_1 = OCH$_3$ R_2 = OCH$_3$ **8**: R_1 = OCH$_3$ R_2 = OCH$_3$
4: R_1 = OCH$_3$ R_2 = H   **9**: R_1 = OCH$_3$ R_2 = H
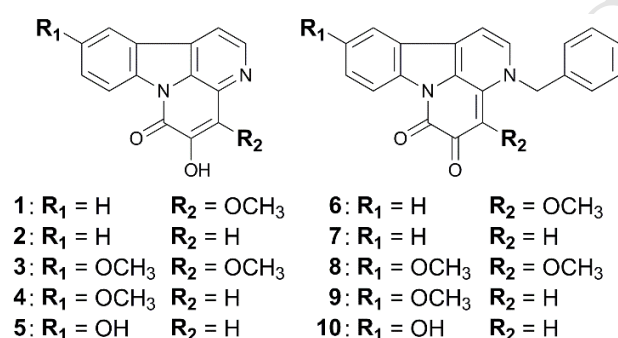**5**: R_1 = OH R_2 = H   **10**: R_1 = OH R_2 = H

Fig. 2 Structures of synthesized canthin-5,6-dione compounds.

Although several canthine compounds have been reported, most of them were extracted from plants,[8] and there have been few synthetic studies.[9] Among the compounds shown in Fig. 2, compounds **2** and **7** were synthesized by reported procedures,[9b,10] while the other compounds were synthesized by modified procedures, as shown in Scheme 1. Tryptamine (**11a**) and 5-methoxytryptamine (**11b**) were used as starting materials, and they were reacted with methoxyacetyl chloride (**12a**) or acetic anhydride (**12b**) to obtain acylated tryptamines (**13**), which were transformed to 1-alkyl-β-carbolines (**14**) with Hendrickson reagent[11] followed by oxidation. The construction of the canthin-5,6-dione moiety was performed by the reaction of **14** and dibenzyl oxalate to obtain compounds **6** - **9**, and then reductive debenzylation yielded **1** – **4**. Demethylation of the 10-methoxy group of **4** and **9** was performed with tribromoborane, affording **5** and **10**, respectively.
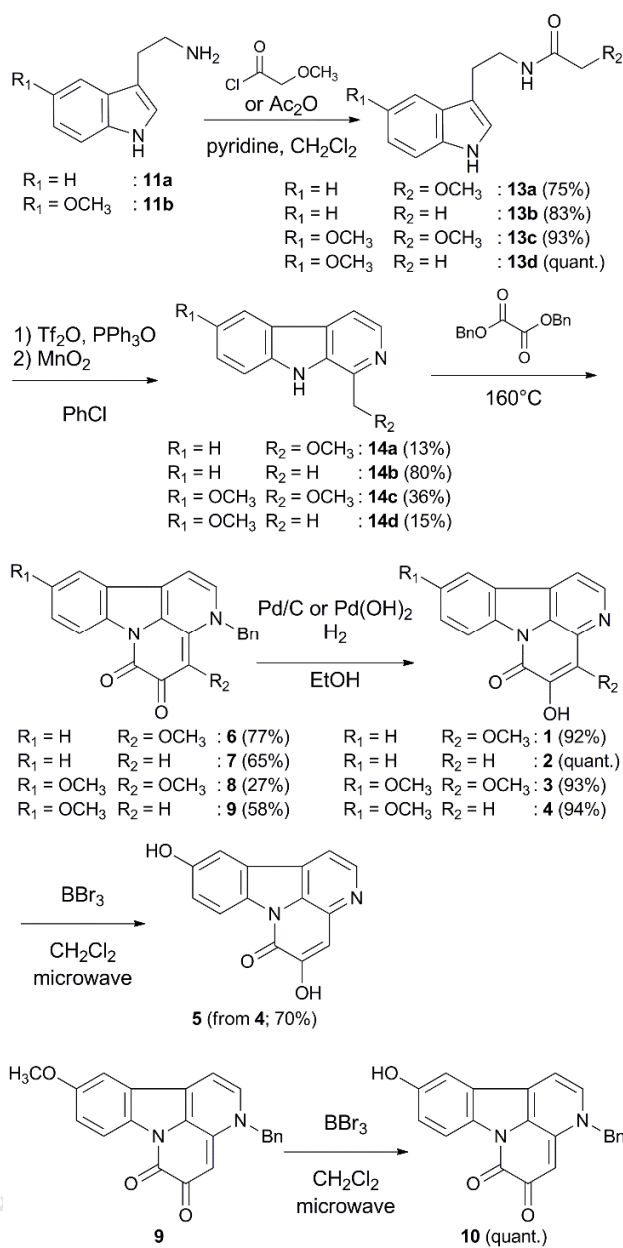
Scheme 1 Synthesis of compounds **1 – 10.**

2.2. Study of fluorescence properties of canthin-5,6-dione compounds.

The fluorescence properties of compounds **1** – **10** were evaluated in chloroform, acetonitrile, DMF, DMSO, methanol and H_2O (100 mM sodium phosphate buffer, pH 7.4), (Fig. S1 – S4), and the results are summarized in Table 1. As regards the absorption spectra, compounds **1** – **5** with no substituent group at the N^3-position, showed maxima at around 350 – 370 nm, whereas compounds with a N^3-benzyl group showed maxima at over 450 nm. Introduction of a functional group at the N^3-position appears to hinder the tautomerization between 5,6-dione and 5-hydroxyl-6-one forms, and shorten the absorption maximum wavelength. The 5,6-dione form might contribute predominantly to the absorption at over 450 nm.

The parent compound nigakinone (**1**) showed moderate fluorescence in chloroform with two emission maxima at 432 and 616 nm; the longer-wavelength emission reached more than 600 nm, and its Stoke's shift was up to 258 nm. Although its quantum yield was relatively low, emission at longer wavelength would be favorable for application to living cells or tissues, minimizing the phototoxic effect of the excitation light and interference from autofluorescence due to biomolecules such as nucleotides. In addition, fluorophores with a large Stoke's shift would also be useful in applications such as multicolor imaging and dual-color fluorescence cross-correlation spectroscopy (FCCS), like a fluorescent protein "Keima".[12] Focusing on the effects of 4-subsituents, removal of the methoxy group enhanced the fluorescence, as indicated by the quantum yields of **1** vs **2**, **3** vs **4**, **6** vs **7** and **8** vs **9** (Fig. 3, Table 1). In these compounds, the Stoke's shift was still large, up to 186 nm in the case of compound **2** (chloroform), and the quantum yield at this wavelength was relatively high (Φ = 0.103). Although further optimization might be necessary, those compounds appear to be good candidates as novel platforms for fluorescent dyes.
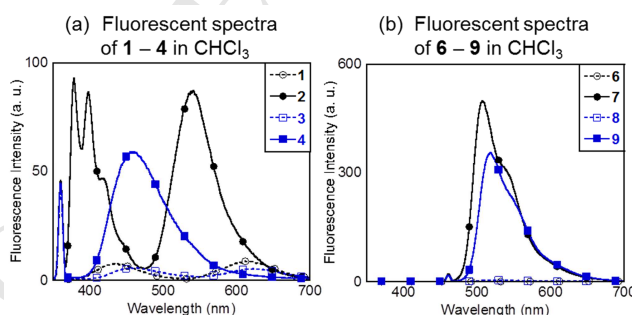

Fig. 3 The effect of 4-substituents on the fluorescence spectra. (a) The fluorescence spectra in chloroform (excitation at 360 nm) of 4-OCH_3 compounds (**1** and **3**) are shown as dashed lines and open symbols, and those of 4-H compounds (**2** and **4**) as solid lines and closed symbols. (b) Fluorescence spectra (excitation at 460 nm) of N^3-benzyl compounds, **6** - **9**.

Table 1. Photophysical Properties of Compounds **1** – **10**.

Compound **1** (R_1 = H; R_2 = OCH$_3$)				Compound **6** (R_1 = H; R_2 = OCH$_3$)			
Solvent	λ_{abs} (nm)	λ_{em} (nm)	[a]Φ_{fl}	Solvent	λ_{abs} (nm)	λ_{em} (nm)	[a]Φ_{fl}
CHCl$_3$	358	432, 616	0.021, 0.016	CHCl$_3$	500	[c]n. d.	[d]n. d.
CH$_3$CN	357	444	0.147	CH$_3$CN	499	[c]n. d.	[d]n. d.
DMF	474	[c]n. d.	[d]n. d.	DMF	500	[c]n. d.	[d]n. d.
DMSO	363	[c]n. d.	[d]n. d.	DMSO	501	[c]n. d.	[d]n. d.
CH$_3$OH	358	[c]n. d.	[d]n. d.	CH$_3$OH	488	[c]n. d.	[d]n. d.
[b]H$_2$O	358, 457	[c]n. d.	[d]n. d.	[b]H$_2$O	478	[c]n. d.	[d]n. d.

Compound **2** (R_1 = H; R_2 = H)				Compound **7** (R_1 = H; R_2 = H)			
Solvent	λ_{abs} (nm)	λ_{em} (nm)	[a]Φ_{fl}	Solvent	λ_{abs} (nm)	λ_{em} (nm)	[a]Φ_{fl}
CHCl$_3$	356	378, 542	0.145, 0.103	CHCl$_3$	456	513	0.814
CH$_3$CN	355	377, 545	0.229, 0.034	CH$_3$CN	455	509	0.688
DMF	450	535	0.168	DMF	457	514	0.669
DMSO	360	404, 533	0.053, 0.223	DMSO	460	518	0.682
CH$_3$OH	375	509	0.229	CH$_3$OH	447	513	0.711
[b]H$_2$O	407	507	0.313	[b]H$_2$O	444	517	0.536

Compound **3** (R_1 = OCH$_3$; R_2 = OCH$_3$)				Compound **8** (R_1 = OCH$_3$; R_2 = OCH$_3$)			
Solvent	λ_{abs} (nm)	λ_{em} (nm)	[a]Φ_{fl}	Solvent	λ_{abs} (nm)	λ_{em} (nm)	[a]Φ_{fl}
CHCl$_3$	360	462, 616	0.007, 0.012	CHCl$_3$	505	517	0.020
CH$_3$CN	358	467	0.031	CH$_3$CN	502	519	0.016
DMF	361	[c]n. d.	[d]n. d.	DMF	504	524	0.018
DMSO	364	478, 635	0.005, 0.003	DMSO	505	539	0.020
CH$_3$OH	362	564	0.004	CH$_3$OH	497	526	0.016
[b]H$_2$O	364	[c]n. d.	[d]n. d.	[b]H$_2$O	484	[c]n. d.	[d]n. d.

Compound **4** (R_1 = OCH$_3$; R_2 = H)				Compound **9** (R_1 = OCH$_3$; R_2 = H)			
Solvent	λ_{abs} (nm)	λ_{em} (nm)	[a]Φ_{fl}	Solvent	λ_{abs} (nm)	λ_{em} (nm)	[a]Φ_{fl}
CHCl$_3$	357	461	0.214	CHCl$_3$	461	518	0.689
CH$_3$CN	357	475	0.093	CH$_3$CN	459	518	0.595
DMF	450	514	0.113	DMF	463	521	0.596
DMSO	361	505	0.116	DMSO	463	524	0.617
CH$_3$OH	360	489	0.214	CH$_3$OH	453	518	0.353
[b]H$_2$O	388	508	0.213	[b]H$_2$O	395	532	0.022

Compound **5** (R_1 = OH; R_2 = H)				Compound **10** (R_1 = OH; R_2 = H)			
Solvent	λ_{abs} (nm)	λ_{em} (nm)	[a]Φ_{fl}	Solvent	λ_{abs} (nm)	λ_{em} (nm)	[a]Φ_{fl}
CHCl$_3$	357	485	0.044	CHCl$_3$	461	516	0.467
CH$_3$CN	356	491	0.035	CH$_3$CN	456	516	0.603
DMF	358	511	0.079	DMF	462	519	0.558
DMSO	362	511	0.020	DMSO	463	529	0.588
CH$_3$OH	358	492	0.112	CH$_3$OH	400	515	0.011
[b]H$_2$O	392	503	0.081	[b]H$_2$O	397	506	[d]n. d.

[a]Quantum yields of fluorescence were determined using that of quinine sulfate (0.577, in 0.1 M H$_2$SO$_4$) or fluorescein (0.90, in 0.1 M NaOH) as a standard.[1a] [b]Measured in sodium phosphate buffer (100 mM. pH 7.4). [c]No emission peak was detected. [d]Not determined.

Next, the effects of 10-substituents on the fluorescence properties were evaluated. In compounds **1** - **5**, the emission maximum wavelengths varied depending upon the 10-substituent, especially in aprotic solvents. For example, in chloroform, compound **2** (10-H) showed emission maxima at 378 and 542 nm, while **4** (10-OCH$_3$) showed a maximum at 461 nm, and **5** (10-OH) showed a maximum at 485 nm (Fig. 4a). Similar differences were seen in other aprotic solvents, acetonitrile, DMF and DMSO, whereas the differences in protic solvents such as methanol and water were relatively small (Fig. 4b). However, in the case of N^3-benzyl compounds, **7** (10-H), **9** (10-OCH$_3$) and **10** (10-OH), the influence of 10-substituents on the emission maximum wavelength was almost lost in both aprotic and protic solvents (Fig. 4c, 4d).
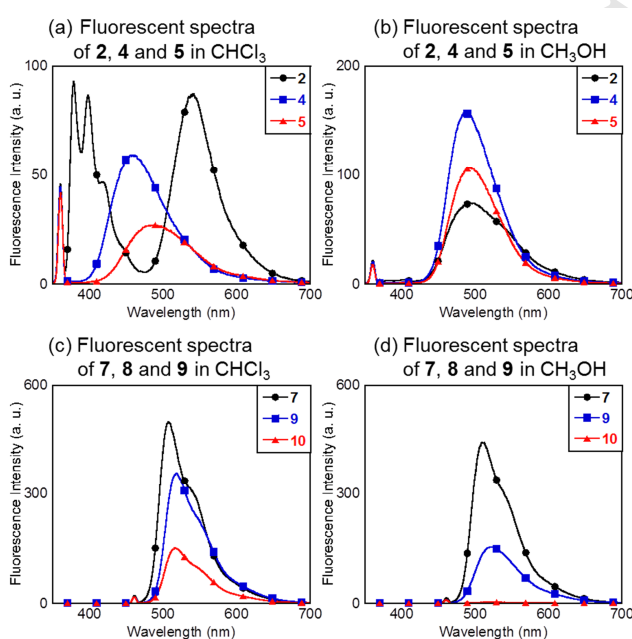

Fig. 4 Effects of 10-substituents on fluorescence spectra. The fluorescence spectra of **2** (10-H, black line), **4** (10-OCH$_3$, blue line) and **5** (10-OH, red line) in (a) chloroform and (b) methanol with excitation at 360 nm are shown, together with those of N^3-benzyl compounds (**7** (10-H, black line), **9** (10-OCH$_3$, blue line) and **10** (10-OH, red line)) with excitation at 460 nm in (c) chloroform and (d) methanol.

The presence of an N^3-benzyl group greatly affected the fluorescence properties. For example, solvent-dependent changes in the florescence spectra of **2** and N^3-benzylated **7** are shown in Fig. 5. Compound **2** showed peaks at around 400 nm in acetonitrile and chloroform, small peaks at this position in DMF and DMSO, and almost no peak in methanol and water (Fig. 5a). Changes of maximum wavelength, as well as intensity, at over 500 nm were also observed, that is, 542 nm in chloroform, 535 nm (DMF), 509 nm (methanol) and 507 nm (water) (Fig. 5a and Table 1). On the other hand, in N^3-benzylated **7**, only small changes of fluorescence at about 500 nm were induced by change of solvent, with little change of intensity (Fig. 5b). Thus, the fluorescence

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properties of the 5-hydroxy-6-one form appear to be little affected by the solvent. On the other hand, compound **2**, which can undergo tautomerization between 5-hydroxy-6-one and 5,6-dione forms, showed large solvent-dependent fluorescence changes (Fig. 5c).
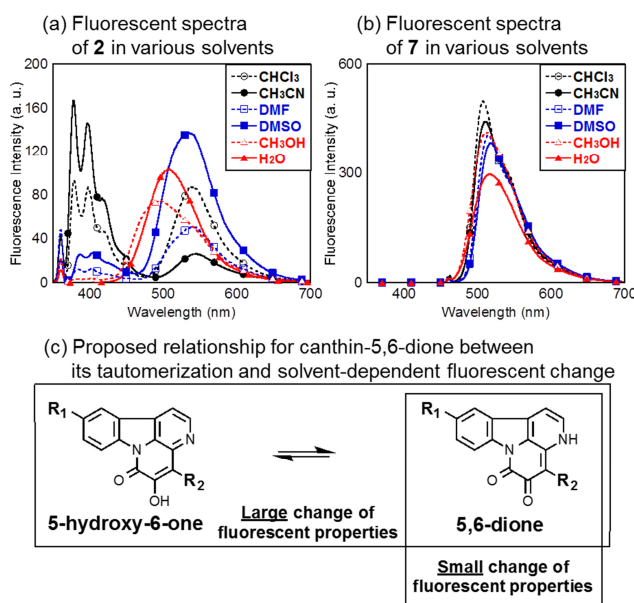

Fig. 5 Solvent-dependent fluorescence change of canthin-5,6-dione derivatives. Fluorescence spectra of (a) **2** (Ex. 360 nm) and (b) of **7** (Ex. 460 nm) in various solvents are shown. (c) Proposed relationship of tautomerization and fluorescence change.

2.3. The sensor function of the compounds.

We expected that the tautomerization equilibrium would be influenced by pH, because of the possibility of deprotonation of the hydroxyl group of the 5-hydroxy-6-one, and in addition, compound **5** has a hydroxyl group on the benzene moiety. Therefore, we evaluated the pH-dependent fluorescence changes of compounds **2**, **4** and **5**, (Fig. 6). A switch from acidic to neutral conditions increased the fluorescence intensities of all compounds, in addition to decreasing the absorbance at around 450 nm and 370 nm, and increasing that at around 400 nm (Fig. 6, S5, S6 and S7), presumably due to deprotonation of the 5-hydroxy-6-one moiety. A change from neutral to basic conditions had little effect on the spectra of **2** and **4**, whereas the 10-hydroxy compound, **5**, showed a decrease of fluorescence as well as an increase of absorption at 390 nm, presumably due to deprotonation of the 10-hydroxy group. Thus, apparently due to sequential deprotonations of the two hydroxyl groups, compound **5** showed an "OFF-ON-OFF"-type fluorescence change with increase of pH. So, like our previously reported sensor based on a coumarin scaffold bearing two hydroxyl groups[13] and similar sensors developed by other groups,[14] **5** could be

utilized as a fluorescent sensor for a specific range of pH (Fig. 7). Such a sensor should be useful, compared with most conventional pH sensors, which sense only whether the pH is below or above a specific value.[2]
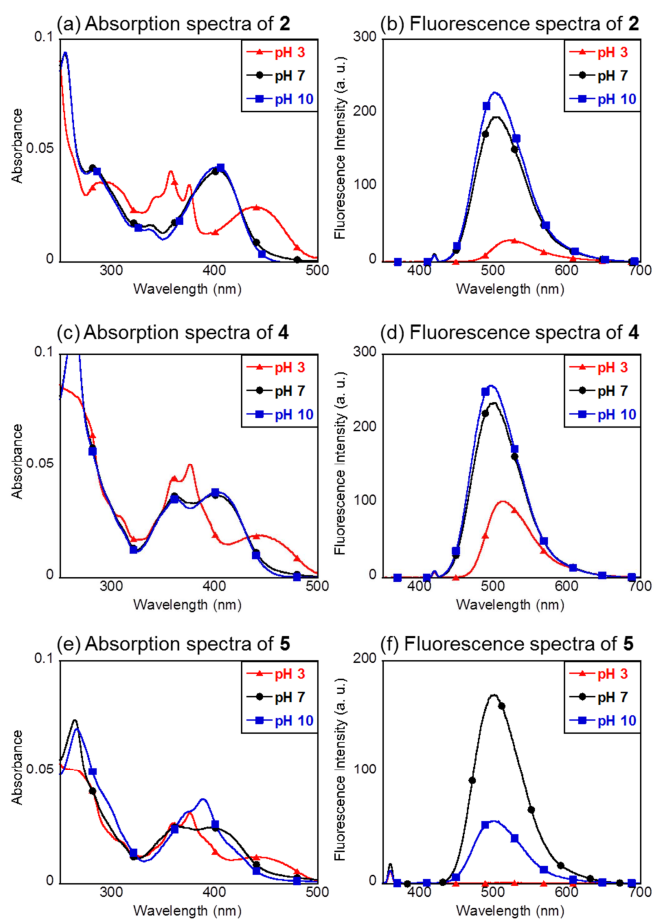

Fig. 6 Absorption and fluorescence spectra of **2**, **4** and **5** at various pH values. Absorption spectra and fluorescence spectra of (a and b) **2**, (c and d) **4** and (e and f) **5** were measured in a mixture of methanol and 100 mM sodium phosphate buffer (50/50 = v/v).
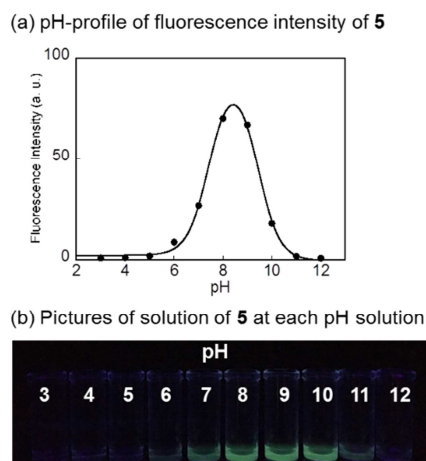
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(a) pH-profile of fluorescence intensity of **5**

(b) Pictures of solution of **5** at each pH solution

Fig. 7 pH-dependent fluorescent change of **5**. (a) The pH profile of fluorescence intensity (excitation at 360 nm; emission at 500 nm) of **5** is shown. Those data were obtained in 100 mM sodium phosphate buffer (pH 3.0 – 12.0) containing 0.3% DMSO as a co-solvent. (b) Photographs of each solution.

Many canthine compounds, mostly natural products, have been reported, but only limited information is available about their biological properties, such as cytotoxicity.[15] Here, we synthesized several canthin-5,6-dione derivatives and evaluated their structure-fluorescence relationship. We found that the fluorescence properties of these compounds are influenced by substitution at the N^3-, 4- and 10-positions. In particular, N^3-benzylated compounds showed little solvent-dependent fluorescent change. Many fluorescent dyes with solvent-dependent fluorescent change, such as dansyl and NBD,[16] show a longer emission maximum wavelength in more polar solvents such as DMSO and water. Among our compounds, **4** showed such behavior (emission at 461 nm in chloroform and 508 nm in water); this change is thought to be due to solvent relaxation. On the other hand, **2** showed two emission maximum wavelengths, at 378 nm and 542 nm in less polar chloroform, and one at 507 nm in water (Fig. 8). The longer-wavelength maximum in chloroform is thought to involve a state other than a locally excited (LE) state, such as an internal charge transfer (ICT) state and excited-state intramolecular proton transfer (ESIPT),[1a,17] and the difference between **2** and **4** should be due to the presence of the 10-methoxy group. Another interesting effect of the 10-methoxy group was seen on comparing N^3-benzyl compounds **7** and **9**. In aqueous solution, fluorescence of **9** was weak (quantum yield 0.022), whereas **7** showed strong fluorescence (0.536). As we previously reported, amarastelline A showed similar properties to compound **9**, that is, its quantum yield values were 0.78 in CHCl$_3$, 0.44 in CH$_3$OH and <0.001 in H$_2$O.[6] Interestingly, amarastelline A does not possess a 10-methoxy group, and its N^3-group, 9-methoxy-β-carboline, is different from those of **7** and **9**, which have a benzyl group. Thus the combination of substituent groups at the N^3- and 10-positions can regulate the fluorescence properties of canthin-5,6-diones, especially in aqueous solution. For example, we showed that **5** could be used as a sensor responding to a specific range of pH, based on its "OFF-ON-OFF"-type fluorescence change with increase of pH. The insights presented here should be valuable for the development of a range of

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novel fluorescent sensors by the introduction of recognition sites for various analytes into the canthin-5,6-dione structure. Work along this line is in progress.
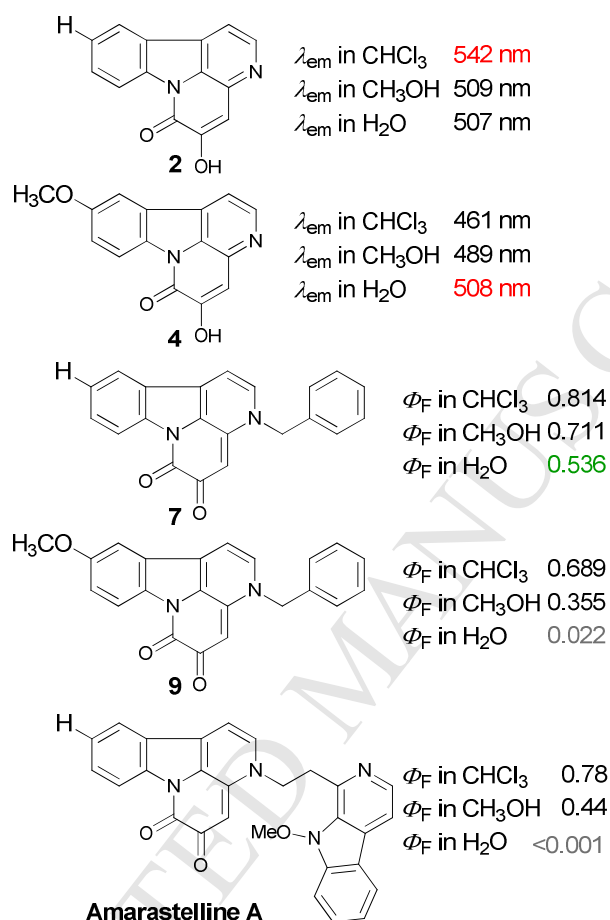

Fig. 8 Effects of a 10-methoxy group.

3. Conclusion

We synthesized several derivatives of canthin-5,6-dione, which is a common structural component of the fluorescent natural products amarastelline A and nigakinone, to evaluate substituent effects on the fluorescence properties. We found that the combination of N^3- and 10-substituted groups can regulate the fluorescence properties, and we showed that this behavior could be utilized to obtain a fluorescent sensor responding to a specific range of pH. Our findings indicate that the canthin-5,6-dione moiety could be a valuable scaffold for a range of fluorescent sensors and probes.

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4. Experimental Section

4.1. General

All reagents were purchased from Sigma-Aldrich Chemical, Tokyo Kasei Kogyo, Wako Pure Chemical Industries, and Kanto Kagaku. Silica gel for column chromatography was purchased from Kanto Kagaku. NMR spectra were recorded on Bruker AVANCE 400 or Bruker Advance 500 spectrometer. Mass spectral data was obtained on Brucker Daltonics microTOF-2focus in the positive and negative ion detection modes. Melting points were taken on a Yanagimoto micro melting point apparatus and are uncorrected. UV spectra were recorded with JASCO V-550, and fluorescence spectra were recorded with JASCO FP-6600.

4.2. Synthesis

4.2.1. Preparation of 13

A solution of triptamine (**11a**) (3.0 g, 19 mmol), methoxyacetyl chloride (**12a**) (2.6 ml, 28 mmol) and pyridine (4.7 mL, 56 mmol) in dry dichloromethane (90 ml) was stirred for 23h at room temperature, and saturated hydrochloric acid was added. The mixture was extracted with dichloromethane. The organic solution was washed with brine and concentrated. Purification of the residue by column chromatography (silica gel, AcOEt) gave **13a** (3.3 g, 75%) as brown oil. ^{1}H NMR (400 MHz, CDCl$_3$) δ 8.20 (brs, 1 H), 7.62 (d, J = 8.0, 1.2 Hz, 1 H), 7.38 (dd, J = 8.0, 1.2 Hz, 1 H), 7.21 (td, J = 8.0, 1.2 Hz, 1 H), 7.13 (td, J = 8.0, 1.2 Hz, 1 H), 7.04 (s, 1 H), 6.66 (brs, 1 H), 3.88 (s, 2H), 3.65 (q, J = 6.8 Hz, 2 H), 3.32 (s, 3 H), 3.01 (t, J = 6.8, 0.8 Hz, 2 H); ^{13}C NMR: (125 MHz, CDCl$_3$): δ 169.8, 136.5, 127.4, 122.3, 122.1, 119.6, 118.8, 113.0, 111.4, 72.1, 59.3, 39.2, 25.5; HRMS (ESI) calcd for C$_{13}$H$_{17}$N$_2$O$_2$ (M+H)$^+$ 233.1285. Found 233.1280.

13b[18] and **13d**[19] were prepared according to a reported procedure. **13c** were similarly prepared from **11b** and **12a** according to the procedure described for **13a**. Analytical data are given below.

13c: ^{1}H NMR (400 MHz, CDCl$_3$) δ 8.11 (brs, 1 H), 7.26 (d, J = 8.8 Hz, 1 H), 7.05 (d, J = 2.4 Hz, 1 H), 7.02 (s, 1 H), 6.87 (dd, J = 8.8, 2.4 Hz, 1 H), 6.69 (brs, 1 H), 3.89 (s, 2H), 3.87 (s, 3H), 3.64 (q, J = 6.8 Hz, 2 H), 3.32 (s, 3 H), 2.97 (t, J = 6.8 Hz, 2 H); ^{13}C NMR: (125 MHz, CDCl$_3$): δ 169.9, 154.2, 131.6, 127.8, 122.9, 112.7, 112.5, 112.1, 100.6, 72.1, 59.3, 56.0, 39.2, 25.5; HRMS (ESI) calcd for C$_{14}$H$_{19}$N$_2$O$_3$ (M+H)$^+$ 263.1390. Found 263.1384.

4.2.2. Preparation of 14

Triflic anhydride (1.1 ml, 6.6 mmol) was added to a solution of triphenylphosphine oxide (3.6 g, 13 mmol) in anhyd chlorobenzene (30 ml) at 0°C. The reaction mixture was stirred for 15 min at 0°C, and a solution of **13a** (1.0 g, 4.4 mmol) in dry chlorobenzene (4 ml) was added. The reaction mixture was stirred for 1 h at room tempreture, and then the mixture was allowed to warm to 130°C. Stirring was continued for another 1h, then manganese dioxide (1.1 g, 13 mmol) was added. Stirring was continued for 4 h, then the reaction mixture was cooled to room temperature, and was diluted with chloroform (100 mL). The resulting mixture was filtered through celite. The filtrate was washed with saturated aqueous ammonium chloride and brine and concentrated.

Purification of the residue by column chromatography (silica gel, CHCl$_3$/AcOEt = 1/1) gave **14a** (0.12 g, 13%) as brown crystals. Mp 119.7-120.5°C (hexane/CH$_2$Cl$_2$); ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.4 (s, 1 H), 8.27 (d, J = 5.2 Hz, 1 H), 8.22 (d, J = 8.0 Hz, 1 H), 8.07 (d, J = 5.2 Hz, 1 H), 7.65 (d, J = 8.0 Hz, 1 H), 7.54 (t, J = 8.0, 1.2 Hz, 1 H), 7.24 (t, J = 8.0, 1.2 Hz, 1H), 4.88 (s, 2 H), 3.91 (s, 3 H); ^{13}C NMR: (125 MHz, CDCl$_3$): δ 141.6, 140.5, 137.8, 134.1, 129.7, 128.7, 121.9, 121.3, 120.1, 114.2, 111.8, 76.1, 59.1; HRMS (ESI) calcd for C$_{13}$H$_{13}$N$_2$O (M+H)$^+$ 213.1022. Found 213.1025.

14b[9a] and **14d**[20] were prepared according to a reported procedure. **14c** were similarly prepared from **13c** according to the procedure described for **14a,** and analytical data is given below.

14c: Mp 122.3-123.9°C; ^{1}H NMR (400 MHz, CDCl$_3$) δ 9.02 (brs, 1 H), 8.32 (d, J = 5.4 Hz, 1 H), 7.88 (d, J = 5.4 Hz, 1 H), 7.56 (d, J = 2.5 Hz, 1 H), 7.46 (d, 1 H, J = 8.8 Hz), 7.23 (dd, J = 8.8, 2.5, 1 H), 5.10 (s, 2H), 3.94 (s, 3H), 3.57 (s, 3H); ^{13}C NMR: (125 MHz, CDCl$_3$): δ 154.3, 141.8, 137.4, 135.4, 134.7, 129.5, 121.6, 118.9, 114.1, 112.6, 103.6, 59.1, 56.2, 29.9; HRMS (ESI) calcd for C$_{14}$H$_{15}$N$_2$O$_2$ (M+H)$^+$ 243.1128. Found 243.1127.

4.2.3. Preparation of 6 - 9

14a (57 mg, 0.27 mmol) and dibenzyl oxalate (0.32 g, 1.2 mmol) was stirred at 160°C for 7.5 h. Purification of the reaction mixture by column chromatography (silica gel, AcOEt/MeOH = 10/1, CHCl$_3$/MeOH = 10/1) gave **6** (65 mg, 77%) as red crystals. A small amount of the compound was recrystallized to give an analytical sample. Mp 223-231°C (decomp.) (hexane/CH$_2$Cl$_2$); ^{1}H NMR:(400 MHz, DMSO-d_6) δ 8.44 (d, J = 8.0 Hz, 1 H), 8.23 (d, J = 8.0 Hz, 1 H), 8.12 (d, J = 6.8 Hz, 1 H), 7.69 (t, J = 8.0 Hz, 1 H), 7.60 (d, J = 6.8 Hz, 1 H), 7.54 (t, J = 8.0 Hz, 1 H), 7.37 (t, J = 7.6 Hz, 2 H), 7.29 (t, J = 7.6 Hz, 1H), 7.18 (d, J = 7.6 Hz, 1 H), 5.95 (s, 2H), 3.51 (s, 3H); ^{13}C NMR: (125 MHz, DMSO-d_6): δ 165.4, 157.2, 139.5, 138.7, 137.5, 132.9, 132.8, 130.3, 128.8, 127.6, 126.0, 126.0, 125.8, 124.8, 122.9, 122.7, 116.1, 105.2, 59.2, 58.3; HRMS (ESI) calcd for C$_{22}$H$_{17}$N$_2$O$_3$ (M+H)$^+$ 357.1234. Found 357.1227.

7 was prepared according to a reported procedure.[9b,10] **8** and **9** were similarly reported from the corresponding 1-alkyl-β-carboline moieties, **14c** and **14d**, respectively, and analytical data are given below.

8: Mp 250°C (decomp.) (hexane/CH$_2$Cl$_2$/MeOH); ^{1}H NMR (500 MHz, DMSO-d_6) δ 8.30 (d, J = 9.0 Hz, 1 H), 8.11 (d, J = 6.5 Hz, 1 H), 7.84 (d, J = 2.5 Hz, 1 H), 7.59 (d, J = 6.5 Hz, 1 H), 7.37 (t, J = 7.5 Hz, 2 H), 7.29 (t, J = 7.5 Hz, 1 H), 7.26 (dd, J = 9.0, 2.5 Hz, 1 H), 7.18 (d, J = 7.5 Hz, 2 H), 5.93 (s, 2H), 3.89 (s, 3H), 3.49 (s, 3H); ^{13}C NMR (125 MHz, DMSO-d_6): δ 165.5, 157.6, 156.8, 138.5, 137.5, 133.8, 132.8, 132.6, 128.9, 128.8, 127.6, 126.0, 123.0, 117.7, 116.8, 106.5, 105.3, 59.2, 58.3, 55.8; HRMS (ESI) calcd for C$_{23}$H$_{19}$N$_2$O$_4$ (M+H)$^+$ 387.1339. Found 387.1336.

9: Mp >300°C (CH$_2$Cl$_2$); ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.32 (d, J = 8.8 Hz, 1 H), 8.24 (d, J = 6.8 Hz, 1 H), 7.87 (d, J = 2.4 Hz, 1 H), 7.59 (d, J = 6.8 Hz, 1 H), 7.39 (m, 2 H), 7.30 (m, 4 H), 5.96 (s, 1 H), 5.58 (s, 2 H), 3.90 (s, 3 H); ^{13}C NMR (125 MHz, DMSO-d_6) δ 171.1, 157.9, 156.9, 140.5, 136.8, 135.7, 134.2, 129.3, 128.4, 127.4,

126.2, 125.9, 125.5, 118.2, 117.3, 107.0, 105.2, 94.4, 56.3, 56.2; HRMS (ESI) calcd for $C_{22}H_{17}N_2O_3$ (M+H)$^+$ 357.1234. Found 357.1224.

4.2.4. Preparation of 2 - 4

8 (15.9 mg, 0.04 mmol) and hydrogenated over palladium on carbon (8.1 mg) in ethanol (6 ml) was stirred for 23h. The reaction mixture was filtrated, and concentrated. Purification of the residue mixture by column chromatography (silica gel, AcOEt/MeOH/NH$_3$ aq. = 10/1/0.1, CH$_2$Cl$_2$/MeOH/NH$_3$ aq. = 10/1/0.1) gave **3** (11.0 mg, 93%) as a brown solid. Mp 230°C (decomp.) (AcOEt/MeOH); ^{1}H NMR (500 MHz, DMSO-d_6) δ 9.98 (s, 1H), 8.77 (d, J = 5.0 Hz, 1 H), 8.37 (d, J = 9.0 Hz, 1 H), 8.17 (d, J = 5.0 Hz, 1 H), 7.97 (d, J = 2.5 Hz, 1 H), 7.33 (dd, J = 9.0, 2.5 Hz 1 H), 4.19 (s, 3 H), 3.91 (s, 3H); ^{13}C NMR (125 MHz, DMSO) δ 157.4, 156.9, 145.0, 143.2, 140.6, 134.4, 132.5, 129.0, 126.4, 126.0, 117.9, 116.7, 114.9, 107.4, 60.9, 55.9; HRMS (ESI) calcd for $C_{16}H_{13}N_2O_4$ (M+H)$^+$ 297.0870. Found 297.0863.

2 was prepared according to a reported procedure.[9b,10] **1** was similarly prepared from **6**, and it was identified by the comparison with reported NMR data. **4** was similarly prepared from **9**, and its analytical data is given below.

4: Mp 254°C (decomp.) (CH$_2$Cl$_2$/MeOH); ^{1}H NMR (500 MHz, DMSO-d_6) δ 8.67 (d, J = 5.0 Hz, 1 H), 8.40 (d, J = 9.0 Hz, 1 H), 8.03 (d, J = 5.0 Hz, 1 H), 7.95 (d, J = 2.5 Hz, 1 H), 7.33 (dd, J = 9.0, 2.5 Hz 1 H), 7.11 (s, 1 H), 3.92 (s, 3 H); ^{13}C NMR (125 MHz, DMSO-d_6) δ 158.0, 156.3, 153.8, 145.7, 138.3, 133.0, 128.5, 127.2, 126.9, 118.0, 117.4, 113.8, 110.7, 107.7, 56.2; HRMS (ESI) calcd for $C_{15}H_{11}N_2O_3$ (M+H)$^+$ 267.0764. Found 267.0760.

4.2.5. Preparation of 5 and 10

In a microwave vaial equipped a stir bar, boron tribromide (1.0 M solution in dichloromethane, 1.4 ml, 1.4 mmol) was added to a solution of **4** (49 mg, 0.14 mmol) in dry dichloromethane (2.3 ml) at room tempreture under an atmosphere of argon. The reaction mixture was stirred in a microwave resctor at for 10 min at 100°C, and added to iced water and sturated aqueous sodium bicarbonate was added. The mixture was extracted with dichloromethane. The organic phase was washed with brine and concentrated. Purification of the reaction mixture by column chromatography (silica gel, CH$_2$Cl$_2$/MeOH = 10/1) gave **5** (25 mg, 70%) as yellow crystals. A small amount of the compound was recrystallized to give an analytical sample. Mp >300°C (EtOH/H$_2$O); ^{1}H NMR (400 MHz, MeOD) δ 8.59 (d, J = 5.2 Hz, 1 H), 8.39 (d, J = 8.8 Hz, 1 H), 7.95 (d, J = 5.2 Hz, 1 H), 7.59 (d, J = 2.4 Hz, 1 H), 7.18 (dd, J = 8.8, 2.4 Hz, 1 H), 7.11 (s, 1 H); ^{13}C NMR (125 MHz, DMSO-d_6) δ 156.1, 155.6, 152.8, 145.4, 137.4, 131.7, 128.6, 127.0, 126.7, 118.2, 117.2, 113.9, 110.6, 109.2; HRMS (ESI) calcd for $C_{14}H_9N_2O_3$ (M+H)$^+$ 253.0608. Found 253.0607.

10 was similarly prepared from **9** according to the procedure described for **5**, and analytical data is given below.

10: Mp >300°C (MeOH); ^{1}H NMR (500 MHz, DMSO-d_6) δ 9.91 (s, 1 H), 8.24 (d, J = 8.5 Hz, 1 H), 8.22 (d, J = 6.5 Hz, 1 H), 7.56 (d, J = 6.5 Hz, 1 H), 7.55 (d, J = 2.5 Hz, 1 H), 7.39 (t, J = 7.5 Hz, 2 H), 7.33 (t, J = 7.5 Hz, 1

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H), 7.28 (d, J = 7.5 Hz, 2 H), 7.12 (dd, J = 8.5, 2.5 Hz, 1 H), 5.94 (s, 1 H), 5.58 (s, 2 H); ^{13}C NMR (125 MHz, DMSO-d_6)δ 170.8, 156.5, 155.7, 140.1, 136.3, 135.4, 132.9, 128.9, 128.0, 127.0, 125.9, 125.4, 125.2, 118.2, 117.0, 108.4, 104.8, 94.0, 55.9; HRMS (ESI) calcd for $C_{21}H_{15}N_2O_3$ (M+H)$^+$ 343.1077. Found 343.1071.

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