Communications to the Editor

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CW Laser Fluorometry Using an Optical Fiber —An Application to High Performance Liquid Chromatography—

The fluorescence detection sensitivity in high performance liquid chromatography can substantially be increased by the use of a continuous wave laser as an excitation source combining with an optical fiber as a wave guide. Sensitivity is discussed for dansyl-alanine and compared to that of a conventional HPLC fluorescence detector. This technique has a linear responce over four order of magnitude by the quantity of sample.

Keywords——laser fluorometry; liquid chromatography detector; fluorescence cell; dansyl-alanine; optical fiber; ultratrace analysis

Laser fluorometry has proven to be a powerful method for detecting ultratrace amounts of fluorescent materials.[1-4] We report here a new method of laser fluorometry, which is applicable to the fluorescence detection of conventional high performance liquid chromatography (HPLC).

A schematic of the experimental set-up is shown in Fig. 1. The ultraviolet light, 351.1 and 363.8 nm from Ar ion laser (A) transmits along an optical fiber (B) which is axially introduced into a flow cell system and excites fluorescence of a flowing sample in capillary cell (C), without irradiating cell walls and/or liquid surfaces. The fiber is a silica core optical fiber with a core diameter of 200 μm and a numerical aperture (N.A.) of 0.20. A graded index type micro-lens (SELFOC LENS of Nippon Sheet Glass Co. Ltd.) (D) of 1 mm in diameter is attached to the exit end of the fiber to reduce the numerical aperture of the transmitted laser beam. The beam has a spot 5 mm in diameter at a distance of 10 cm from the exit end of the fiber (N.A.=0.05). The laser beam is collimated and focused by a quartz lens of f=3 cm (E) onto the entrance end of the optical fiber through a pass filter (TOSHIBA UV-33S) (F), which removes visible background emission from the plasma tube. The capillary cell is constructed from 1.5 mm i.d. quartz tubing with a sample inlet of about 0.5 mm i.d. quartz tube which is connected to the outlet of the column of HPLC (G). The

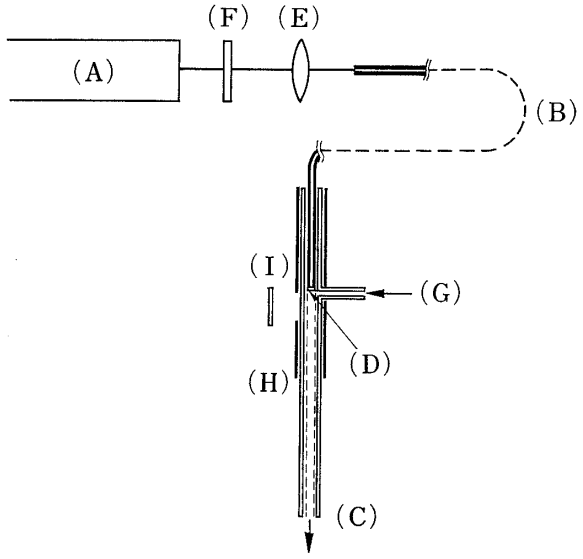

Fig. 1. Schematic Diagram of the Laser Fluorescence Flow-through Cell

(A) CW laser; (B) Optical fiber of 200 μm core; (C) Capillary cell of 1.5 mm i.d.; (D) SELFOC LENS; (E) Quartz lens; (F) Pass filter; (G) Column of HPLC; (H) Cell holder; (I) Interference filter.

1) M.R. Berman and R.N. Zare, *Anal. Chem.*, **47**, 1200 (1975); G.J. Diebold and R.N. Zare, *Science*, **196**, 1439 (1977).
2) T. Imasaka, H. Kadone, T. Ogawa, and N. Ishibashi, *Anal. Chem.*, **49**, 667 (1977).
3) G.J. Diebold, N. Karny, and R.N. Zare, *Anal. Chem.*, **51**, 67 (1979).
4) N. Ishibashi, T. Ogawa, T. Imasaka, and M. Kunitake, *Anal. Chem.*, **51**, 2096 (1979).

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exit end of the optical fiber is inserted and sealed in the tube. Thus, the laser beam emitted from the fiber directly irradiates the effluents of HPLC along the capillary cell, advantageously to get a stronger fluorescence signal from the flowing sample with a reduced amount of scattered radiation. The sample emission is viewed by a photomultiplier at right angle to the laser beam through a slit in the cell holder (H) and appropriate optics. This laser fluorescence cell can be set up in a conventional HPLC fluorescence detection system without changing any optics and/or electronics. A seven cavity interference filter (DITRIC Co.) (I) is placed in front of the photomultiplier to remove both scattered excitation light and Raman scattering from the solvent.

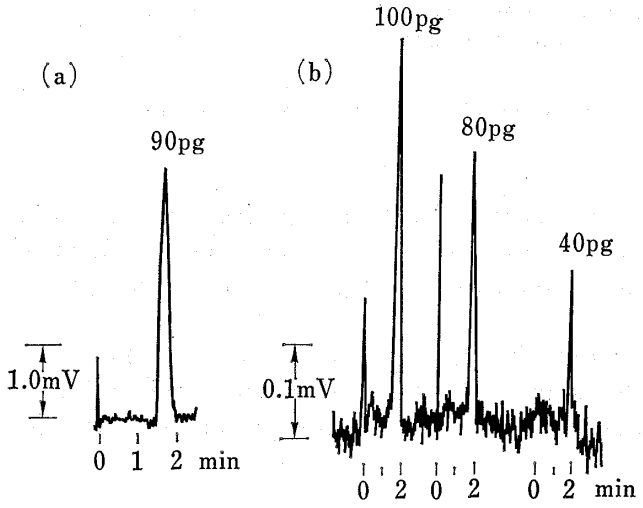

Fig. 2. Fluorescence Respose of Fluorometer (JASCO FP-110) to Various Quantities of Dansyl-alanine Standard Injected onto the HPLC Column

The JASCO ODS-A column (4ϕ × 300 mm) is operated at a flow rate of 1 ml/min with the eluting solvent of methanol, at 25°.
(a) Obtained by the use of laser fluorescence cell excited with 351.1+363.8 nm laser of 0.85 mw.
(b) Obtained by the use of flow-through cell for JASCO FP-110, with 365 nm light of a 130 w medium pressure mercury lamp.

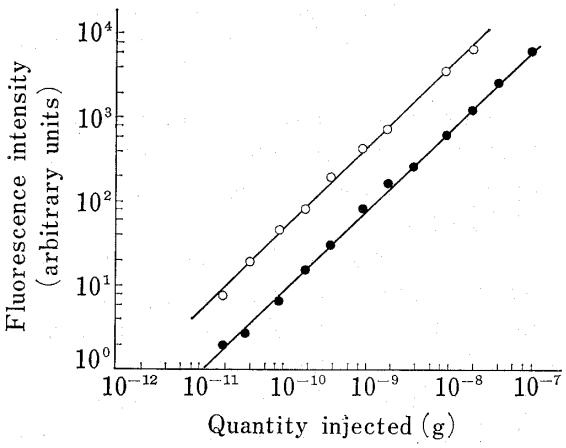

Fig. 3. Liner Quantitation of Dansyl-alanine Standard Using Laser Fluorometry, and Its Dependence on Laser Power

—○—, 0.80 mw; —●—, 0.15 mw

Fig. 2 presents elution peaks of dansyl-alanine (DNS-Ala) standard (Sigma Inc.) obtained (a) by using the laser fluorescence cell combined with a JASCO (Japan Spectroscopic Co. Ltd.) FP-110 spectrophotometer, and (b) by using a conventional fluorescence cell for the medium pressure mercury lamp, with the same spectrophotometer. The detection limit for DNS-Ala by the former method with laser power of 0.85 mw[5] is 2—3 pg (at S/N of 2), which is more sensitive by one order of magnitude than that by the latter conventional method with a 130 w mercury lamp. The linear quantitation of DNS-Ala as well as laser power intensities are shown in Fig. 3. Our tests show that the fluorescence intensity is linearly increased in the range from 10^{-11} to 10^{-7} g by the quantity of injected DNS-Ala, and it is linearly increased with the laser power in the range of 0.1—1 mw also, that the detection limit could be improved, if the sample is excited by a higher power laser.

Importantly, by the use of an optical fiber a high sensitivity laser fluorescence analysis can be made with a simple widely used fluorometer for general use.

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5) This value is the output power of the fiber, measured by a laser power meter (SCIENTECH Model 36001).

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A New Synthetic Method of 3-Formylcephalosporins

Cephalosporin 3′-bromolactones (**3**) were prepared in good yields *via* silyl ethers (**2**) starting from readily available cephalosporin lactones (**1**). Treatment of **3** with dimethyl sulfoxide yielded 3-formylcephalosporins (**4**), a useful intermediate for the chemical modification at the C_3-position of cephalosporins.

Keywords——cephalosporin lactone derivatives; silylation; bromination; 3-formylcephalosporins; silica gel chromatography; separation of epimers

The preceding paper described a new and efficient synthesis of cephalosporins bearing a thiadiazole ring directly attached to the C_3-position starting from 3-formylcephalosporin derivatives.[1] 3-Formylcephalosporins have been obtained from the corresponding 3-hydroxymethyl derivatives by oxidation with DMSO–Ac_2O[2a] or CrO_3–sulfuric acid.[2b,c] However, these oxidation reactions have often been accompanied by side reactions, such as double bond migration ($\Delta^3 \rightarrow \Delta^2$) and lactonization.

This report describes a new and useful synthetic method of 3-formylcephalosporins starting from the readily available cephalosporin lactones.

Direct bromination of 7-phthalimidocephalosporin lactone with NBS has been reported by Bohme *et al.*, but the bromine atom was introduced at the C_2-position.[3] In order to introduce a halogen atom selectively into the $C_{3'}$-position, it was designed to prepare bromolactones (**3**) *via* silyl ethers (**2**) starting from lactones (**1**).

A mixture of a cephalosporin lactone derivative (**1a**), trimethylsilyl chloride (4—8 mol. equiv.) and triethylamine (3—5 mol. equiv.) in DMF was stirred at room temperature to yield the corresponding trimethylsilyl ether (**2a**).[4] Subsequent addition of bromine (1.2 mol. equiv.) at 0° to the reaction mixture afforded the bromolactone (**3a**) as a mixture of

1) T. Sugawara, H. Masuya, T. Matsuo, and T. Miki, *Chem. Pharm. Bull.*, **27**, 2544 (1979).
2) *a*) H. Peter and H. Bickel, *Helv. Chim. Acta*, **57**, 2044 (1974); *b*) Eli Lilly Co., Japan. Patent Provisional Publication, 46-20707 (1971); *c*) Takeda Chem. Co., Japan. Patent Provisional Publication, 49-80097 (1974).
3) E.H. Bohme and J.E. Dolfin, *Chem. Commun.*, **1972**, 941; the methine proton of the C_2-position was observed as a singlet (1H) at 3.53 ppm (DMSO-d_6).
4) *Cf.* Yoshii *et al.* reported that the treatment of but-2-enolides with trimethylsilyl chloride and triethylamine gave 2-trimethylsilyloxyfuranes. E. Yoshii, T. Koizumi, E. Katatsuji, and T. Kaneko, *Heterocycles*, **4**, 1663 (1976).

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