1-(Ethylthio)-3-(dihalo-1,3,5-triazinyl)-2-*n*-propylisoindole as Fluorescent Labeling Reagent for Estrogens

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Recently we reported that 1-(dichloro-1,3,5-triazinyl)-2-methylisoindole (DTMI) was useful as a fluorescent labeling reagent for estrogens.¹ This paper describes the preparation of 1-(ethylthio)-3-(dichloro-1,3,5-triazinyl)-2-*n*-propylisoindole (ECPI) and 1-(ethylthio)-3-(difluoro-1,3,5-triazinyl)-2-*n*-propylisoindole (EFPI) and their applicabilities to fluorescent labeling of estrogens in high performance liquid chromatography (HPLC).

Experimental

Instrumental conditions

Proton and fluorine nuclear magnetic resonance (¹H- and ¹⁹F-NMR) spectra were recorded on a JNM-GX400 spectrometer at 400 MHz for ¹H and at 376 MHz for ¹⁹F (JEOL Ltd., Tokyo). Chemical shifts were reported in parts per million relative to tetramethylsilane (δ 0.00) for ¹H-NMR and benzotrifluoride (ϕ 67.75) for ¹⁹F-NMR as an internal standard, respectively. Mass spectra were taken with a JMS-DX303HF spectrometer (JEOL Ltd., Tokyo). A 655A-11 high performance liquid chromatograph (Hitachi Ltd., Tokyo) was used with a F-1000 fluorescence detector (Hitachi Ltd., Tokyo) operating at Ex 415 nm and Em 445 nm. The column was stainless steel (250×4.6 mm i.d.) packed with a TSKgel ODS-120 A (particle size 5 μ m, TOSOH, Tokyo). Methanol-water (15:1) was used as a mobile phase at a flow rate of 1.0 ml/min at room temperature.

Synthesis of ECPI

o-Phthalaldehyde (268 mg), ethanethiol (150 μ l), and *n*-propylamine (165 μ l) were mixed in the stated order in 10 ml of anhydrous ether at room temperature; then the mixture was cooled in ice-water. To this solution, an ethereal solution (20 ml) of cyanuric chloride (368 mg) was added drop by drop with stirring. After stirred for 1 min, the solvent was removed under reduced pressure. The red-black oily residue was dissolved in 10 ml of acetone and applied to preparative

silica gel thin-layer plates using benzene as eluent. The band of ECPI was extracted with acetone and evaporated to dryness *in vacuo*. The residue was purified by precipitation from a concentrated acetone solution by the dropwise addition of water, followed by drying under reduced pressure to yield 120 mg as orange needles (mp 108°–109°C). Anal. Calcd. for C₁₆H₁₆Cl₂N₄S: 52.32% C, 4.39% H, 15.25% N; found: 52.20% C, 4.47% H, 15.15% N. MS *m/z*: 366(M⁺). ¹H-NMR (CDCl₃) δ : 1.05 (3H, t, C₁₄-H), 1.24 (3H, t, C₁₁-H), 1.81 (2H, tq, C₁₃-H), 2.92 (2H, q, C₁₀-H), 5.00–5.10 (2H, m, C₁₂-H), 7.29 (1H, t, C₅-H), 7.44 (1H, t, C₆-H), 7.84 (1H, d, C₄-H), 8.71 (1H, d, C₇-H).

Synthesis of EFPI

The starting material was cyanuric fluoride and the method was the same as for ECPI. EFPI (100 mg) was obtained as orange needles (mp 105°–106°C). Anal. Calcd. for C₁₆H₁₆F₂N₄S: 57.47% C, 4.82% H, 16.75% N; found: 57.14% C, 4.68% H, 16.53% N. MS *m/z*: 334 (M⁺). ¹H-NMR (CDCl₃) δ : 1.04 (3H, t, C₁₄-H), 1.24 (3H, t, C₁₁-H), 1.82 (2H, tq, C₁₃-H), 2.93 (2H, q, C₁₀-H), 5.03–5.14 (2H, m, C₁₂-H), 7.29 (1H, t, C₅-H), 7.45 (1H, t, C₆-H), 7.85 (1H, d, C₄-H), 8.76 (1H, d, C₇-H). ¹⁹F-NMR (CDCl₃) ϕ : 93.32 (s).

Derivatization procedure

To an acetone solution of estrogens (each 25 pmol, 20 μ l) was added ECPI or EFPI in acetone (3 mM, 20 μ l), followed by 10 μ l of 50 mM aqueous NaOH for ECPI or 10 mM aqueous NaOH for EFPI, respectively. After being kept for 5 s at room temperature, 10 μ l of 174 mM acetic acid for ECPI or 17.4 mM acetic acid for EFPI was added; the components were mixed thoroughly. Then 10 μ l of the final solution was injected into the HPLC apparatus.

Results and Discussion

We have studied the DTMI analogues which were

Fig. 1 Chromatogram of estrogens labeled with ECPI. Peaks: 1, ethinylestradiol; 2, 17α -estradiol; 3, estrone; 4, 17β -estradiol.

Fig. 2 Chromatogram of estrogens labeled with EFPI. Peaks: 1, estrone; 2, ethinylestradiol; 3, 17α -estradiol; 4, 17β -estradiol.

more reactive and sensitive than DTMI. The insertion of triazinyl groups to 1-(ethylthio)-2-*n*-propylisoindole^{2,3} afforded ECPI and EFPI. EFPI was more stable than DTMI and could be used for 1 week in acetone solution when protected from light at room temperature. ECPI and EFPI readily reacted with the phenolic hydroxyl group of estrogens in alkaline medium at room temperature to give the corresponding fluorescent derivatives. The structures of the derivatives were confirmed by fast atom bombardment mass spectrometry. The reactivities of the two reagents were investigated with estrone as a model compound. Based upon our preliminary examinations, reaction time of 5 s at 25°C, a 1200-fold excess of ECPI or EFPI for estrone, and 50 mM NaOH for ECPI or 10 mM NaOH for EFPI were employed throughout the study. The alkaline reaction mixture was neutralized by the addition of 174 mM acetic acid for ECPI or 17.4 mM acetic acid for EFPI to protect the column. The product in the final solution was stable for 48 h. The detection

limit (signal/noise=2) for estrone was 1.1 pmol with ECPI and 270 fmol with EFPI per injection. The detection sensitivity of EFPI was about 10 times higher than that with DTMI.¹ Figures 1 and 2 show the separation profiles of 25 pmol each of the derivatized mixture of estrogens with ECPI and EFPI, respectively. Further studies on the utilities of ECPI and EFPI for clinical applications are in progress.

References

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