Synthesis of 2,3-Disubstituted 6-Aminoquinoxalines and Their Application to New Fluorescence Derivatization Reagents for Carboxylic Acids

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(Received February 1, 1996)

Fluorescent 2,3-disubstituted 6-aminoquinoxalines were synthesized by reaction of 2,3-dichloro-6-nitroquinoxaline with some nucleophiles and subsequent catalytic hydrogenation of the nitro group. Further, two of them were demonstrated to be new high-sensitive fluorescence derivatization reagents (1 fmol/1 μl injection volume) for long-chain carboxylic acids.

There are many kinds of trace amounts of bioactive substances such as carboxylic acids, alcohols, and amines in the body. The quantitative determination of them is quite important for elucidation of the physiological function, the diagnosis of sickness, the explication of the cause of disease, and the chemotherapeutic treatment. Recently, high-performance liquid chromatography (HPLC) equipped with a fluorescence detector has received much attention owing to high-sensitive determination of bioactive substances as mentioned above.[1] Indeed, 6,7-dimethoxy-1-methyl-2(1*H*)-quinoxalinone,[2] benzofurazan,[3] coumarin,[4] 4-dimethylaminobenzofuran,[5] acridine,[6] 1-oxo-2-phenyl-1,3-dihydroisoindole[7] derivatives are commercially available as fluorescence derivatization reagents. On the other hand, quinoxaline derivatives have been demonstrated to utilize as fluorescent whiteners and disperse dyes for polyester fibres.[8] However, no paper concerning the application of quinoxaline to the fluorescence derivatization reagent has been reported, to the best of our knowledge. As a part of our studies on 2,3-dichloro-6-nitroquinoxaline,[9] in this paper we describe the synthesis of fluorescent 2,3-disubstituted 6-aminoquinoxalines and their application as new fluorescence derivatization reagents for long-chain carboxylic acids.

The synthetic procedure of 2,3-disubstituted 6-aminoquinoxalines (**3a-d**) is depicted in Scheme 1. 2,3-Dichloro-6-nitroquinoxaline (**1**) was allowed to react with some nucleophiles such as amines and an alcohol to give 2,3-disubstituted 6-nitroquinoxalines (**2a-d**)[9b] in high yields. The catalytic hydrogenation of compounds (**2a-d**) with 10% Pd-C in MeOH and subsequent purification by recrystallization from AcOEt-hexane mixture or column chromatography on silica gel with $CHCl_3$:acetone-EtOH (100:5:1) mixture gave the corresponding 6-aminoquinoxaline derivatives (**3a-d**),[10] which emitted strong fluorescence.

Uv-vis and fluorescent spectral properties are summarized in Table 1. The fluorescence intensity of 2,3-dimorpholino-6-aminoquinoxaline (**3a**) in aqueous MeCN was maximal when H_2O concentration was 40% (v/v) (Figure 1). The fluorescence intensity of 2,3-dimethoxy-6-aminoquinoxaline (**3b**) gradually decreased in proportion to H_2O content, while those of other 6-aminoquinoxalines (**3c,d**) rapidly decreased. Further, the pH of the solution had greatly influence upon fluorescence intensities of 6-aminoquinoxalines. The quantum yield of fluorescence of compound (**3b**) was estimated to be about 0.8[11] in EtOH.

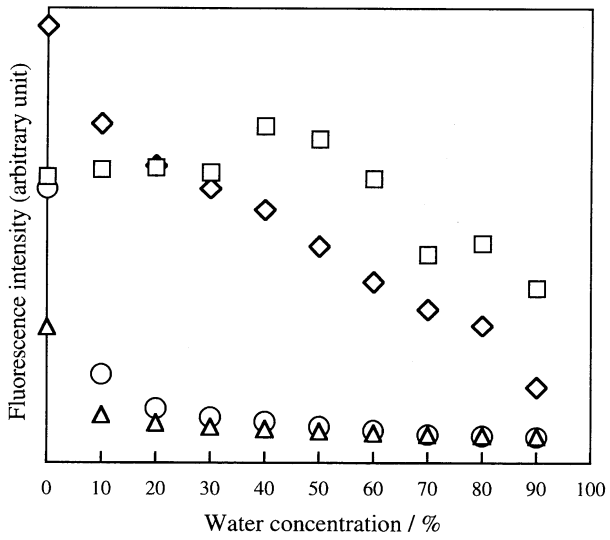
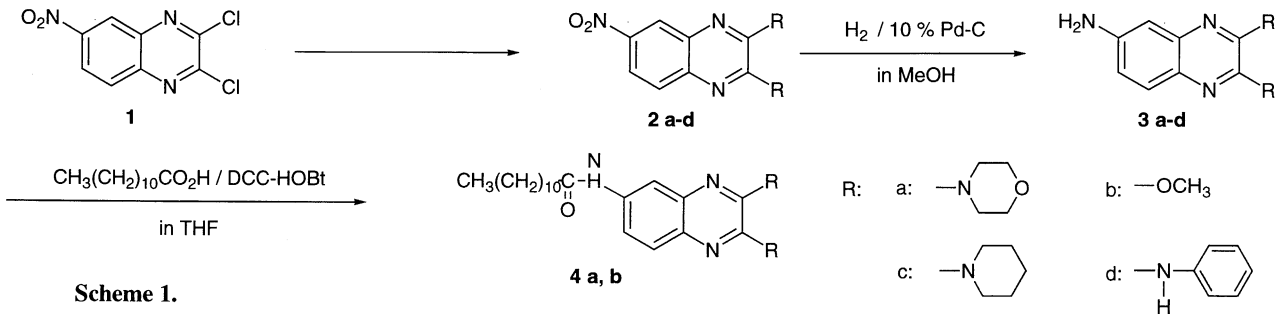


Figure 1. Effect of water concentration in aqueous MeCN on the fluorescence intensity of 6-aminoquinoxalines: compounds 3a (□); 3b (◇); 3c (△); 3d (○).

6-Aminoquinoxalines (**3a,b**) were coupled with dodecanoic acid by means of the dicyclohexylcarbodiimide (DCC)-1-hydroxybenzotriazol (HOBt) method to give 6-(dodecanoyl)-aminoquinoxalines (**4a,b**)[12] in high yields, which also emitted strong fluorescence (Scheme 1). Compounds (**4a,b**) were monitored by reversed phase HPLC equipped with a



Scheme 1.

Table 1. Absorption and fluorescence spectral data of 2,3-disubstituted 6-aminoquinoxalines

Compd.	Solvent	UV-VIS	Fluorescence	
		λ max / nm (log ε)	λ ex / nm	λ em / nm
3a	MeCN	385 (4.0)	385	468
	MeOH	382 (4.1)	385	514
3b	MeCN	356 (3.9)	355	445
	MeOH	353 (3.6)	355	477
3c	MeCN	385 (4.1)	385	469
	MeOH	385 (4.1)	385	512
3d	MeCN	390 (4.2)	390	475
	MeOH	391 (4.2)	390	520

fluorescence detector (λ_{ex} 330 nm and λ_{em} 395 nm) by using a column packed with a Finepak SIL $C_{12}S$. The detection limit of these compounds were found to be 1.0 fmol/1 μl (injection volume). Further, the coupling of a mixture of equimolar amounts of decanoic, dodecanoic, tetradecanoic, hexadecanoic, and octadecanoic acids with 2,3-dimethoxy-6-aminoquinoxaline (**3b**) by means of the DCC-HOBt method afforded the corresponding N-acyl derivatives. All the peaks were completely separated within 40 min when these N-acyl derivatives were subjected to chromatography on the reverse-phase HPLC with MeCN:H_2O (75:25) mixture as a mobile phase (Figure 2).

In conclusion, 2,3-disubstituted 6-aminoquinoxalines (**4a,b**) have been demonstrated to be applicable to new fluorescence derivatization reagents of the carboxylic acids. In addition, 6-aminoquinoxalines have the following advantages; 1) the derivatization proceeds at room temperature and 2) 6-aminoquinoxalines are quite stable toward moisture, heat, and light.

The present work was supported by the Grant-in Aid for Scientific Research No. 07651038 from the Ministry of Education, Science and Culture.

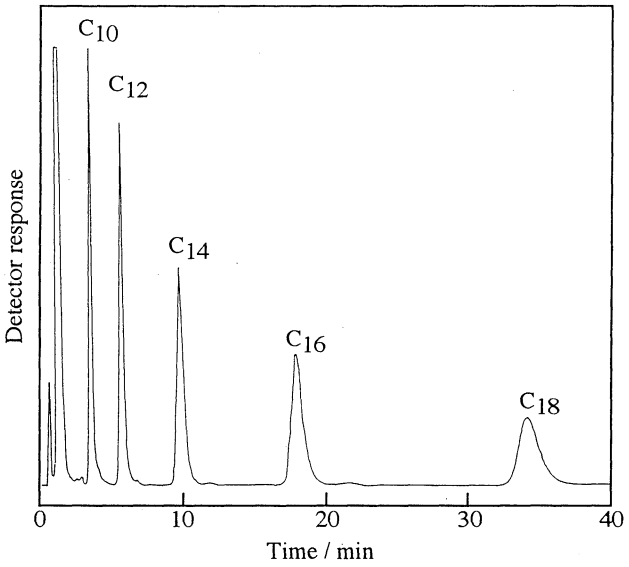


Figure 2. Chromatogram of a mixture of derivatization products; C_{10}:decanoic acid, C_{12}:dodecanoic acid, C_{14}: tetradecanoic acid, C_{16}:hexadecanoic acid, C_{18}:octadecanoic acid.

References and Notes

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10 Data of melting point, NMR, IR, and elemental analysis for (**3a-d**) are as follows. *6-Amino-2,3-dimorpholinoquinoxaline* (**3a**): yield 88%; mp 222-224 °C; ^{1}H NMR (DMSO-d_6, 270 MHz): δ 3.3 (m, 4H), 3.5 (m, 4H), 3.8 (m, 8H), 5.40 (br s, 1H), 6.71 (d, 1H, *J* = 3.1 Hz), 6.85 (dd, 1H, *J* = 3.1 and 7.4 Hz),7.38 (d, 1H,*J* = 7.4 Hz); IR (KBr, cm^{-1}): 3480 and 3380 (υNH_2); Found: C, 60.94; H, 6.71; N, 21.96%. Calcd for $C_{16}H_{21}N_5O_2$: C, 60.95; H, 6.67; N, 22.22%. *6-Amino-2,3-dimethoxyquinoxaline* (**3b**): yield 73%; mp 121-123 °C; ^{1}H NMR ($CDCl_3$, 270 MHz): δ 3.82 (br s, 2H), 4.08 (s, 3H) 4.12 (s, 3H), 6.90 (dd, 1H, *J* = 2.8 and 8.9 Hz), 6.98 (d, 1H, *J* =2.8 Hz), 7.57 (d, 1H, *J* = 8.9 Hz); IR (KBr, cm^{-1}): 3440 and 3330 (υNH_2); Found: C, 58.64; H, 5.44; N, 20.64%. Calcd for $C_{10}H_{11}N_3O_2$: C, 58.53; H, 5.40; N, 20.48%. *6-Amino-2,3-dipiperidinoquinoxaline* (**3c**): yield 89%; mp 130-132 °C; ^{1}H NMR (DMSO-d_6, 270 MHz): δ 1.56-1.78 (m, 12H), 3.39 (t, 4H,*J* =5.4 Hz), 3.52 (t, 4H, *J* =5.6 Hz), 6.81 (dd, 1H, *J* = 2.7 and 8.5 Hz), 6.92 (d, 1H, J =2.7 Hz), 7.50 (d, 1H,*J* = 8.5 Hz); IR (KBr, cm^{-1}): 3450 and 3345 (υNH_2); Found: C, 69.22; H, 8.18; N, 22.31%. Calcd For $C_{18}H_{25}N_5$: C, 69.42; H, 8.09; N, 22.49%. *6-Amino-2,3-dianilinoquinoxaline* (**3d**): yield 60%; mp 84-86 °C; ^{1}H NMR (DMSO-d_6, 270 MHz): δ 6.58 (d, 1H, *J* =2.6 Hz), 6.75 (dd, 1H, *J* = 2.6 and 8.7 Hz), 6.97 (t, 1H, *J* =7.8 Hz), 7.04 (t, 1H, *J* = 7.8 Hz), 7.28 (d, 1H, *J* = 8.7 Hz), 7.34 (t, 2H, *J* =7.8 Hz), 7.37 (t, 2H, *J* = 7.8 Hz), 7.79 (d, 2H,*J* =7.8 Hz), 7.88 (d, 2H, *J* =7.8 Hz), 8.61 (br s, 1H), 8.80 (br s, 1H); IR (KBr, cm^{-1}): 3363 and 3217 (υNH_2), 3344 (υNH); Found: C, 73.32; H, 5.23; N, 21.26%. Calcd for $C_{20}H_{17}N_5$:C, 73.38; H, 5.23; N, 21.39%.

11 The emission spectra of the sample and standard solutions were recorded at an excitation wavelength of 356 nm. The value (0.3) for anthracene in EtOH was used as the standard.

12 *6-(Dodecanoyl)amino-2,3-dimorpholinoquinoxaline* (**4a**): yield 81%; mp 140-141 °C; ^{1}H NMR ($CDCl_3$, 270 MHz): δ 0.88 (t, 3H,*J* = 8.5 Hz), 1.28 (m, 14 H), 1.67 (m, 2H), 2.40 (t, 2H,*J* =8.5 Hz), 3.55 (t, 4H,*J* =6.4 Hz), 3.62 (t, 4H, *J* = 6.4 Hz), 3.85 (m, 8H), 7.35 (br s, 1H), 7.50 (dd, 1H, *J* = 2 and 11 Hz), 7.67 (d, 1H,*J* =11 Hz), 8.02 (d, 1H, *J* =2 Hz); IR (KBr, cm^{-1}): 3269 (υNH), 657 (υCO); Found: C, 67.49; H, 8.89; N, 13.81%. Calcd for $C_{28}H_{43}N_5O_3$: C, 67.57; H, 8.71; N, 14.07%. *6-(Dodecanoyl)amino-2,3-dimethoxyquinoxaline* (**4b**): yield 87%; mp 125-127 °C; ^{1}H NMR ($CDCl_3$, 270 MHz): δ 0.83 (t, 3H, *J* =6.8 Hz), 1.26 (m, 16H), 1.73 (m, 2H) 2.40 (t, 2H,*J* =6.8 Hz), 4.11 (s, 3H), 4.17 (s, 3H), 7.56 (br s, 1H), 7 .58 (dd, 1H,*J* =2.3 and 9.0 Hz), 7.67 (d, 1H,*J* = 9.0Hz) 8.15 (d, 1H,*J* =2.3 Hz); IR (KBr, cm^{-1}): 3269 (υNH), 1656 (υCO); Found: C, 67.90; H, 8.88; N, 10.52%. Calcd for $C_{22}H_{33}N_3O_3$: C, 68.21; H, 8.53; N, 10.85%.