Photophysical Properties of the 2-Hydroxytryptanthrin and Its Sodium Salt as Near-infrared Dyes for Fluorescent Imaging

Jun Kawakami,*† Masahiro Takahashi,* Shunji Ito,* and Haruo Kitahara**

*Graduate School of Science and Technology, Hirosaki University, 3 Bunkyo-cho, Hirosaki, Aomori 036-8561, Japan
**Kitahara Laboratory Co. Ltd, 3 Bunkyo-cho, Hirosaki, Aomori 036–8561, Japan

2-Hydroxytryptanthrin (T2OH) and its sodium salt (sodium tryptanthrin-2-olate, T2ONa) were synthesized as near-infrared (NIR) dyes for fluorescent imaging. The absorption maxima ($\lambda_{a,max}$) of T2OH under a pH range from 1.3 to 7.2 and from 8.5 to 10.6 were ca. 410 nm and ca. 495 nm, respectively. Moreover, the fluorescence maxima ($\lambda_{f,max}$) were ca. 660 nm regardless of the pH range. T2ONa was water soluble and the $\lambda_{f,max}$ were ca. 660 nm in both aprotic and protic solvents.

Keywords Near-infrared fluorescence, fluorescent dye, 2-hydroxytryptanthrin, pH-dependent

(Received September 9, 2015; Accepted September 14, 2015; Published February 10, 2016)
was well washed by acetone. Pure T2OH (1.18 g) in 89.7% yield was obtained. The electron-spray ionization mass spectra of T2OH showed corresponding molecular ion peaks, and the 1H and 13C NMR spectra showed that the compound was synthesized correctly. T2ONa was obtained by adding excess sodium carbonate to a methanolic solution of T2OH. After filtration of the insoluble sodium carbonate, the purple solid of T2ONa precipitated upon adding hexane and some acetone to the methanolic solution.

**Measurements**

Stock solutions of T2OH and T2ONa were prepared by dissolving a weighed amount of each compound in different solvents. The UV-vis spectra (between 250 and 800 nm) of the resulting solutions were recorded at room temperature with a Jasco V-670 spectrophotometer. The fluorescence spectra were measured at between 500 and 850 nm with a Hitachi F-4500 fluorometer using an excitation wavelength (λex) of the absorption maxima or the isosbestic point. The concentration of all samples was 10 μM (M = mol dm−3), where no intermolecular interactions were observed.

**Results and Discussion**

UV-vis absorption and fluorescence spectra of T2OH in a DMSO/H2O (1/9, v/v) solution containing 0.1 M HEPES at different pH values are shown in Fig. 3. The pH was modulated...
by adding 0.1 M HCl or 0.1 M NaOH in a DMSO/H2O (1/9, v/v) solution containing 0.1 M HEPES. Under a pH range from 1.3 to 7.2, the λ\text{max} values of T2OH were ca. 410 nm, and the colors of the solutions were yellow. These absorption bands are from undissociated T2OH. On the other hand, under a pH range from 8.5 to 10.6, the λ\text{max} values were ca. 495 nm, and the colors of the solutions were red. These absorption bands are from the proton-dissociated tryptanthrin-2-olate anion (T2O⁻). However, the λ\text{max} values were ca. 660 nm, regardless of the pH. These fluorescence bands are from proton-dissociated T2O⁻. T2OH showed a strong acidity in the excited state. The absorption and fluorescence spectra of T2ONa and the photophysical properties of T2ONa are shown in Fig. 4 and Table 1. The absorption bands at ca. 410 nm are from undissociated T2ONa (T2O⁻H). On the other hand, the absorption bands at more than 450 nm are from ion-dissociated T2O⁻. The λ\text{max} of T2O⁻ in 1-butanol (1-ButOH), 2-propanol (2-ProOH), ethanol (EtOH), methanol (MeOH), and water (H2O) were between 488 and 550 nm, whereas those in N,N-dimethylformamide (DMF) and dimethylsulfoxide (DMSO) were ca. 600 nm. The λ\text{max} values were observed at longer wavelengths in aprotic solvents compared with protic solvents, and the molar absorbance coefficients (c) in aprotic solvents were larger than those in protic solvents. These results may be attributed to a lack of stabilization in the ground states by hydrogen bonding in the case of aprotic solvents. The λ\text{max} of T2ONa were ca. 660 nm in both protic and aprotic solvents. These fluorescence bands are from ion-dissociated T2O⁻ and the emitted colors were purplish-red. T2ONa demonstrated a strong ionic dissociation in the excited state both in protic and aprotic solvents. The molecular structure of T2O⁻ was calculated using density functional theory calculations. The electron densities of the corresponding HOMO and LUMO surfaces of T2O⁻ are shown in Fig. 5. The electrons are localized in the olate anion in the HOMO and in the carbonyl group of the five-membered ring in the LUMO. The electronic transitions are π–π*, originating from the olate anion. These results show that T2O⁻ has ICT characteristics. Both the λ\text{max} and λ\text{max} of T2ONa were observed at more than 597 nm in aprotic solvents. Note that T2ONa is strongly polarized in both the excited state and the ground state. The Φ values of T2ONa were between 0.04 and 0.36, and the Φ values in aprotic solvents were higher than those in protic solvents. T2ONa is water soluble, and the λ\text{max} was ca. 660 nm in both aprotic and protic solvents.

### Table 1 Absorption maxima (λ\text{max}), molar absorption coefficients (c), emission maxima (λ\text{em}), fluorescence quantum yields (Φf), and Stokes shifts of T2ONa

| Solvent  | λ\text{max} (nm) | logε (cm² mol⁻¹) | λ\text{em} (nm) | Φf | Stokes shift (cm⁻¹) |
|----------|-----------------|----------------|-----------------|----|-------------------|
| DMF      | 600             | 4.24           | 661             | 0.36 | 1538              |
| DMSO     | 597             | 4.24           | 664             | 0.33 | 1690              |
| 1-ButOH  | 533             | 3.11           | 656             | 0.11 | 3518              |
| 2-ProOH  | 550             | 3.33           | 655             | 0.12 | 2915              |
| EtOH     | 524             | 3.15           | 658             | 0.09 | 3886              |
| MeOH     | 496             | 3.10           | 658             | 0.10 | 4964              |
| H2O      | 487             | 3.01           | 662             | 0.04 | 5428              |

a. λ\text{max} of ion-dissociated T2O⁻.
b. The Φf values were determined at room temperature relative to the absolute Φf of 2-(N,N-dimethylamino)tryptanthrin in dichloromethane (Φf = 0.89) using solutions of matched absorbance (0.1 L mol⁻¹ cm⁻¹) at the excitation wavelength.

The fluorescence of T2OH and T2ONa was observed in the longer-wavelength NIR region, which is referred to as the “optical window” of cells and tissues. The existing fluorescent dyes, by expanding of π-conjugated systems, possess large molecular size and low solubility in water. Those large molecules might inhibit the biological reaction. On the other hand, T2OH and T2ONa are small organic fluorescent dyes. T2ONa is also water soluble. We expect that T2OH and T2ONa can be used as an organic fluorescent dye for fluorescent imaging probes in biological research.

### Conclusions

The fluorescence of T2OH and T2ONa was observed in the longer-wavelength NIR region, which is referred to as the 'optical window' of cells and tissues. The existing fluorescent dyes, by expanding of π-conjugated systems, possess large molecular size and low solubility in water. Those large molecules might inhibit the biological reaction. On the other hand, T2OH and T2ONa are small organic fluorescent dyes. T2ONa is also water soluble. We expect that T2OH and T2ONa can be used as an organic fluorescent dye for fluorescent imaging probes in biological research.

### Acknowledgements

This work was supported by JSPS KAKENHI Grant Number 25410137.

### References

1. A. Witt and J. Bergman, *Curr. Org. Chem.*, 2003, 7, 659.
2. L. A. Mitscher, W. C. Wong, T. De Meulenere, J. Sulko, and S. Drake, *Heterocycles*, 1981, 15, 1017.
3. J. Kawakami, N. Matsushima, Y. Ogawa, H. Kakinami, A. Nakane, H. Kitahara, M. Nagaki, and S. Ito, *Trans. Mater. Res. Soc. Japan*, 2011, 36, 603.
4. J. Kawakami, Japan Patent, 2014, 5448046.
5. J. Kawakami, H. Kawaguchi, K. Kikuchi, A. Yamaya, S. Ito, and H. Kitahara, *Trans. Mater. Res. Soc. Japan*, 2013, 38, 123.
6. J. Kawakami, A. Soma, K. Kikuchi, Y. Kikuchi, S. Ito, and H. Kitahara, *Anal. Sci.*, 2014, 30, 949.
7. K. König, *J. Microscopy*, 2000, 200, 83.
8. **T2OH**: ¹H NMR (500 MHz, DMSO-d₆) δ 7.38 (1H, dd, H₁, J = 2.8 Hz, 8.7 Hz), 7.47 (1H, td, H₄, J = 0.8 Hz, 7.5 Hz), 7.64 (1H, d, H₁, J = 2.79 Hz), 7.81 (1H, d, H₆, J = 8.7 Hz), 7.83 – 7.89 (2H, m, H₂, H₃), 8.48 (1H, dd, H₁, J = 0.8 Hz, 8.7 Hz) 10.85 (1H, s, –OH). ¹³C NMR δ 125.29, 127.18, 132.41, 138.99, 142.50, 146.12, 157.82 ppm. ¹H NMR (500 MHz, DMSO-d₆) δ 7.34 (1H, dd, H₁, J = 2.79 Hz, 8.7 Hz), 7.81 (1H, d, H₆, J = 8.7 Hz), 7.83 – 7.89 (2H, m, H₂, H₃), 8.48 (1H, dd, H₁, J = 0.8 Hz, 8.7 Hz) 10.85 (1H, s, –OH); ¹³C NMR δ 125.29, 127.18, 132.41, 138.99, 142.50, 146.12, 157.82 ppm. EI-MS m/z (M+H) calcd. 265.0613, found 265.0692.
9. **T2ONa**: ¹H NMR (500 MHz, DMSO-d₆) δ 6.59 (1H, dd, H₁, J = 2.8 Hz, 9.2 Hz), 6.81 (1H, d, H₁, J = 2.8 Hz), 7.29 – 7.43 (2H, m, H₂, H₃), 7.67 – 7.79 (2H, m, H₄, H₅), 8.48 (1H, d, H₁, J = 8.4 Hz).
10. Submitted for publication.
11. Density functional theory calculations at the B3LYP/6-31G* level were performed using the SPARTAN '10 software package (Wavefunction Inc., Irvine, CA, 2000).