Fluorescence Properties of 2-Substituted 6-, 7- or 8-Methoxyquinoline-4-carboxylic Acid Derivatives[1)]

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2-Substituted 6-, 7- or 8-methoxyquinoline-4-carboxylic acid derivatives were synthesized. The fluorescence quantum yield (Φ) of these compounds increased in the order, 2-thioxo-, 2-methylthio-, 2-methylsulfinyl-, 2-methylsulfonyl derivatives of 6-methoxyquinoline-4-carboxylic acid. Ethyl 6-methoxy-2-methylsulfonylquinoline-4-carboxylate ($\Phi_{CH_3CN}=0.74$ or $\Phi_{EtOH}=0.69$) showed a much higher fluorescence quantum yield than those ($\Phi_{CH_3CN}=0.19$ or $\Phi_{EtOH}=0.19$ and $\Phi_{CH_3CN}=0.16$ or $\Phi_{EtOH}=0.05$) of the 7- and 8-methoxy derivatives in both acetonitrile and ethanol.

Keywords 2-substituted 6-methoxyquinoline-4-carboxylic acid; 6-methoxy-2-methylsulfonylquinoline-4-carboxylic acid; ethyl 6-methoxy-2-methylsulfonylquinoline-4-carboxylate; fluorescence property; quantum yield

Several fluorescent derivatization reagents have a moiety such as naphthol (dansyl chloride[2)] or 2-dansylethyl chloroformate hydrochloride[3)]), coumarin (7-methoxycoumarin-4-carbonyl chloride[4)] or fluoride[5)]) and quinoxaline (6,7-dimethoxy-1-methyl-2(1*H*)-quinoxalinone-3-carbonyl chloride[6)] or azide[7)]). These reagents have been developed for the determination of alcohols by high-performance liquid chromatography (HPLC). On the other hand, quinine sulfate has been widely used as a standard substance for measuring fluorescence quantum yield and fluorescence life time, and for fluorometry. But, as far as we know, no systematic examination of the fluorescence properties of quinoline derivatives has been reported.

We have already synthesized a number of 1,2-dihydro-2-thioxoquinoline-4-carboxylic acid (TQC) derivatives and described the potentiometric determination of silver protein.[8)] We found that although these TQC derivatives are almost nonfluorescent, the methylthio derivative was oxidized to form an intensely fluorescent methylsulfonyl derivative. Then, we synthesized a number of 2-substituted 6-, 7- or 8-methoxyquinoline-4-carboxylic acid derivatives (Chart 1) and measured their fluorescence quantum yields. These results indicated that ethyl 6-methoxy-2-methylsulfonylquinoline-4-carboxylate showed the highest fluorescence quantum yield in acetonitrile and ethanol. Thus, substitution of a methylsulfonyl group and a methoxyl group at the 2- and 6-positions of quinoline-4-carboxylic acid (QCA), respectively, greatly increased the fluorescence quantum yield of QCA.

Experimental

Measurements of the physical and spectral properties were performed as indicated in the footnotes of Table I. The fluorescence quantum yield was measured with a Hitachi F-4000 fluorescence spectrophotometer. All solvents (Luminazol) except EtOH for measuring fluorescence quantum yield were purchased from Wako Pure Chemical Industries.

Syntheses of QCA Derivatives 1,2-Dihydro-6-, 7- or 8-methoxy-2-thioxoquinoline-4-carboxylic Acids (**6a**, **6b** or **6c**): First, 1,2-dihydro-6-methoxy-2-oxoquinoline-4-carboxylic acid (**4a**) was synthesized according to the method of Halberkann[9)] as shown in Chart 1. However, the 7- or 8-methoxyl derivatives (**4b** or **4c**) could not be obtained by this method. Thus, **4b** or **4c** were synthesized by the oxidation of 1,2-dihydro-7- or 8-methoxy-4-methyl-2-oxoquinoline (**2b** or **2c**), respectively, with SeO_2,[10)] followed by the oxidation of **3b** or **3c** with tetrabutylammonium permanganate.[11)] Next, **4a**—**c** were refluxed in $POCl_3$ to give **5a**—**c** and then **5a**—**c** were boiled with thiourea in isopropanol to give **6a**—**c** in a modification of the previous method.[8)]

6-, 7- or 8-Methoxy-2-methylthioquinoline-4-carboxylic Acid (**7a**,**7b** or **7c**): Methyl iodide (2.3 g) was added to 1 N NaOH solution (10 ml) containing **6a**, **6b** or **6c** (1 g) and the mixture was refluxed for 1.5 h. After cooling, the reaction mixture was acidified with 10% HCl solution to give **7a**, **7b** or **7c**.

6-Methoxy-2-methylsulfinylquinoline-4-carboxylic Acid (**8a**): This compound was prepared from **7a** (0.4 g) and *m*-chloroperbenzoic acid (*m*-Cl-PBA) (0.35 g) as described for 2-methylsulfinylquinoline.[12)] The product was purified by silica gel column chromatography. The eluate was evaporated to dryness *in vacuo* and the residue was recrystallized

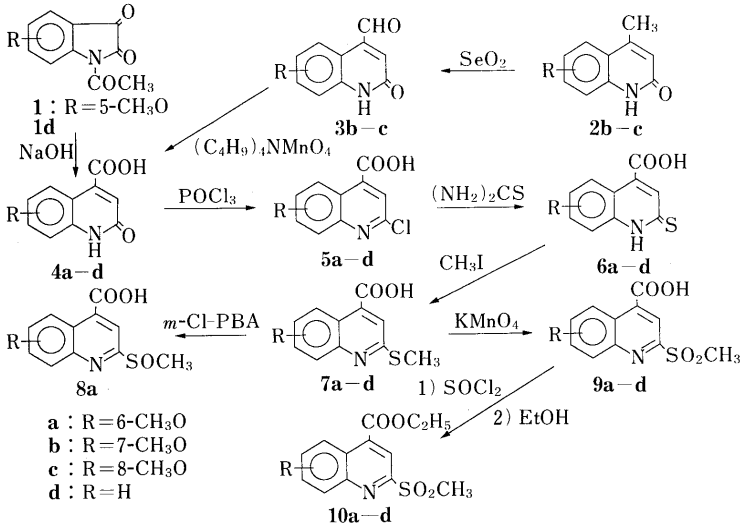

Chart 1

TABLE I. Analytical and Spectral Data of QCA Derivatives

Compd. No.	mp[a] (°C)	Yield (%)	Formula	Analysis (%) Calcd (Found) C	H	N	MS[b] m/z (M^+)	IR[c] ν_{max}^{KBr} (cm^{-1})	^{1}H-NMR (DMSO-d_6)[d] δ, ppm
6a	271—273 (dec.)	76	$C_{11}H_9NO_3S$	56.16 (56.01)	3.86 (3.75)	5.95 (5.87)	235	1676 (C=O)	3.82 (3H, s, OCH_3), 7.37, 7.62, 7.65 and 7.87 (each $1H_{arom.}$), 13.96 (2H, br, NH and COOH)
7a	205—206	85	$C_{12}H_{11}NO_3S$	57.82 (57.57)	4.45 (4.18)	5.62 (5.63)	249	1694 (C=O)	2.65 (3H, s, SCH_3), 3.88 (3H, s, OCH_3), 7.44, 7.75, 7.89 and 8.06 (each $1H_{arom.}$), 13.80 (1H, br, COOH)
8a	201—202	37	$C_{12}H_{11}NO_4S$	54.33 (54.23	4.17 4.06	5.27 5.19)	265	1712 (C=O), 1030 (SO)	2.90 (3H, s, $SOCH_3$), 3.95 (3H, s, OCH_3), 7.62, 8.08, 8.27 and 8.40 (each $1H_{arom.}$), 14.15 (1H, br, COOH)
9a	215—216	42	$C_{12}H_{11}NO_5S$	51.24 (51.35	3.94 3.86	4.98 4.93)	281	1700 (C=O), 1134, 1312 (SO_2)	3.42 (3H, s, SO_2CH_3), 3.98 (3H, s, OCH_3), 7.69, 8.19, 8.29 and 8.43 (each $1H_{arom.}$), 14.26 (1H, br, COOH)
10a[e]	133—134	75	$C_{14}H_{15}NO_5S$	54.36 (54.31	4.89 4.92	4.53 4.48)	309	1722 (C=O), 1128, 1314 (SO_2)	1.49 (3H, t, CH_2CH_3), 3.37 (3H, s, SO_2CH_3), 4.02 (3H, s, OCH_3), 4.53 (2H, q, CH_2CH_3), 7.54, 8.14, 8.35 and 8.64 (each $1H_{arom.}$)
10b[e]	161—162	65	$C_{14}H_{15}NO_5S$	54.36 (54.26	4.89 4.86	4.53 4.51)	309	1730 (C=O), 1136, 1306 (SO_2)	1.48 (3H, t, CH_2CH_3), 3.37 (3H, s, SO_2CH_3), 4.00 (3H, s, OCH_3), 4.53 (2H, q, CH_2CH_3), 7.46, 7.56, 8.45 and 8.80 (each $1H_{arom.}$)
10c[e]	139—140	68	$C_{14}H_{15}NO_5S$	54.36 (54.17	4.89 4.83	4.53 4.44)	309	1728 (C=O), 1126, 1302 (SO_2)	1.45 (3H, t, CH_2CH_3), 3.44 (3H, s, SO_2CH_3), 4.09 (3H, s, OCH_3), 4.53 (2H, q, CH_2CH_3), 7.21, 7.73, 8.40 and 8.60 (each $1H_{arom.}$)
10d[e]	91—92	85	$C_{13}H_{13}NO_4S$	55.90 (55.85	4.69 4.68	5.01 5.00)	279	1732 (C=O), 1156, 1300 (SO_2)	1.49 (3H, t, CH_2CH_3), 3.41 (3H, s, SO_2CH_3), 4.55 (2H, q, CH_2CH_3), 7.81—8.93 ($5H_{arom.}$ m)

a) Uncorrected, measured with a Yanagimoto melting point apparatus. *b*) Recorded on a JEOL DX302 spectrometer. *c*) Recorded on a Hitachi 270-30 spectrophotometer. *d*) Recorded on a JEOL JNM-GX400 spectrometer using tetramethylsilane as an internal standard. The following abbreviations are used: s, singlet; t, triplet; q, quartet; m, multiplet. *e*) ^{1}H-NMR spectra were measured in $CDCl_3$.

from CH_2Cl_2–hexane to give **8a**.

6-, 7- or 8-Methoxy-2-methylsulfonylquinoline-4-carboxylic Acids (**9a**, **9b** or **9c**): These compounds were obtained by oxidation of **7a**, **7b** or **7c** (0.2 g) with 6.6% $KMnO_4$ solution in a modification of the preparation for 2-methylsulfonylquinoline.[13] The product was recrystallized to give **9a**, **9b** or **9c**.

Ethyl 6-, 7- or 8-Methoxy-2-methylsulfonylquinoline-4-carboxylate (**10a**, **10b** or **10c**): Compound **9a**, **9b** or **9c** (0.1 g) was added to $SOCl_2$ (1 ml) and refluxed for 30 min at 80 °C. The excess $SOCl_2$ was removed by evaporation under a stream of N_2 *in vacuo*. The residue was dissoled in anhydrous benzene and this solution was added to anhydrous benzene solution containing EtOH (1 ml) and anhydrous pyridine (2 drops). The mixture was allowed to stand for 30 min at room temperature and then washed with H_2O. The separated benzene layer was dried over Na_2SO_4, the solvent was distilled off, then the residue was purified by silica gel column chromatography. The eluate was evaporated to dryness *in vacuo* to give **10a**, **10b** or **10c**.

Ethyl 2-Methylsulfonylquinoline-4-carboxylate (**10d**): This compound was synthesized from isatin (**1d**) as shown in Chart 1 according to the method described for **10a**.

Analytical and spectral data of QCA derivatives containing an S-atom at the 2-position of the quinoline ring are shown in Table I.

Measurement of Fluorescence Quantum Yield The relative fluorescence quantum yield was measured with excitation at 366 nm by using quinine sulfate solution in 0.1 N H_2SO_4 as a standard solution.[14]

Results and Discussion

Fluorescence Properties of QCA Derivatives The fluorescence spectral data of QCA derivatives are shown in Table II. The fluorescence quantum yields of 6-methoxyquinoline-4-carboxylic acid (MQC) derivatives containing a S-atom at the 2-position of the quinoline ring increased in the sequence **6a**<**7a**<**8a**<**9a** in all the solvents examined, except ethanol. From the above results, we found that **9a** showed a much higher fluorescence quantum yield than the other 2-substituted MQC derivatives. Therefore, substitution of a methylsulfonyl group at the 2-position of MQC is favorable for the fluorescence emission of MQC.

TABLE II. Fluorescence Spectral Data of QCA Derivatives in Various Solvents

Compd. No.	Quantum yield (F_{max}, nm)[a] Acetonitrile	Ethanol	Benzene	Chloroform	Cyclohexane
6a	0.01 (436)	—[b]	0.01 (436)	0.07 (444)	—[b]
7a	0.05 (460)	0.03 (425)	0.06 (456)	0.07 (465)	0.04 (440)
8a	0.21 (454)	0.04 (441)	0.29 (437)	0.23 (439)	0.05 (426)
9a	0.36 (457)	0.07 (448)	0.48 (437)	0.33 (420)	0.17 (420)
10a	0.74 (447)	0.69 (456)	0.66 (432)	0.45 (431)	0.38 (406)
10b	0.19 (464)	0.19 (470)	0.15 (435)	0.11 (437)	—[b]
10c	0.16 (535)	0.05 (545)	0.52 (495)	0.61 (497)	0.69 (460)
10d	0.11 (436)	0.04 (453)	0.06 (424)	0.05 (413)	—[b]

a) Excited at 366 nm and corrected by using rhodamine B. *b*) Not calculated.

Next, we investigated the effects of a methoxyl group located at various positions on the quinoline ring of 2-methylsulfonylquinoline-4-carboxylic acid (SQC) on the fluorescence quantum yield. Compounds **10a**, **10b** and **10c** showed much stronger fluorescence than that of **10d** in all the solvents examined, as shown in Table II. These results indicated that the substitution of a methoxyl group on the quinoline ring of SQC strongly enhanced the fluorescence of SQC derivatives. Compound **10a** or **10c** showed a higher fluorescence quantum yield than that of **10b** in benzene, chloroform and cyclohexane, in accordance with the relationship between **9a** or **9c** and **9b**. Compound **10a** showed the highest fluorescence quantum yield among these three isomers in acetonitrile and alcohol, which are widely used in reversed-phase HPLC. Therefore the substitution of a methylsulfonyl group and a methoxyl group at the 2- and 6-positions of QCA, respectively, enhanced the fluorescence quantum yield of QCA. We concluded that the acid chloride or azide of 6-methoxy-2-

methylsulfonylquinoline-4-carboxylic acid (**9a**) is likely to be a useful fluorescent derivatization reagent for alcohols.

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References and Notes

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2) K. Yamada and Y. Aizawa, *J. Pharmacol. Methods*, **9**, 1 (1983); C, F. Saller and M. J. Czupryna, *J. Chromatogr.*, **487**, 167 (1989).
3) A. Takadate, M. Iwai, H. Fujino, K. Tahara, and S. Goya, *Yakugaku Zasshi*, **103**, 962 (1983).
4) S. Goya, A. Takadate, T. Tanaka, and F. Nakashima, *Yakugaku Zasshi*, **100**, 289 (1980).
5) H. Fujino and S. Goya, *Yakugaku Zasshi*, **110**, 693 (1990).
6) T. Iwata, M. Yamaguchi, S. Hara, M. Nakamura, and Y. Ohkura, *J. Chromatogr.*, **362**, 209 (1986).
7) M. Yamaguchi, T. Iwata, M. Nakamura, and Y. Ohkura, *Anal. Chim. Acta*, **193**, 209 (1987).
8) S. Nakano, T. Yoshida, H. Taniguchi, and M. Yasuki, *Chem. Pharm. Bull.*, **28**, 49 (1980).
9) J. Halberkann, *Chem. Ber.*, **54**, 3079 (1921).
10) C. E. Kwartler and H. G. Lindwall, *J. Am. Chem. Soc.*, **59**, 524 (1937).
11) T. Sala and M. V. Sargent, *J. Chem. Soc., Chem. Commun.*, **1978**, 253.
12) B. Barlin and W. V. Brown, *J. Chem. Soc.*, **1968**, 1435.
13) E. Hayashi and T. Saito, *Yakugaku Zasshi*, **89**, 74 (1969).
14) Y. Nishikawa and K. Hiraki, "Analytical Method of Fluorescence and Phosphorescence," Kyoritu Press, Inc., 1984; C. A. Parker and W. T. Rees, *Analyst*, **85**, 587 (1960).