Fluorometric Determination of Aromatic Aldehydes with 1,4-Dimethyl-3-carbamoylpyridinium Chloride

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1,4-Dimethyl-3-carbamoylpyridinium chloride (DCP·Cl) has been synthesized as a new fluorogenic reagent for aromatic aldehydes. Aldehydes gave a green fluorescence (λ_{ex} 420–439 nm and λ_{em} 504–519 nm) after reaction with DCP·Cl at 37°C for 50 min in the presence of alkali and sodium sulfite. The method is simple and sensitive for aromatic aldehydes except for those having hydroxy or dimethylamino group. Aliphatic and arylaliphatic aldehydes and substances other than aldehydes showed no or weak fluorescence. By the proposed method, benzaldehyde, furfural and 4-methoxybenzaldehyde could be determined in the ranges 0.06–3 nmol/ml, 0.08–3 nmol/ml and 0.3–6 nmol/ml, respectively, with relative standard deviations of 0.5–2.5%.

Keywords Aromatic aldehyde, 1,4-dimethyl-3-carbamoylpyridinium chloride, fluorometry, benzaldehyde

Some aromatic aldehydes are often found in foodstuffs[1] and environmental materials such as waste by-products of manufactures.[2] In biological fluids there are many substances such as vanillylmandelic acid[3] and *p*-hydroxyphenylpyruvic acid[4] which can be easily converted to aromatic aldehydes. Enzymes such as monoamine oxidase[5] have been assayed by measuring aromatic aldehydes formed from substrates. Therefore, a sensitive and selective method for determination of aromatic aldehydes is needed. Several fluorogenic reagents such as 2-aminobenzenethiol (ABT)[6], 1,2-diaminonaphthalene (DAN)[7], 2,2'-dithiobis(1-aminonaphthalene) (DTAN)[8] and 4,5-dimethoxy-1,2-diaminobenzene (DDB)[9] have been proposed for selective determination of aromatic aldehydes at sub-nanomole levels. These reagents react with aldehydes in acidic medium to produce fluorophore; but some additional treatments are required for the fluorescence development; *e.g.*, solvent extraction[6] and addition of alkali and/or 2-mercaptoethanol.[7–9] These methods also have some defects. The reaction condition in ABT method is drastic. DAN should be handled with extreme care because of its carcinogenicity. DTAN is itself fluorescent. DDB solution must be used within 3 h after the preparation because of its low stability. Therefore we searched for a fluorogenic reaction suitable for the determination of aromatic aldehydes.

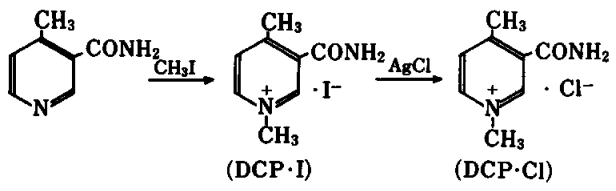

Fig. 1 Synthesis of DCP·I and DCP·Cl.

1,4-Dimethyl-3-carbamoylpyridinium chloride (DCP·Cl) and iodide (DCP·I) (Fig. 1) were designed and synthesized as a new fluorogenic reagent for our purpose from analogy with a fluorogenic reaction of α-methylene carbonyl compounds with N[1]-methylnicotinamide[10,11] and related compounds.[12] DCP halide molecules have three functional groups. These are considered to act as follows: 1) methyl group at N1 activates the reactivity of methyl group at C4 on pyridine ring[13]; 2) methyl group at C4 conjugates with aldehyde group selectively[13]; and 3) carbamoyl group at C3 makes it possible to transform the intermediate to fluorophore. The present work aimed to establish a sensitive and selective fluorometric method for determining aromatic aldehydes by using the proposed reagents.

Experimental

Chemicals and apparatus

Aldehydes used were obtained from Kanto Chemical Co., Tokyo Kasei Co. and Nakarai Chemicals Co. Stock standard solutions of aldehydes were prepared at 1 mM concentration in water whenever possible or in ethanol. Diluted solutions were prepared by using water. Water used was purified on a Milli RO–Milli Q system (Millipore Ltd.). All chemicals used were of analytical-reagent grade.

^{1}H-NMR spectra were recorded on a JEOL JNM FX-100 spectrometer operated at 100 MHz using tetramethylsilane as an internal standard (abbreviation: s, singlet; d, doublet). MS spectra were obtained with a Hitachi M-80 double-focusing mass spectrometer. A Hitachi F-3000 fluorescence spectrometer was used with a 1-cm quartz cell at room temperature. The spectral bandwidths were 5 nm for both excitation and emission. All fluorescence excitation and emission spectra were uncorrected.

Synthesis of DCP·I and DCP·Cl

DCP·I was prepared from 4-methylnicotinamide[14] (12 g) by the method of Girgis-Takla *et al.*[11] for the preparation of N^1-methylnicotinamide iodide. The product obtained was washed with small amounts of ethanol and diethyl ether. DCP·I was used without further purification (white powder, yield 77%): mp 202–204°C (dec., uncorrected); ^{1}H-NMR (in CF_3COOD) δ 2.28 (3H, s), 4.49 (3H, s), 7.96 (1H, d, J=6.3 Hz), 8.62 (1H, d, J=6.3 Hz), 8.96 (1H, s); MS m/z: 150 ([M-HI]$^+$); Anal. Calcd for $C_8H_{11}N_2OI$: 34.55% C, 3.99% H, 10.07% N, 45.63% I; found: 34.60% C, 4.05% H, 9.91% N, 45.74% I.

DCP·Cl was prepared from DCP·I by the method of Karrer *et al.*[15] for the preparation of N^1-methylnicotinamide chloride. To a solution of DCP·I (10 g) in water (50 ml) was added silver chloride (15.4 g); the mixture was stirred for 2 h at room temperature. After removing the precipitate, the aqueous solution was evaporated to dryness under reduced pressure. DCP·Cl obtained was used without further purification (white powder, yield 95%): mp 209–211°C (dec., uncorrected); ^{1}H-NMR (in CF_3COOD) δ 2.86 (3H, s), 4.48 (3H, s), 7.98 (1H, d, J=6.3 Hz), 8.63 (1H, d, J=6.3 Hz), 8.92 (1H, s); MS m/z: 150 ([M-HCl]$^+$); Anal. Calcd for $C_8H_{11}N_2OCl$: 51.48% C, 5.94% H, 15.01% N, 19.00% Cl; found: 50.63% C, 5.95% H, 14.93% N, 18.66% Cl.

Recommended procedure

To 1 ml of sample solution in a 1.5-ml glass-stoppered test tube are added 50 µl of 0.4 M DCP·Cl in water and 100 µl of 1.5 M aqueous sodium hydroxide solution containing 20 mM sodium sulfite. The mixture is then incubated for 50 min at 37°C. After cooling to room temperature, the fluorescence intensity was measured with excitation at 436 nm and emission at 506 nm.

Results and Discussion

Reaction conditions

In preliminary experiments using formaldehyde, benzaldehyde, acetophenone, benzoic acid and benzyl alcohol as test compounds, it was found that only benzaldehyde gave an intense green fluorescence under 365 nm UV light after reaction with DCP·Cl or DCP·I in the presence of alkali. Formaldehyde gave a weak fluorescence. Other substances and a reagent blank gave a negligible faint blue fluorescence.

When 1,2-dimethyl-3-carbamoylpyridinium iodide, which was prepared from 2-methylnicotinamide[16] and methyl iodide was used as a reagent, benzaldehyde showed no fluorescence. Benzaldehyde gave also no fluorescence by reaction with 1,4-dimethylpyridinium iodide.[17] The presence of methyl group at C4 and carbamoyl group at C3 on the pyridine ring is considered essential for the fluorescence development of benzaldehyde, though details on the reaction product are not clear.

Thus DCP halides seemed applicable to the determination of aromatic aldehydes with good sensitivity and selectivity. Reaction conditions for determining them were examined by taking benzaldehyde as a standard.

In the reaction of benzaldehyde, the fluorescence intensity obtained with DCP·Cl was about twice as much as that obtained with DCP·I. This phenomenon can probably be attributed to the quenching effect of iodide ion, because addition of iodide ion (sodium iodide) corresponding to DCP·I concentration to the final reaction mixture obtained from benzaldehyde with DCP·Cl caused a similar decrement of the fluorescence intensity. Thus DCP·Cl was used in the present study.

Benzaldehyde reacted with DCP·Cl in dilute alkaline solution but not in neutral and acidic solutions. The reaction was also observed to occur in the presence of organic amines such as piperidine and diethylamine,

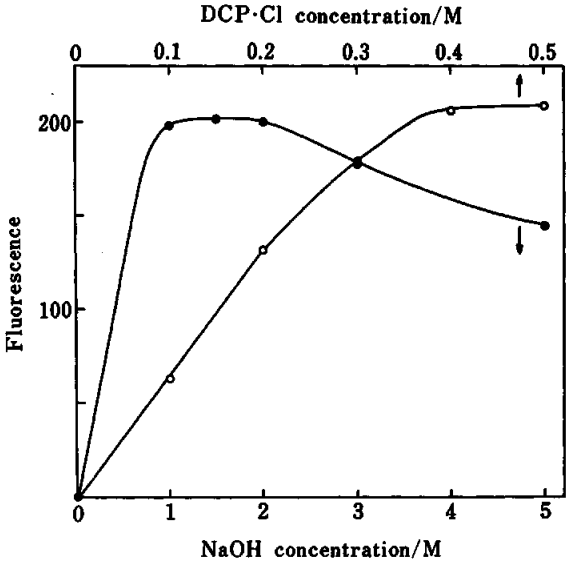

Fig. 2 Effects of sodium hydroxide and DCP·Cl concentrations on the fluorescence reaction of benzaldehyde: (●) sodium hydroxide, (○) DCP·Cl. Benzaldehyde (2 nmol/ml) was treated by the recommended procedure, except that 1.5 M sodium hydroxide or 0.4 M DCP·Cl was replaced by varying concentrations of the reagents.

but these amines gave a higher blank fluorescence intensity. Thus sodium hydroxide was selected. Figure 2 shows the effects of sodium hydroxide and DCP·Cl concentrations. Constant fluorescence intensities were obtained over the concentration ranges from 1 to 2 M for the former and 0.4 to 0.5 M for the latter. Therefore the use of 1.5 M sodium hydroxide and 0.4 M DCP·Cl was recommended.

Reducing agents such as sodium sulfite, sodium bisulfite and sodium pyrosulfite decreased the blank fluorescence intensity. The potency of these reagents for this effect was almost the same; so sodium sulfite was arbitrarily used. The blank fluorescence intensity decreased with an increase in sodium sulfite concentration, but the fluorescence intensity of benzaldehyde also decreased at more than 50 mM concentration; so 20 mM sodium sulfite was used. The blank fluorescence intensity under this condition was about 50% of the value obtained in its absence. Sodium hydroxide and sodium sulfite could be used conveniently in a mixture.

Next, the effects of reaction temperature and time were studied. The results obtained are shown in Fig. 3. Although the reaction of benzaldehyde occurred even at room temperature, the reaction was accelerated with the increase of temperature. However above 50°C, the fluorescence decay was also accelerated, and higher temperatures gave higher blank fluorescence intensities. At 37°C, the fluorescence intensity reached a constant value after 40 - 60 min. Thus the reaction at 37°C for 50 min was selected.

The effect of pH on the fluorescence properties of the reaction mixture resulting from benzaldehyde was studied. When varying pH values of buffers were added to the reaction mixture, three types of fluorescence characteristics were observed. Fluorescence maxima were found at λ_{ex} 390 nm and λ_{em} 460 nm in strongly acidic media (below pH 0.7), at λ_{ex} 390 nm and λ_{em} 506 nm at around pH 2 - 4, and at λ_{ex} 436 nm and λ_{em} 506 nm in alkaline region above pH 7. To select the best analytical condition, the fluorescence properties of the reaction mixture itself (recommended conditions) were compared with those obtained by using various inorganic and organic acids (5 M solution, 500 µl) in place of buffers for convenience. In general, higher fluorescence intensity and more stability were obtained by addition of acids. But the optimal condition was determined as the alkaline condition because it gave the highest ratio of test to blank in the fluorescence; the alkaline condition was simpler as well. The fluorescence was stable for 30 min at room temperature. If desired, the use of acetic acid was able to prevent the fluorescence decrement over several hours. The determination range of benzaldehyde by using acetic acid (λ_{ex} 390 nm and λ_{em} 506 nm) was comparable to that of the recommended condition.

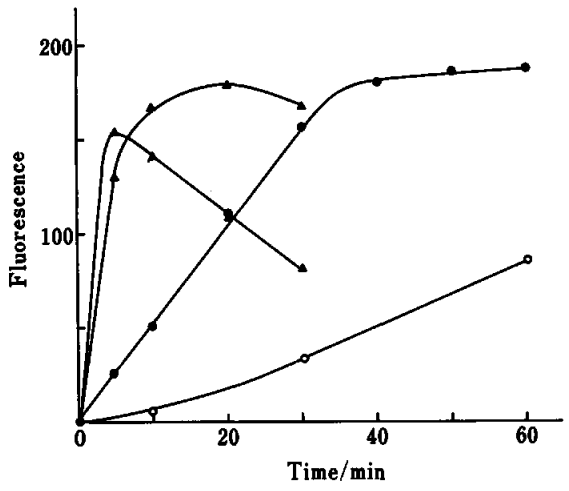

Fig. 3 Effects of reaction temperature and time on the fluorescence reaction of benzaldehyde: (○) room temperature, (●) 37°C, (△) 50°C, (▲) 70°C. Benzaldehyde (2 nmol/ml) was incubated at various temperatures and times. Other conditions are the same as in the recommended procedure.

Fluorescence from aldehydes and other substances

The fluorescence characteristics and intensities of 27 aldehydes obtained by the recommended procedure are summarized in Table 1. All aldehydes listed had similar spectra as to the maximal wavelengths for fluorescence excitation and emission, but with pronounced differences in relative fluorescence intensity. In general, the proposed method is sensitive for aromatic aldehydes but not for aliphatic and arylaliphatic aldehydes. Although benzaldehyde derivatives having a dimethylamino or hydroxy group such as 4-dimethylaminobenzaldehyde, 2-, 3- and 4-hydroxybenzaldehydes, 3-hydroxy-4-methoxybenzaldehyde and 4-hydroxy-3-methoxybenzaldehyde gave no fluorescence at a concentration of 1 µmol/ml, it was found that addition of methanesulfonyl chloride was effective for the fluorescence development of benzaldehydes having hydroxy group. When the reactions of these aldehydes were performed in the presence of 3 µl of methanesulfonyl chloride, the fluorescence intensities obtained were 3.6 - 58% of the value for benzaldehyde obtained by the recommended procedure (Table 2).

Benzoic acid, benzyl alcohol, acetophenone, phenol, 11 different amino acids (asparagine, aspartic acid, cysteine, histidine, leucine, lysine, methionine, phenylalanine, proline, threonine and tryptophan), glutathione, pyruvic acid, oxalacetic acid, α-ketoglutaric acid and saccharose showed no fluorescence at a concentration of 1 µmol/ml. Glucose and maltose gave a fluorescence; the intensities of 0.3 µmol/ml solution were only twice the blank value.

Calibration curves

Calibration curves were constructed for benzaldehyde, 4-methoxybenzaldehyde and furfural by the recommended procedure. The fluorescence intensities

Table 1 Relative fluorescence intensities and maximal wavelengths for some aldehydes

Aldehyde[a]	λ_{max}/nm		RFI[b]
	Ex	Em	
Benzaldehyde	436	506	100
2-Chlorobenzaldehyde	435	504	108
3-Chlorobenzaldehyde	437	505	105
4-Chlorobenzaldehyde	437	506	90
2-Methylbenzaldehyde	438	507	93
3-Methylbenzaldehyde	437	507	94
4-Methylbenzaldehyde	438	508	73
2-Methoxybenzaldehyde	437	506	78
3-Methoxybenzaldehyde	438	507	89
4-Methoxybenzaldehyde	438	509	20
o-Phthalaldehyde	420	507	0.4
Terephthalaldehyde	437	508	92
2,6-Dichlorobenzaldehyde	442	517	4.0
2,3-Dimethoxybenzaldehyde	438	509	26
3,4-Dimethoxybenzaldehyde	437	507	0.1
1-Naphthaldehyde	437	507	84
2-Naphthaldehyde	438	507	84
Pyridine-3-aldehyde	437	504	106
Pyridine-4-aldehyde	437	504	103
Furfural	436	506	67
Phenylacetaldehyde	437	510	1.2
trans-Cinnamaldehyde	438	511	7.7
Formaldehyde	439	519	0.1
Acetaldehyde	435	510	0.4
Propionaldehyde	437	510	0.5
Acrolein	437	510	1.4
Crotonaldehyde	437	510	2.0

a. Aldehydes (2 - 100 nmol/ml) were treated by the recommended procedure.
b. Relative fluorescence intensity at maximal wavelengths; benzaldehyde is arbitrarily taken as 100. Each value represents the mean of three runs.

Table 2 Relative fluorescence intensities and maximal wavelengths for benzaldehydes having hydroxy group

Aldehyde[a]	λ_{max}/nm		RFI[b]
	Ex	Em	
2-Hydroxybenzaldehyde	431	502	3.6
3-Hydroxybenzaldehyde	435	505	58
4-Hydroxybenzaldehyde	438	507	43
3-Hydroxy-4-methoxybenzaldehyde	432	502	30
4-Hydroxy-3-methoxybenzaldehyde	438	507	28

a. Aldehydes (2 nmol/ml) were treated by the recommended procedure in the presence of 3 μl of methanesulfonyl chloride.
b. Relative fluorescence intensity at maximal wavelength. The value obtained from benzaldehyde by the recommended procedure is arbitrarily taken as 100. Each value represents the mean of three runs.

were proportional to the sample concentrations in the range 0.06 - 3 nmol/ml for benzaldehyde, 0.3 - 6 nmol/ml for 4-methoxybenzaldehyde and 0.08 - 3 nmol/ml for furfural. The relative standard deviations (n=9) were 1.4% (at 0.1 nmol/ml) and 2.1% (at 2 nmol/ml) for benzaldehyde, 2.5% (at 0.5 nmol/ml) and 0.6% (at 4 nmol/ml) for 4-methoxybenzaldehyde and 2.1% (at 0.2 nmol/ml) and 0.5% (at 2 nmol/ml) for furfural. When the reaction was carried out in the presence of methanesulfonyl chloride (3 μl), 4-hydroxybenzaldehyde could be determined in the range 0.25 - 10 nmol/ml with the relative standard deviations (n=9) of 5.4% (at 0.5 nmol/ml) and 3.8% (at 6 nmol/ml).

The DCP·Cl method is one of the best methods for determining aromatic aldehydes because of its high sensitivity, good reproducibility and simplicity, although 4-hydroxybenzaldehyde can also be determined by DTAN method[8] with high sensitivity. Selectivity of the proposed method for aromatic aldehydes is comparable to the methods with ABT[6], DTAN[8] and DDB[9], and higher than that of the method with DAN[7] which gives positive results with many aliphatic aldehydes. Although the fluorophore stability of the present method is inferior to that for other methods such as ABT and DAN method, it suffices for usual measurements. If desired, the fluorescence can be stabilized by addition of acetic acid.

References

1. W. R. LaCourse and I. S. Krull, *Anal. Chem.*, **59**, 49 (1987).
2. H. T. Hoffman, Jr., *J. Chromatogr.*, **194**, 228 (1980).
3. J. J. Pisano, J. R. Crout and D. Abraham, *Clin. Chim. Acta*, **7**, 285 (1962).
4. I. J. Holcomb, D. S. McCann and A. J. Boyle, *Anal. Chem.*, **37**, 1657 (1965).
5. C. M. McEwen and J. D. Cohen, *J. Lab. Clin. Med.*, **62**, 766 (1962).
6. T. Uno and H. Taniguchi, *Bunseki Kagaku*, **21**, 76 (1972).
7. Y. Ohkura and K. Zaitsu, *Talanta*, **21**, 547 (1974).
8. Y. Ohkura, K. Ohtsubo, K. Zaitsu and K. Kohashi, *Anal. Chim. Acta*, **99**, 317 (1978).
9. M. Nakamura, M. Toda, H. Saito and Y. Ohkura, *Anal. Chim. Acta*, **134**, 39 (1982).
10. H. Nakamura and Z. Tamura, *Anal. Chem.*, **50**, 2047 (1978).
11. P. Girgis-Takla and I. Chroneos, *Analyst* [London], **104**, 117 (1979).
12. A. Sano, Y. Asabe, M. Suzuki and S. Takitani, *Bunseki Kagaku*, **32**, E93 (1983).
13. A. P. Phillips, *J. Org. Chem.*, **14**, 302 (1949).
14. J. M. Bobbitt and D. A. Scola, *J. Org. Chem.*, **25**, 560 (1960).
15. P. Karrer, G. Schwarzenbach, F. Benz and U. Solmssen, *Helv. Chim. Acta*, **19**, 811 (1936).
16. A. Dornow, *Ber.*, **73**, 78 (1940).
17. E. A. Covlson and J. I. Jones, *J. Soc. Chem. Ind.*, **65**, 169 (1946).

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