

Fluorogenic derivatization of aryl halides based on the formation of biphenyl by Suzuki coupling reaction with phenylboronic acid

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Abstract

The fluorogenic derivatization method for aryl halide was developed for the first time. This method was based on the formation of fluorescent biphenyl structure by Suzuki coupling reaction between aryl halides and non-fluorescent phenylboronic acid (PBA). We measured the fluorescence spectra of the products obtained by the reaction of *p*-substituted aryl bromides (*i.e.*, 4-bromobenzonitrile, 4-bromoanisole, 4-bromobenzoic acid ethyl ester and 4-bromotoluene) with PBA in the presence of palladium (II) acetate as a catalyst. The significant fluorescence at excitation maximum wavelength of 275-290 nm and emission maximum wavelength of 315-350 nm was detected in all the tested aryl bromides. This result demonstrated that non-fluorescent aryl bromides could be converted to the fluorescent biphenyl derivatives by the coupling reaction with non-fluorescent PBA. We tried to determine these aryl bromides by HPLC-fluorescence detection with pre-column derivatization. The aryl bromide derivatives were detected on the chromatogram within 30 min without any interfering peak derived from the reagent blank. The detection limits ($S/N=3$) for aryl bromides were 13-157 fmol/injection.

Keywords: Fluorescence derivatization, Phenylboronic acid, Aryl halide, Fluorogenic, Suzuki coupling reaction

1. Introduction

Fluorescence detection is applied to analyze trace amount of compounds in combination with high-performance liquid chromatography (HPLC) or capillary electrophoresis (CE) because it has high sensitivity and selectivity. However, most of compounds are non-fluorescent or weakly fluorescent. Therefore, fluorescence derivatization reagents have been developed for conversion of non- or weakly fluorescent compounds to highly fluorescence compounds. Up till now, various fluorescence derivatization reagents for a wide variety of functional groups, such as amine, phenol, thiol, aldehyde, ketone and carbonyl groups have been developed [1-4]. However, it is difficult to derivatize the compounds that do not have derivatizable functional group with a typical fluorescence derivatization reagent. We recently developed the fluorescent aryl boronic acid, 4-(4,5-diphenyl-1*H*-imidazol-2-yl)phenylboronic acid (DPA, a lophine derivative) as a fluorescence derivatization reagent for aryl halides [5]. The derivatization reaction with DPA is based on Suzuki coupling reaction [6], which is a palladium-catalyzed cross-coupling reaction of aryl halides with aryl boronic acids. DPA can convert compounds having an aryl halide in their structures into fluorescent compounds, even in the absence of the derivatizable functional groups for a conventional derivatization reagent. Furthermore, we found that Suzuki coupling proceeded well without the interference with biological compound and this was the practical reaction for biomedical analysis. By using DPA, we have developed the determination method for aryl halide drugs including clofibrate [5], haloperidol [7] and hydroxyzine [8] in

biological fluids. However, DPA has strong fluorescence itself and sometimes interfere the detection of target derivatives

On the other hand, fluorescence derivatization reagents are categorized in two groups *i.e.*, fluorescence labeling reagent and fluorogenic derivatization reagent [9]. The fluorescent labeling reagent is composed of strong fluorophore and reactive group for reacting with the functional group of the target compounds. In contrast to fluorescent labeling reagent like DPA, fluorogenic derivatization reagent is non-fluorescence itself; however, it reacts with target compound and form fluorescent structure. The fluorogenic derivatization reagent can greatly reduce the interference from fluorescence of the reagent compared with fluorescence labeling reagent. Therefore, we attempted to develop the fluorogenic derivatization reagent for aryl halides based on Suzuki coupling reaction. We selected phenylboronic acid (PBA) as a fluorogenic derivatization reagent for aryl halides. PBA is non-fluorescent mono substituted benzene and the boronic acid works for derivatization reaction. It was expected that the aryl halides reacted with PBA to form fluorescent biphenyl derivatives (Fig. 1). In this study, we employed *p*-substituted aryl bromide as a model compound of aryl halide. The fluorescence spectra of the products obtained by the reaction of *p*-substituted aryl bromide with PBA were measured. Subsequently, we established pre-column derivatization HPLC method for aryl bromides to evaluate the analytical performance of the proposed derivatization reaction.

2. Experimental

2.1 Material and reagents

PBA, 4-bromobenzonitrile (BrPhCN), 4-bromoanisole (BrPhOCH₃), 4-bromobenzoic acid ethyl ester (BrPhCOOC₂H₅) and 4-bromotoulene (BrPhCH₃) were purchased from Tokyo Chemical Industries (Tokyo, Japan). 4-Phenylbenzonitrile and 4-methoxybiphenyl were from Sigma (St. Louis, MO, USA). Palladium (II) acetate (Pd(OAc)₂), potassium fluoride (KF), *N,N*-dimethylformamide (DMF) were from Nacalai Tesque (Osaka, Japan). Acetonitrile and dioxane were from Kanto Chemical (Tokyo, Japan) and Wako (Tokyo, Japan), respectively. Water was distilled and passed through a Pure Line WL21P system (Yamato, Tokyo, Japan). All other chemicals were the highest purity and quality available.

2.2 Fluorescence spectra measurement of the products by the reaction of aryl bromides with PBA

To the dioxane solution including aryl bromides (50 μL), 7.5 mM PBA in dioxane (50 μL), 3.0 mM Pd(OAc)₂ in dioxane and aqueous solution of 30 mM KF (50 μL) were successively added and mixed well. After purging with N₂ (5.0 mL/s) for 5 s, the reaction mixture was heated at 100 °C for 30 min. After cooling, the reaction mixture was diluted 10 times with a mixture of acetonitrile and 5 mM phosphate buffer (pH 5.5) (50:50, v/v) for the fluorescence measurement. Fluorescence spectra and intensities were measured with a 650-10S fluorescence spectrophotometer (Hitachi, Tokyo, Japan).

2.3 Pre-column derivatization HPLC method for aryl bromides

The HPLC system consisted of an pump LC-6A (Shimadzu, Kyoto, Japan), a Shimadzu RF-550 fluorescence detector, a 7125 injector with a 20- μ L loop (Rheodyne, Cotati, CA, USA), and a FBR-1 recorder (Tosoh, Tokyo, Japan). Chromatographic separation was performed on a Cosmosil 5C18MS (250 mm x 4.6 mm, i.d., Nacalai tesque) by an isocratic elution with a mixture of acetonitrile and 5 mM phosphate buffer (pH 5.5) (60:40, v/v) at a flow rate of 1.0 ml/min. The fluorescence wavelength of the detector was changed according to the time program to detect each derivative at a maximum wavelength.

For the derivatization for HPLC analysis, DMF was employed to prepare each solution of aryl bromides, PBA and Pd(OAc)₂ because the peak heights of derivatives obtained using DMF were slightly higher than those obtained using dioxane. A 20- μ L portion of the resultant reaction mixture after passing through a membrane filter (0.45 μ m, HLC-DISK 3, Kanto chemical) was injected into the HPLC system.

Calibration curves were prepared by analyzing five or six different concentrations of standard aryl bromide solutions in triplicate. The detection limits were defined as the concentration corresponding to a signal three times the noise level.

3. Results and Discussion

Fig. 2 shows the fluorescence spectra of the reaction mixture of BrPhCOOC₂H₅ with PBA. The fluorescence was observed at excitation and emission maximum of 290 nm and 350 nm, respectively. Hence, this result presented that BrPhCOOC₂H₅ was

converted to fluorescent compound by the reaction with PBA. On the other hand, the significant fluorescence was not observed from the reagent blank. Table 1 shows the fluorescence characteristics of the products obtained by the reaction of four aryl bromide with PBA. All of the products emit the fluorescence at excitation of 275-290 nm and emission of 315-350 nm. Because these excitation and emission wavelengths were very similar to those of biphenyl, it was strongly suggested that aryl bromides were converted to biphenyls by Suzuki coupling reaction with PBA.

BrPhCN and BrPhCOOC₂H₅, which have electron-withdrawing group at *p*-position of bromo group, emit stronger fluorescence at relatively longer wavelength regions than BrPhOCH₃ and BrPhCH₃, having electron-donating group. To clarify whether the different fluorescence characteristics was due to the difference of reaction yield or the fluorescence intensity of the products, we measured the fluorescence intensities of standard solution of 4-phenylbenzotrile and 4-methoxybiphenyl, that are possible reaction products of BrPhCN and BrPhOCH₃ with PBA, respectively. As a result, the fluorescence intensity of 4-phenylbenzotrile was 3 times higher than that of 4-methoxybiphenyl. Therefore, the fluorescence intensity of the products can be attributed to the fluorescence characteristics of each product.

Fig. 3(A) and (B) show the chromatograms obtained by the injection of the reagent blank (PBA) and reaction mixture of aryl bromides with PBA into HPLC system, respectively. Under the HPLC conditions described in experimental section, all the peaks derived from aryl bromides were detected within 30 min. On the other hand, no reagent blank peaks significantly interfering the detection of the aryl bromides were observed on the chromatogram because the PBA is non-fluorescent. In order to confirm that the peaks

at 12 and 17 min in Fig. 3(B) were derived from BrPhCN and BrPhOCH₃, authentic 4-phenylbenzotrile and 4-methoxybiphenyl that corresponds to each derivative were injected into the HPLC system. As shown in Fig. 3(C), the retention times of 4-phenylbenzotrile and 4-methoxybiphenyl were identical with those of the derivative of BrPhCN and BrPhOCH₃, respectively, which revealed that aryl bromide was converted to biphenyl by the reaction with PBA. The reaction yields for BrPhCN and BrPhOCH₃, were 100 and 97%, respectively, calculated by comparing the peak heights of the reaction products and authentic 4-phenylbenzotrile and 4-methoxybiphenyl. The derivatization reaction for aryl bromides proceeded with excellent yield. On the other hand, the reaction yields for 4-iodobenzotrile and 4-chlorobenzotrile, both of which produce 4-phenylbenzotrile by reaction with PBA, were 100 and 50%, respectively. The tendency of the reactivity was agreed with the results of our previous report by using DPA [5]. The proposed derivatization method can also be applied to aryl iodide and even aryl chloride.

Calibration curves were prepared with a standard mixture of aryl bromides and good linearities ($r^2 > 0.999$) were observed between the fluorescence intensity as peak height and concentration of aryl bromides up to 50 μM (Table 2). The detection limits of standard aryl bromides ranged from 13 to 157 fmol/injection at a signal-to-noise ratio (S/N) of 3. The sensitivities of BrPhCH₃ and BrPhOCH₃ of the proposed method were 3 and 10 times higher than those of the fluorescence labeling method with DPA [5]. The repeatability of the proposed method was examined at 1 μM and 5 μM of aryl bromides. The relative standard deviations for within-day ($n = 5$) and between-day ($n = 5$) runs were less than 7.8 and 8.6%, respectively, for all the analytes.

4. Conclusion

In this study, we reported a novel fluorogenic derivatization reaction for aryl halides based on the formation of biphenyl derivative by Suzuki coupling reaction of aryl halides with PBA. Because PBA is non-fluorescent compounds, the interference derived from the reagent can be negligible. The pre-column derivatization HPLC based on the proposed reaction allowed the sensitive detection of aryl bromides with the detection limits of 13-157 fmol/injection. We previously demonstrated that Suzuki coupling reaction was well applied to biomedical analysis [5, 7-8], and therefore the fluorogenic derivatization reaction will become a promising tool for the development of sensitive determination method for aryl halides.

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Figure captions

Fig. 1. Scheme of the proposed fluorogenic derivatization reaction for aryl bromide.

Fig. 2. Fluorescence spectra obtained from the reaction mixture of $\text{BrPhCOOC}_2\text{H}_5$ with PBA and the reagent blank. Excitation spectra with the emission wavelength at 350 nm of the product (A, solid line) and the reagent blank (B, dashed line). Emission spectra with the excitation wavelength at 290 nm of the product (C, solid line) and the reagent blank (D, dashed line).

Fig. 3 Chromatograms of (A) reagent blank, (B) reaction mixture and (C) authentic biphenyls. Peaks: 1, BrPhCN derivative; 2, BrPhOCH_3 derivative; 3, $\text{BrPhCOOC}_2\text{H}_5$ derivative; 4, BrPhCH_3 derivative; 1' 4-phenylbenzotrile; 2', 4-methoxybiphenyl. Sample concentration: 25 μM for all compounds.

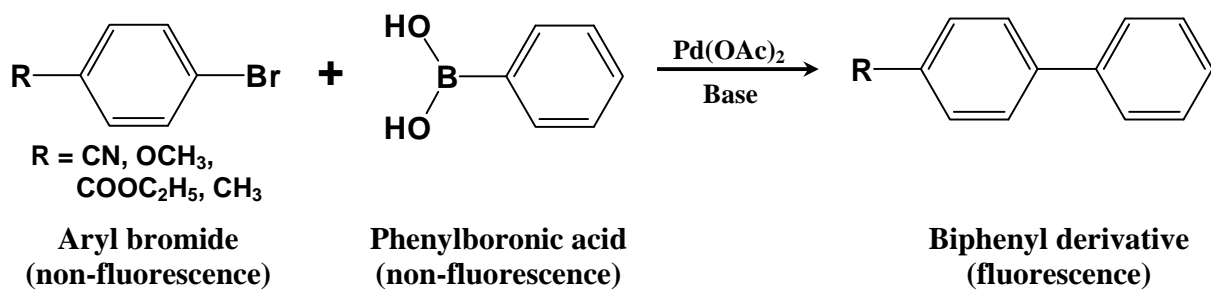


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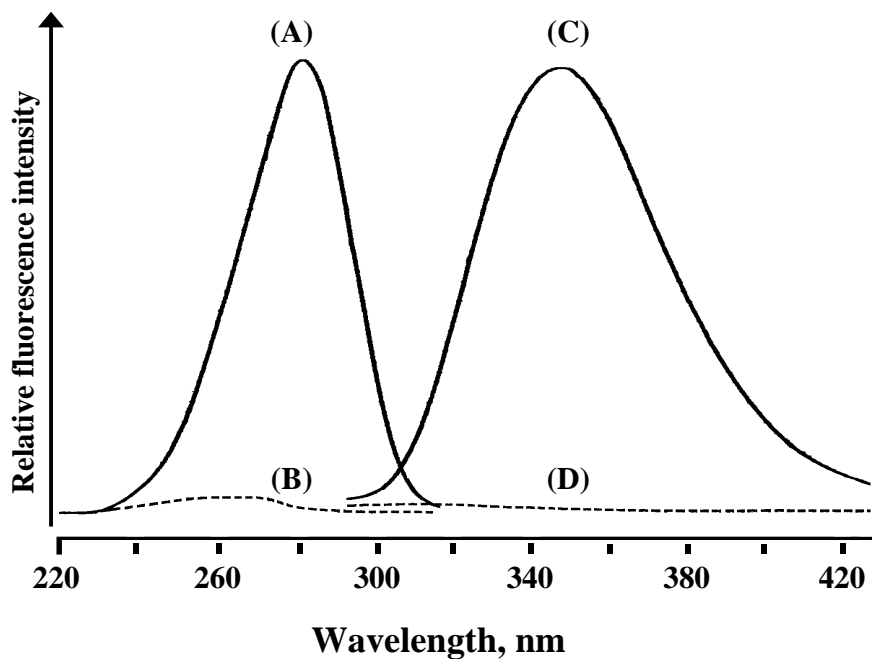


Fig. 2. Fluorescence spectra obtained from the reaction product of $\text{BrPhCOOC}_2\text{H}_5$ with PBA and the reagent blank. Excitation spectra with the emission wavelength at 350 nm of the product (A, solid line) and the reagent blank (B, dashed line). Emission spectra with the excitation wavelength at 290 nm of the product (C, solid line) and the reagent blank (D, dashed line).

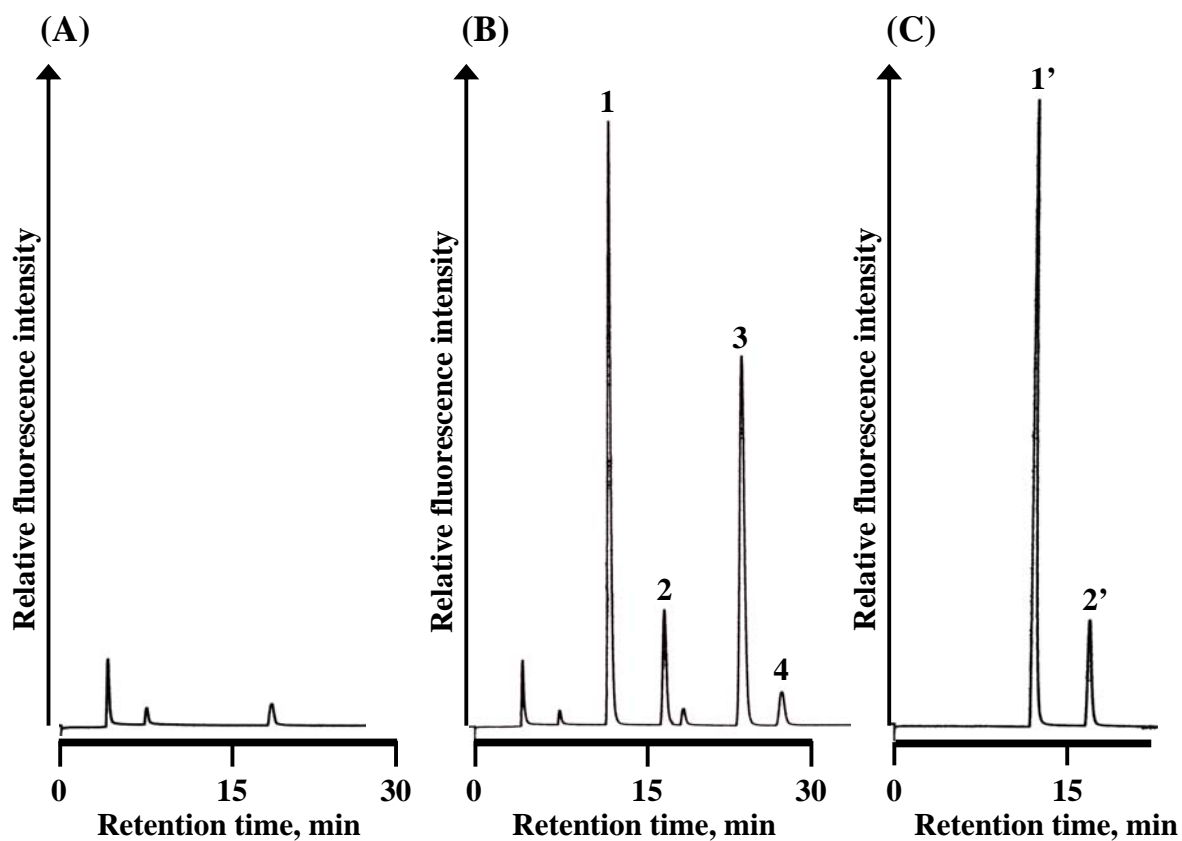


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Table 1 Fluorescence characteristics of the reaction products of aryl bromides with PBA

| Aryl bromide | λ_{ex} | λ_{em} | RFI ^a |
|--------------------------------------|----------------|----------------|------------------|
| BrPhCN | 285 | 335 | 91 |
| BrPhOCH ₃ | 280 | 330 | 36 |
| BrPhCOOC ₂ H ₅ | 290 | 350 | 100 |
| BrPhCH ₃ | 275 | 315 | 33 |

^aRelative fluorescence intensity; BrPhCOOC₂H₅ taken as 100

Table 2 Calibration curves and detection limits for aryl bromides

| Aryl bromide | Calibration curve ^a | | | | Detection limit ^b , μM (fmol/injection) |
|--------------------------------------|--------------------------------|--------------------|------------------------|-------|---|
| | Range, μM | Slope ^c | Intercept ^c | r^2 | |
| BrPhCN | 0.008 - 50 | 25.12 ± 0.007 | 8.38 ± 0.52 | 0.999 | 0.003 (13) |
| BrPhOCH ₃ | 0.06 - 50 | 6.54 ± 0.53 | 0.65 ± 0.70 | 1.000 | 0.013 (67) |
| BrPhCOOC ₂ H ₅ | 0.02 - 50 | 20.38 ± 0.36 | 7.30 ± 0.85 | 1.000 | 0.007 (33) |
| BrPhCH ₃ | 0.1 - 50 | 1.08 ± 0.03 | 0.44 ± 0.10 | 1.000 | 0.03 (157) |

^a Peak height (cm) *versus* concentration (μM).

^b Detection limit at a S/N ratio of 3.

^c Data presented as mean ± S.E. of three experiments.