4-(2-Phthalimidyl)benzoyl Cyanide as Fluorescent Labeling Reagent for Alcohols in High-Performance Liquid Chromatography

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4-(2-Phthalimidyl)benzoyl cyanide (Phibyl-CN) was designed as a fluorescent labeling reagent for primary and secondary alcohols in high-performance liquid chromatography. Reaction conditions were optimized with hexanol, benzylalcohol, cyclopentanol and 1-phenyl-1-propanol as model compounds of primary and secondary alcohols. Phibyl-CN reacted quantitatively with alcohols on heating at 50 °C for 30 min in the presence of 4-dimethylaminopyridine to give the corresponding fluorescent esters which were separated on a reversed-phase column with fluorescence detection. The detection limits for alcohols labeled with the reagent were 0.24—0.38 pmol per injection. The extents of conversion of primary and secondary alcohols into fluorescent esters were approximately 100%.

Keywords high-performance liquid chromatography; fluorescent labeling reagent; 4-(2-phthalimidyl)benzoyl cyanide; acyl cyanide; alcohol; ester; fluorescent derivative

Acyl cyanides are well known to react with hydroxyl compounds under mild conditions to form the corresponding esters. Some cyanides having a fluorophore such as naphthalene,[1,2] anthracene[3] or pyrene[4] were used as labeling reagents for alcohols in high-performance liquid chromatography (HPLC). Previously, we noted that phenylphthalimidine was useful as a fluorophore for labeling reagents, and developed phthalimidylbenzoyl chloride (Phibyl-Cl) for alcohols and amino compounds,[5] phthalimidylbenzene sulphonyl chloride for amino compounds,[6] phthalimidylphenylmaleimide for thiols[7] and phthalimidylbenzoyl azide (Phibyl-N_3) for alcohols.[8] In these labeling reagents, Phibyl-Cl had a low efficiency of conversion of alcohols to fluorescent derivatives and the reactions of alcohols with Phibyl-N_3 occurred at a higher temperature than 120 °C.

This paper deals with 4-(2-phthalimidyl)benzoyl cyanide (Phibyl-CN) as a fluorescent labeling reagent for alcohols.

Experimental

Synthesis of Phibyl-CN To the suspension of Phibyl-Cl (300 mg) prepared as described previously[5] and zinc iodide (*ca.* 50 mg) in dichloromethane (35 ml), trimethylsilyl cyanide (6 ml) was dropped at room temperature. After being stirred at room temperature for 30 h, the solvent was evaporated under reduced pressure. The resulting residue was washed with hexane (10 ml, 3 times) and recrystallized from dichloromethane added with hexane. Yield 174 mg. Pale yellow needles. mp 293 °C. Infrared spectrum ν cm^{-1} (KBr): 1660 (COO); 1690 (C=O in phthalimidine); 2225 (CN). *Anal.* Calcd for $C_{16}H_{10}N_2O_2$: C, 73.28; H, 3.84; N, 10.68. Found: C, 73.38; H, 4.04; N, 10.60. Proton nuclear magnetic resonance (^{1}H-NMR) ($CDCl_3$) δ: 8.19 (4H, s, benzene ring-H of phenylphthalimidine), 7.40—8.08 (4H, m, aromatic H of phthalimidine), 4.95 (2H, s, $-CH_2-$ of phthalimidine). Mass spectrum (MS) *m/z*: 262 (M^+).

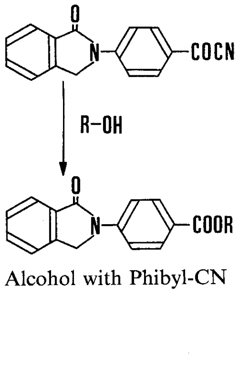

Chart 1. Reaction of Alcohol with Phibyl-CN

Preparation of Phibyl-Derivatives To the solution of Phibyl-CN (55 mg) and alcohol (5 ml) in dichloromethane (25 ml) was added 4-dimethylaminopyridine (55 mg). After heating at 60 °C for 60 min, the solvent was removed by distillation and the precipitate was filtered off and washed with hexane. The precipitate dissolved in chloroform chromatographed on a silica gel column (Wakogel C-200, 20 × 2 cm i.d.) with chloroform. The main fraction was evaporated to dryness under reduced pressure. The residue was recrystallized from cyclohexane, except for Phibyl-derivatives of benzylalcohol and cyclopentanol from methanol and 1-phenyl-1-propanol from ethanol. Phibyl-derivatives (I—XIII) shown in Table I were colorless crystals.

Labeling Reaction Procedure To a test solution of alcohols (100 nmol/ml in dichloromethane each, 50 μl) placed in a reaction vial, 4-dimethylaminopyridine (100 mM in dichloromethane, 30 μl) and Phibyl-CN (5 mM in dichloromethane, 180 μl) were successively added and mixed well. The reaction mixture was heated at 50 °C for 30 min. After cooling, the residue dissolved in methanol (1.0 ml) was sonicated for about 10 s. An aliquot (20 μl) of the mixture was injected into the HPLC.

HPLC Conditions High-performance liquid chromatograph consists of an ECCP (Tosoh, Tokyo) equipped with a Rheodyne 7161 injector (a 20 μl-loop) and a RF 535 fluorescence detector (Shimadzu, Tokyo), set at an excitation wavelength (λ_{ex}) of 305 nm and an emission wavelength (λ_{em}) of 420 nm. An ERC-ODS-1161 column (100 × 6 mm i.d., Erma, Tokyo) was employed at an amibient temperature. As a mobile phase, acetonitrile–water (70 : 30) was used at a flow rate of 1.0 ml/min.

Results and Discussion

As acyl cyanides react with alcohols to form esters, the reaction products from methanol, ethanol, propanol, butanol, pentanol, hexanol, heptanol, octanol, nonanol, decanol, benzylalcohol, cyclopentanol and 1-phenyl-1-propanol should be the corresponding Phibyl-derivatives, which were confirmed as compounds I—XIII, respectively, from the elemental analyses, MS and ^{1}H-NMR spectral data (Table I).

Phibyl-derivatives (I—XIII) dissolved in 70% (v/v) aqueous acetonitrile showed similar fluorescence spectra with λ_{ex} 305 nm and λ_{em} 417—420 nm.

The labeling reactions were studied with hexanol, benzylalcohol, cyclopentanol and 1-phenyl-1-propanol as model compounds of alcohols. In a typical chromatogram of the reaction mixture (Fig. 1), the peaks due to benzylalcohol, cyclopentanol, 1-phenyl-1-propanol and hexanol appeared at 5.6, 6.5, 8.3 and 11.4 min, respectively. Each retention time coincided with that of each Phibyl-derivative (XI, XII, XIII and VI). The peaks eluted at 1—5 min were

TABLE I. Analytical Data for Phibyl-Derivatives

Compound	R[a]	mp (°C)	Analysis (%) Calcd (Found)			MS m/z (M^+)	^{1}H-NMR[b] δ (ppm) ($CDCl_3$)
			C	H	N		
I	$-CH_3$	211	71.90	4.90	5.24	267	3.92 (3H, s, $-CH_3$)
			(71.94	4.85	5.29)		
II	$-C_2H_5$	179	72.58	5.38	4.98	281	4.38 (2H, q, J=7.1 Hz, $-OCH_2-$), 1.40 (3H, t, J=7.1 Hz, $-CH_3$)
			(72.56	5.30	5.00)		
III	$-C_3H_7$	157	73.20	5.80	4.74	295	4.28 (2H, t, J=7.1 Hz, $-OCH_2-$), 1.60—2.00 (2H, m, $-C\underline{H}_2CH_3$), 1.04 (3H, t, J=7.1 Hz, $-CH_3$)
			(72.77	5.71	4.76)		
IV	$-C_4H_9$	142	73.77	6.19	4.53	309	4.32 (2H, t, J=7.1 Hz, $-OCH_2-$), 1.22—1.96 (4H, m, $-C_2\underline{H}_4-CH_3$), 0.98 (3H, t, J=7.1 Hz, $-CH_3$)
			(73.69	6.17	4.53)		
V	$-C_5H_{11}$	131	74.28	6.55	4.33	323	4.32 (2H, t, J=7.1 Hz, $-OCH_2-$), 1.12—1.92 (6H, m, $-C_3\underline{H}_6-CH_3$), 0.94 (3H, m, $-CH_3$)
			(73.93	6.48	4.32)		
VI	$-C_6H_{13}$	127	74.75	6.87	4.15	337	4.32 (2H, t, J=7.1 Hz, $-OCH_2-$), 1.04—1.92 (8H, m, $-C_4\underline{H}_8-CH_3$), 0.91 (3H, m, $-CH_3$)
			(74.70	6.83	4.17)		
VII	$-C_7H_{15}$	124	75.19	7.17	3.99	351	4.32 (2H, t, J=7.1 Hz, $-OCH_2-$), 1.04—1.94 (10H, m, $-C_5\underline{H}_{10}-CH_3$), 0.90 (3H, m, $-CH_3$)
			(75.11	7.17	3.99)		
VIII	$-C_8H_{17}$	126	75.59	7.45	3.83	365	4.32 (2H, t, J=7.1 Hz, $-OCH_2-$), 1.04—1.94 (12H, m, $-C_6\underline{H}_{12}-CH_3$), 0.89 (3H, m, $-CH_3$)
			(75.62	7.45	3.92)		
IX	$-C_9H_{19}$	123	75.96	7.70	3.69	379	4.32 (2H, t, J=7.1 Hz, $-OCH_2-$), 1.04—1.94 (14H, m, $-C_7\underline{H}_{14}-CH_3$), 0.88 (3H, m, $-CH_3$)
			(75.89	7.66	3.72)		
X	$-C_{10}H_{21}$	125	76.30	7.94	3.56	393	4.32 (2H, t, J=7.1 Hz, $-OCH_2-$), 1.04—1.94 (16H, m, $-C_8\underline{H}_{16}-CH_3$), 0.88 (3H, m, $-CH_3$)
			(76.19	7.96	3.57)		
XI	$-C_7H_7$	190	76.95	4.99	4.08	343	7.18—7.65 (5H, m, Ar-H), 5.37 (2H, s, $-OCH_2-$)
			(77.12	5.10	4.09)		
XII	$-C_5H_9$	223	74.75	5.96	4.36	321	5.43 (1H, m, $-OCH<$), 1.42—2.20 (8H, m, $-C_4H_8-$)
			(74.67	6.02	4.39)		
XIII	$-C_9H_{11}$	209	77.61	5.70	3.77	371	7.20—7.68 (5H, m, Ar-H), 5.91 (1H, t, J=7.1 Hz, $-OCH<$), 1.98 (2H, m, $-CH_2-$), 0.97 (3H, t, J=7.1 Hz, $-CH_3$)
			(77.35	5.75	3.83)		

a) See Chart 1. *b)* All compounds showed signals at 8.10 and 7.98 (2H each, d each, J=8.6 Hz, benzene ring –H), 7.40—8.00 (4H, m, aromatic H of phthalimidine) and 4.90 (2H, s, $-CH_2-$ of phthalimidine). s=singlet; d=doublet; t=triplet; q=quartet; m=multiplet; Ar=aryl.

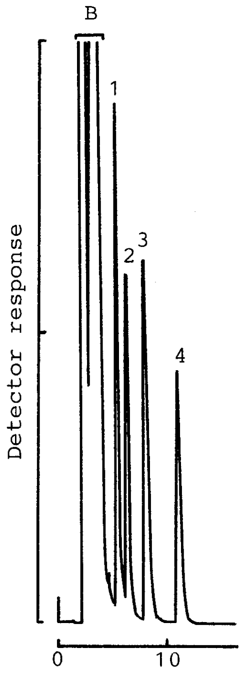

Fig. 1. HPLC Chromatogram of the Reaction Mixture of Alcohols with Phibyl-CN

Peaks: 1, benzylalcohol; 2, cyclopentanol; 3, 1-phenyl-1-propanol; 4, hexanol; B, reagent blank.

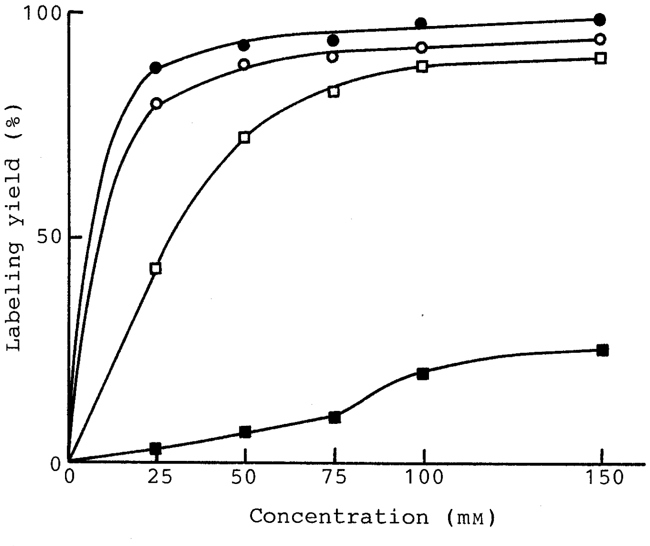

Fig. 2. Effects of Basic Catalyst Concentration on the Labeling Reaction of Hexanol with Phibyl-CN

●, 4-dimethylaminopyridine; □, triethylenediamine; ○, quinuclidine; ■, triethylamine.

due to the reagent blank. Under the same HPLC conditions, the peaks of Phibyl-derivatives of methanol, ethanol, propanol and butanol overlapped with those of the reagent blank.

Phibyl-CN was soluble in dichloromethane, chloroform and acetone, and the labeling reactions of the above four alcohols with the reagent in each solvent were examined in open and sealed vials at 45—100 °C for 30 min. The reaction mixtures in the sealed vials gave only 10% of the peak heights of those in the open vials at each temperature. When the reactions were carried out in the open vials at

50 °C, the solvent (dichloromethane) was distilled away within 20 min and the reactions were completed as mentioned below. However, at a higher temperature than 60 °C the loss of alcohol occurred. Therefore, the labeling conditions were established at 50 °C for 30 min using dichloromethane as a solvent in open vials.

The labeling reactions proceeded effectively when a basic catalyst such as 4-dimethylaminopyridine, triethylenediamine, quinuclidine or triethylamine was added. As shown in Fig. 2, the peak height of the reaction mixture of hexanol reached maximum at the concentration of more than 75 mM of 4-dimethylaminopyridine. Similar results were obtained from other alcohols. A 100 mM (30 μl) solution of 4-dimethylaminopyridine was employed in the present procedure.

The reactions of *tert*-alcohols such as *tert*-butanol and *tert*-amylalcohol (5 mM, 50 μl each) with Phibyl-CN were also examined. Traces of fluorescent peaks were observed at 7.9 min for *tert*-butanol and 14.8 min for *tert*-amylalcohol, indicating that the reagent is not available for the *tert*-alcohols under the present labeling and HPLC conditions.

The peak heights of alcohols labeled with Phibyl-CN were linear up to 50 nmol per test tube of alcohol concentration. The sensitivities ($S/N=3$) of benzylalcohol, cyclopentanol, 1-phenyl-1-propanol and hexanol labeled with the reagent were 0.24, 0.26, 0.28 and 0.38 pmol per injection, respectively. The relative standard deviations ($n=5$) were 3.5% (benzylalcohol), 3.5% (cyclopentanol), 4.1% (1-phenyl-1-propanol) and 1.9% (hexanol) at an amount of 5 nmol per test tube.

The efficiency of conversion of alcohols into the Phibyl-derivatives was examined by comparing the peak heights under the established reaction conditions with those of the prepared Phibyl-derivatives (V—VIII); the extents of conversion (%, mean, $n=4$) were 98.7% (V), 99.0% (VI), 101.1% (VII), 99.4% (VIII), 99.3% (IX), 100.0% (X), 104.1% (XI), 88.9% (XII) and 98.8% (XIII).

Phibyl-Cl also reacted with primary and secondary alcohols under the appropriate conditions to give the same Phibyl-derivatives as those obtained from the reactions with Phibyl-CN. The extents of conversion of hexanol, benzylalcohol, cyclopentanol and 1-phenyl-1-propanol to Phibyl-derivatives were examined by comparing the peak heights of the reaction mixtures. The peaks with Phibyl-Cl were about 40%, 42%, 30% and 41% of those with Phibyl-CN, respectively. This indicates that Phibyl-CN is more suitable for the labeling reagent of alcohols than Phibyl-Cl.

Phibyl-CN was stable for more than half a year at room temperature. Phibyl-derivatives (I—XIII) were stable even if they were heated at 100 °C for 30 min in the reaction mixture.

The new reagent has excellent properties with respect to reactivity and sensitivity for the labeling of primary and secondary alcohols. Phibyl-CN should be useful as a fluorescence labeling reagent in HPLC of primary and secondary alcohols.

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