Letters to the Editor

**7-Methoxycoumarin-3-carbonyl Fluoride as a Fluorescent Labeling Reagent for Primary Amines in High Performance Liquid Chromatography**

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**Keywords** 7-Methoxycoumarin-3-carbonyl fluoride, fluorescent labeling reagent, primary amine, high performance liquid chromatography

In a previous paper 3-(7-methoxycoumarin-3-carbonyl)-2-oxazolone (MCCO) used as a fluorescent labeling reagent for primary amines was reported. However, the procedure for its derivatization requires a long time (90 min) due to the smaller reactivity. We have therefore studied MCCO analogues in order to improve the reagent reactivity and developed 7-methoxycoumarin-3-carbonyl fluoride (MCCF) having fluoride instead of oxazolone as a leaving group toward the amine. This paper describes the preparation of MCCF and the optimum conditions for fluorescent precolumn labeling of primary amines with MCCF in high performance liquid chromatography (HPLC).

**Experimental**

**Instrumental conditions**

Proton-1 and fluorine-19 nuclear magnetic resonance ('H- and 19F-NMR) spectra were recorded on a JNM-GX400 spectrometer (JEOL Ltd., Tokyo) at 400 MHz for 1H and at 376 MHz for 19F. Chemical shifts were reported in parts per million relative to tetramethylsilane (δ 0.00) for 1H-NMR and benzotrifluoride (δ 67.75) for 19F-NMR as an internal standard, respectively. Mass spectra were taken with a JMS-DX303HF mass spectrometer (JEOL Ltd., Tokyo). A Hitachi L-6000 pump was used; it was equipped with a Rheodyne 7125 syringe-loading sample injector valve (20 µl loop) and a Hitachi F-1000 fluorescence spectrophotometer fitted with a 12 µl flow-cell operating at 405 nm emission and 350 nm excitation. The column was stainless steel (250×4.6 mm i.d.) packed with a TSK gel ODS-80TM (particle size 5 µm, TOSOH, Tokyo). Methanol–water (10:3, v/v) was used as a mobile phase at a flow rate of 1.0 ml/min at ambient temperature.

**Synthesis of MCCF**

To a stirred solution of 7-methoxycoumarin-3-carboxylic acid2 (242 mg) and pyridine (90 µl) in acetonitrile (40 ml) was added cyanuric fluoride (60 µl). After being stirred for 2 h at room temperature the solvent was evaporated under reduced pressure to dryness. The resulting residue was recrystallized from hexane to yield MCCF (99 mg) as pale-yellow needles (mp 158–160° C). Anal. Calcd. for C_{11}H_{7}F_{0}O_{4}: 59.46% C, 3.17% H; found: 59.74% C, 3.36% H. MS m/z: 222 (M+). 1H-NMR (CDCl₃) δ: 3.96 (3H, s, 7-OCH₃), 6.85 (1H, d, 8-H), 6.94 (1H, m, 6-H), 7.57 (1H, d, 5-H), 8.62 (1H, s, 4-H). 19F-NMR (CDCl₃) δ: 23.13 (s).

**Labeling procedure**

To 80 µl of a standard mixture of primary amines in acetonitrile (each 1.25 nmol/ml) was added 10 µl of 10 mM triethylamine in acetonitrile, followed by 10 µl of 1 mM MCCF in acetonitrile. After standing at room temperature for 30 s, 10 µl of the reaction mixture was injected into chromatograph.

**Results and Discussion**

MCCF was readily prepared from 7-methoxycoumar-
arin-3-carboxylic acid by the method of Olah et al.\textsuperscript{3} with minor modification. This structure was confirmed by the mass, \(^1\)H- and \(^{19}\)F-NMR spectral and elemental analysis data. In order to investigate the reactivity of MCCF with primary amines, we have used 2-phenylethylamine (PEA) as a model compound. The labeling yield was obtained from the fluorescence response against that of a synthetically prepared derivative mentioned in a previous paper.\textsuperscript{1} This reagent reacted with PEA in the presence of triethylamine (TEA) at room temperature very rapidly to give the corresponding derivative (Fig. 1). In this study, acetonitrile was found to be suitable for labeling with respect to reactivity in contrast to other solvents. It was thus used in evaluating sample/reagent ratios, TEA/reagent ratios and reaction times. Constant peak heights were attained at a concentration of 0.1 mM for MCCF and 1.0 mM for TEA in the reaction solution at room temperature for 30 s; the reaction yield was 100%. In the absence of TEA the reactivity of MCCF was very low and, therefore, TEA was used to facilitate the derivatization. Triethylenediamine, quinuclidine and 4-dimethylaminopyridine also acted to accelerate the reaction, just as TEA. Figure 2 shows the separation profile of 10 pmol each of the derivatized mixture of primary amines with MCCF. The detection limit (S/N=2) of PEA was 100 fmol as the injected amount. Under the HPLC conditions described in the Experimental section, the MCCF amides of secondary amines were not detected because of very weak fluorescence intensities. This work is the first attempt to use acid fluorides as an analytical reagent.

Based on this study, we will try to prepare acid fluorides having other fluorophores and report on the results in forthcoming papers.

Fig. 2 Chromatogram of primary amines labeled with MCCF. Peak identity: a, reagent blank; 1, propylamine; 2, benzylamine; 3, 2-phenylethylamine; 4, cyclohexylamine.

References


(Received February 8, 1990)
(Accepted March 22, 1990)