Isolation of Bright Blue Fluorescent Substances from Sonochemical Hydroxylation of Methyl *p*-Cyanobenzoate

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Sonochemical hydroxylation of methyl *p*-cyanobenzoate (**1a**) in water gave a bright blue fluorescence, which are mainly ascribed to three new fluorescent compounds, 3-hydroxy, 2,3- and 2,5-dihydroxy derivatives of **1a**. Other benzenes substituted with electron-withdrawing groups also gave similar fluorescence from their hydroxylated derivatives. Among the fluorescence substances, methyl 2,5-dihydroxybenzoate was supposed to be applicable for a fluorescent chemosensor.

Importance of fluorescent chemosensors is currently increasing for monitoring of activity or concentration of biologically active substances in vitro, in vivo, inside, or outside cells. Development of new fluorescent compounds suitable for chemosensors are strongly expected. They are required to be intensely fluorescent (excitation wavelength exceeding 320 nm and emission wavelength exceeding 500 nm), chemically stable, and water-soluble.[1]

Years ago, we found that a bright blue fluorescence (λ_{max} ca. 500 nm) was emitted from an aqueous solution of methyl *p*-cyanobenzoate (**1a**) prepared by using an ultrasonic cleaner. Since **1a** itself has only a weak fluorescence at 366 nm, the blue fluorescence was supposed to be sonochemically generated. In various reports of ultrasonic hydroxylation in water,[4] only two groups have observed a fluorescence. Weissler[2] has assigned the fluorescence at 410 nm from a sonicated solution of benzoic acid to the mixture of *m*-, *o*-hydroxybenzoic acid, 3,4- and 2,5-dihydroxybenzoic acid by the comparison of Rf values of a paperchromatogram. Mason and coworkers[3] have assigned the fluorescence at 425 nm from a sonicated solution of terephthalate anion to 2-hydroxyterephthalate anion only by fluorescence spectroscopy.

The blue fluorescence from a sonicated solution of **1a** also might be identified as hydroxylation products of **1a**. However, such fluorescent derivatives of **1a** were never known. In addition, it was notable that the fluorescent property of the solution persisted for a long time at an ambient temperature in the air. In the present paper we first describe the isolation and characterization of the fluorescent substances from **1a** and then examine the formation of fluorescent substances from other simple benzenes under similar conditions to those of **1a**. Finally, we discuss the possibility of the fluorescent substances for the use as chemosensors.

An aqueous solution of **1a** (5 X 10^{-5} mol dm^{-3}) was irradiated by ultrasonic cleaner (HONDA ultrasonic multicleaner W-113, 45 kHz, 100W) at 20 °C with air-bubbling. As shown in Figure 1, a fluorescence emission above 400 nm appears after irradiation. The emission-maximum wavelength as well as the intensity varies with irradiation time. HPLC of the solution showed more than 95% of unreacted **1a** and several kinds of fluorescent and non-fluorescent species. The relative ratio of them also varied with irradiation time. It indicates that **1a** gave several fluorescent and non-fluorescent substances, some of which changed further to other species. An LC-MS total ion chromatogram (Figure 2) of a 15-h irradiated solution of **1a** shows several peaks. The largest peak at 5.0 min is assigned to **1a** from its Ms spectrum (M^+ + 1; m/z 162). Two peaks, (b) and (c), overlapping on the peak of **1a**, show a signal at m/z 194 (162 + 32), respectively and peak (a) shows a signal at m/z 178 (162 + 16), which are assigned to di- and monohydroxyl derivatives of **1a** and confirmed to be fluorescent by HPLC. In order to clarify their fluorescent

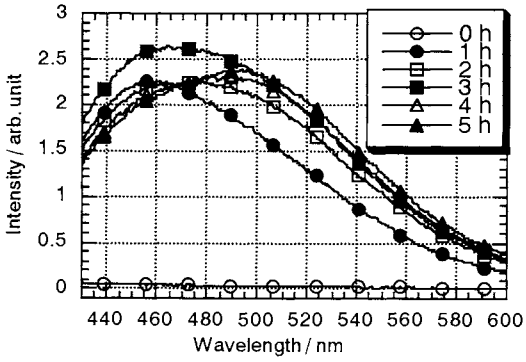


Figure 1. Fluorescence spectra of an aqueous solution of **1a** (5 X 10^{-5} mol dm^{-3}) with ultrasonic irradiation time (excited at 366 nm).

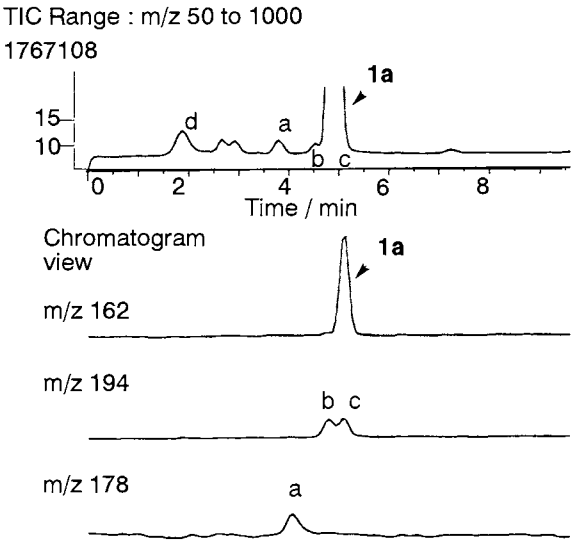


Figure 2. LC-MS (FAB) chromatogram of a supernatant of **1a** (161 mg) in water (100 mL), after 15-h ultrasonic irradiation.

properties, these components were isolated as follows: **1a** (325 mg) was sonicated in water (500 ml) for 12 h. After the removal of water and unreacted **1a**, four major components in the residue (68 mg) were separated by repeated preparative TLC. One non-fluorescent component (6 mg; peak (d) at 2.0 min in Figure 2) was identified as *p*-cyanobenzoic acid with the comparison of an authentic sample. The other three components were identified to be methyl 4-cyano-3-hydroxybenzoate (**2**, less than 1 mg; (a) in Figure 2), methyl 4-cyano-2,3-dihydroxybenzoate (**3**, less than 1 mg; (c) in Figure 2) and methyl 4-cyano-2,5-dihydroxybenzoate (**4**, 2 mg; (b) in Figure 2) from spectral analyses.[5] Compounds **2**, **3**, and **4** are the first direct evidences for the fluorescence generated by ultrasound and are new fluorescent substances, whose fluorescence spectra are shown in Figure 3.

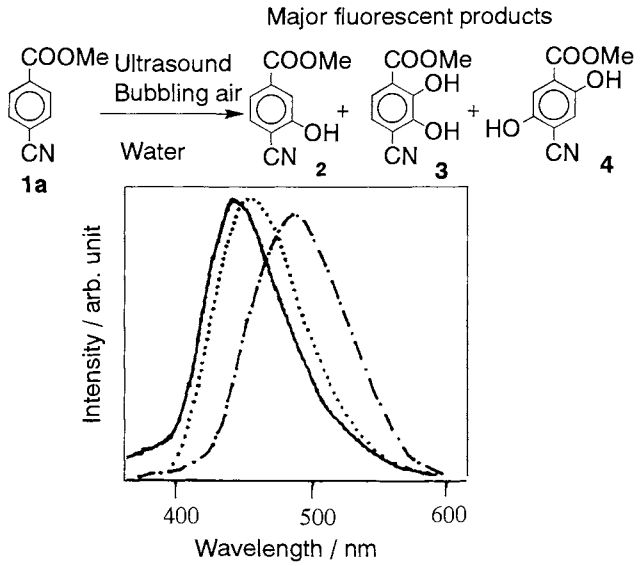
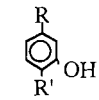
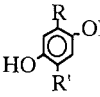


Figure 3. Fluorescence spectra of **2**(·····), **3**(—), and **4** (– · –) in water (Ex. 360 nm).

We then examined the sonochemical hydroxylation of other simple benzenes under the same conditions as in **1a**, expecting to find other new fluorescent substances. Benzenes substituted by electron-donating groups such as Me and OMe (toluene, anisole, *m*- and *p*-xylene, and mesitylene) were rapidly decomposed in several min without showing fluorescence in the visible region, whereas benzenes substituted by electron-withdrawing groups such as COOR and CN (*p*-cyanobenzoic acid (**1b**), monomethyl terephthalate (**1c**), dimethyl terephthalate (**1d**), benzonitrile (**1e**), and *p*-dicyanobenzene (**1f**); **1a** analogues) showed a blue fluorescence similar to that of **1a**, respectively and several known fluorescent hydroxylated derivatives were obtained.[7] These findings can be explained that electron-withdrawing groups deactivate benzene ring itself to the extent of making further degradation impossible.

Although the fluorescence emission maxima of the benzenes substituted by COOR and/or CN were under 400 nm, those are red shifted to the visible region by substitution of hydroxyl group(s). Table 1 shows the absorption and emission maxima of some of the mono- and dihydroxylated derivatives we obtained. Among them, compound **4** shows the largest red-shifted fluorescence at 516 nm with excitation at 360 nm (fluorescence quantum yield; 0.60, lifetime 9.6 ns).[6] In addition, despite a hydroquinone, this hydroquinone derivative was stable over one year in a refrigerator. Furthermore, solvent effect and pH dependence of the fluorescence were observed. These properties of compound **4** satisfy the criteria of fluorescent chemosensors and further investigations of compound **4** for the use as a fluorescent chemosensor are in progress.

Table 1. Emission and absorption maxima of mono- and dihydroxylated derivatives of compounds **1a**, **1c**, and **1d** in water and in ethanol

Compound			Wavelength$_{max.}$/nm	
			H_2O	EtOH
	R	R'	Em. (Abs.)	Em. (Abs.)
	COOMe	CN[a]	458 (316)	458 (316)
	COOMe	COOH	458 (316)	380,460 (324)
	COOH	COOMe	455 (316)	380, 457 (322)
	COOMe	COOMe	442 (318)	390, 454 (327)
	COOMe	CN[b]	516 (365)	430 (368)
	COOMe	COOMe	504 (360)	443 (378)

[a] Methyl 4-cyano-3-dihydroxybenzoate (**2**). [b] Methyl 4-cyano-2,5-dihydroxybenzoate (**4**).

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References and Notes

1 A. W. Czarnik, *Chem. Biol.*, **2**, 423 (1995).
2 A. Weissler, *Nature*, **193**, 1070 (1962).
3 T. J. Mason, J. P. Lorimer, D. M. Bates, and Y. Zhao, *Ultrasonics Sonochem.*, **1**, S91 (1994).
4 C. Pétrier, M-F. Lamy, A. Francony, A. Benahcene, B. David, V. Renaudin, and N. Gondrexon, *J. Phys. Chem.*, **98**, 10514 (1994); N. Serpone, R. Terzian, H. Hidaka, and E. Pelizzetti, *J. Phys. Chem.*, **98**, 2634 (1994); A. Kotronarou, G. Mills, and M. R. Hoffmann, *J. Phys. Chem.*, **95**, 3630 (1991).
5 Spectral data, Methyl 4-cyano-3-hydroxybenzoate (**2**) FAB MS (m/z): 178 (M^+ + 1, 100%), 164 (10), 130 (75); ^{1}H NMR (270 MHz, CD_3OD): δ (ppm) 7.60 (d, J_o= 8.1 Hz, 1H), 7.53 (d, J_m= 1.5 Hz, 1H), 7.48 (dd, J_o= 8.1 Hz, J_m= 1.5 Hz, 1H), 3.91 (s, 3H); ^{13}C NMR (68 MHz, CD_3OD): δ (ppm) 167.2 (-COOMe), 162.7,136.7, 134.5, 120.5, 118.1, 105.0 (ArC), 117.3 (-CN), 53.0 (-$COOCH_3$). Methyl 4-cyano-2,3-dihydroxybenzoate (**3**) FAB MS (m/z): 194 (M^+ + 1, 19%), 176 (20), 162 (100); ^{1}H NMR (270 MHz, $CDCl_3$): δ (ppm) 7.56 (d, J= 8.9 Hz, 1H), 6.90 (d, J= 8.9 Hz, 1H). Methyl 4-cyano-2,5-dihydroxybenzoate (**4**) IR (KBr): 3332 (OH), 2244 (CN), 1688 cm^{-1}(Ar-COOMe); UV (H_2O): λ_{max} (log ε) 365.2 (3.39), 294.0 (2.57), 220.4 nm (4.13); FAB MS (m/z): 194 (M^+ + 1, 100%), 177 (46), 162 (24), 135 (33); ^{1}H NMR (270 MHz, $CDCl_3$): δ (ppm) 10.30 (s, 1H, -OH···O=COMe), 7.46 (s, 1H), 7.26 (s, 1H), 3.99 (s, 3H, CH_3-COO-); ^{13}C NMR (68 MHz, CD_3OD): δ (ppm) 170 (-COOMe), 154, 153, 122, 118, 117, 107 (ArC), 116 (-CN), 53 (-$COOCH_3$).
6 The fluorescence quantum yield (using 9,10-diphenylanthracene as a chemical actinometer) and lifetime were measured in degassed EtOH.
7 Although fluorescent products from **1b** — **g** were less stable than **2**, **3**, and 4 from **1a**, 3-hydroxy-, 2,3-, 2,5-, and 3,5-dihydroxy derivatives from **1b**, **d**, and **e** could be separated in an N_2 atmosphere and identified with comparison of authentic samples by NMR spectra.