Communications to the Editor

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A New Sensitive Derivatization Reagent for Liquid Chromatographic Separation of Hydroxyl Compounds

A new sensitive derivatization reagent for use in liquid chromatographic separation of hydroxyl compounds has been developed. A suitable reagent for this purpose, 4-dimethylamino-1-naphthoyl nitrile, was readily prepared from the corresponding acid chloride by the exchange reaction with trimethylsilyl cyanide. Condensation of a hydroxyl compound with the reagent was effected in the presence of triethylamine under the mild condition. The resulting ester was highly responsible for a fluorescence detector with a detection limit of 200 pg.

Keywords——high-performance liquid chromatography; pre-column derivatization; hydroxyl group; fluorescence labeling; esterification reagent; acyl nitrile; 4-dimethylamino-1-naphthoyl nitrile

In recent years considerable attention has been focused on the development of sensitive and specific derivatization reagents for use in high-performance liquid chromatography (HPLC) with fluorescence detection.[1–4] However, the suitable derivatization reagent for the alcoholic hydroxyl group with respect to reactivity and sensitivity has not yet been developed. In this communication we wish to report a new type of sensitive derivatization reagent for liquid chromatographic separation of hydroxyl compounds.

The design of a promising reagent requires the structural features having both the functional group reactive for hydroxyl compounds and fluorophore responsible for a fluorescence detector. For this purpose an initial project was directed to the synthesis of 4-dimethylamino-1-naphthoyl nitrile, that is a pseudo halogen compound of the acyl chloride.

The acyl nitrile is usually prepared from the corresponding acyl halide by treatment with cupric cyanide or sodium cyanide.[5] However, the hitherto known methods are unsatisfactory owing to the formation of by-products and poor yield. Accordingly, the development of a new synthetic procedure has been undertaken. First, 4-dimethylamino-1-naphthoic acid (I),[6] mp 165°, was prepared from 4-dimethylamino-1-naphthylmagnesium bromide by the Grignard reaction with ethyl chlorocarbonate followed by hydrolysis with methanolic alkali. Treatment of I with oxalyl chloride in methylene chloride–dimethylformamide provided the acyl chloride which on exchange reaction with trimethylsilyl cyanide in the presence of zinc iodide as a catalyst was readily transformed into 4-dimethylamino-1-naphthoyl nitrile (II) in 88% overall yield. mp 130.5—132°. IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 2240 (C≡N), 1730 (C=O), 1680, 1605, 1510, 1460 (C=C). NMR ($CDCl_3$) δ: 3.20 (6H, s, $-N(CH_3)_2$), 6.92 (1H, d, J=8 Hz, 3-H), 7.60 (2H, m, 6- and 7-H), 8.10 (1H, m, 5-H), 8.39 (1H, d, J=8 Hz, 2-H), 9.26 (1H, m, 8-H). MS m/z: 224 (M^+), 198 ([M—CN]$^+$). *Anal.* Calcd for $C_{14}H_{12}N_2O$: C, 74.99; H, 5.38; N, 12.49. Found:

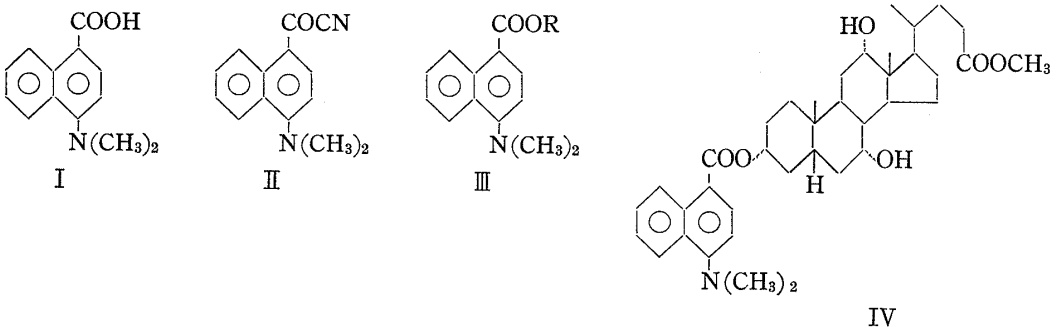

Chart 1

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Table I. Reactivities of Various Hydroxyl Groups with 4-Dimethylamino-1-naphthoyl Nitrile

Compound	Position of hydroxyl group	Esterification rate (%)[a]	
		A	B
Dehydroepiandrosterone	3β (e)	10	100
Testosterone	17β (qe)	0	25
Cortisol	11β (a)	0	0
	17α (tert)	0	0
	21 (prim)	100	—
Estrone	3 (phen)	50	100
Methyl cholate	3α (e)	10	100
	7α (a)	0	0
	12α (a)	0	0

a) Conditions: A) at room temperature for 30 min; B) at 60° for 1 hr. e: equatorial, qe: quasi equatorial, a: axial, tert: tertiary, prim: primary, phen: phenolic. The esterification rate was estimated by comparison of the fluorescence intensity with the authentic ester prepared from the corresponding hydroxyl compound.

C, 74.96; H, 5.47; N, 12.21. It is to be noted that the present method is much more suitable than the known procedures for the preparation of acyl nitrile.

Condensation of a hydroxyl compound with II to form the ester (III) was effected in the presence of triethylamine. The reactivities of II were tested for various hydroxyl functions of steroids (see Table I). A test sample (1 μg) and II (100 μg) were dissolved in acetonitrile–triethylamine (1:1) (150 μl) and the resulting ester (III) was determined by HPLC. The apparatus used was a Waters 6000A solvent delivery system (Waters Assoc., Milford, Mass.) equipped with a 440 absorbance detector (Waters Assoc.) operated at 254 nm and a 650-10LC fluorescence spectrophotometer (Hitachi Ltd., Tokyo). HPLC was carried out employing μPorasil (1 ft.×1/4 inch i.d.) as a column and hexane–ethyl acetate as a mobile phase under the ambient condition.

The primary hydroxyl group was easily converted into the corresponding ester when the reaction mixture was allowed to stand at room temperature for 30 min. Condensation of the secondary hydroxyl group of equatorial nature with II was effected by heating at 60° for 1 hr. On the other hand, the axial hydroxyl groups at 7α and 11β could not be coupled with II under the same condition. This marked difference can be explained in terms of the steric hindrance in a steroid molecule. It has previously been demonstrated that *t*-butyldimethylsilyl chloride which is known as a sterically hindered reagent, reacted with the 3α-hydroxyl group but not with 7α- and 12α-hydroxyl groups of bile acids.[7] The less reactivity was also observed on the 17β-hydroxyl function probably due to the steric hindrance of the 18-methyl group. The tertiary hydroxyl function was inert toward this derivatization reagent.

The resulting ester (excitation maximum 350 nm; fluorescence maximum 530 nm) was highly responsible for a detector monitoring the fluorescence intensity at 530 nm. On the other hand, the reagent itself showed a feeble fluorescence (excitation maximum 370 nm; fluorescence maximum 480 nm), exerting no significant influence on fluorescence detection. For instance, the C-3 ester (IV) formed from methyl cholate could be determined with a detection limit of 200 pg in HPLC.

It is evident from the data that the newly developed reagent is of great use for derivatization of a trace amount of the hydroxyl compound in HPLC. The application of this pre-column derivatization to the separation and determination of physiologically important substances in biological fluids will be a subject in the future communication.

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1,6-Dihydro-3(2*H*)-pyridinones as Synthetic Intermediates. A Convenient Total Synthesis of (±)-Cleavamine

A novel and convenient total synthesis of (±)-cleavamine (**1**) starting from ethyl 1,6-dihydro-3(2*H*)-pyridinone-1-carboxylate (**2**) is described.

Keywords——total synthesis; cleavamine; Claisen rearrangement; 1,3-diaxial interaction; medium-ring closure

The cleavamine-type compounds are of particular interest in that they constitute the indole portion of the bis indole-dihydroindole alkaloids represented by vinblastine, one of clinically important antitumor agents, and that they have provided key intermediates for pentacyclic *Iboga* alkaloids.[1] Therefore, it is of great value to develop convenient synthetic methods for cleavamine and related compounds, and a number of synthetic studies on the cleavamine family, *e.g.* cleavamine or velbanamine, have been reported to data.[2] Now we wish to report here a novel and convenient total synthesis of (±)-cleavamine (**1**) from an easily available starting material, ethyl 1,6-dihydro-3(2*H*)-pyridinone-1-carboxylate[3] (**2**), which has been shown to be a common synthon for various alkaloids.[4]

The allylic alcohol (**3**), obtained in 52% yield by the reaction of **2** with ethylmagnesium bromide,[4b] was heated in ethyl vinyl ether containing mercuric acetate at 200° for 72 hr to provide the aldehyde (**4**). Its ethylene acetal (**5**; 66% yield from **3**) was hydrolyzed with potassium hydroxide in aqueous ethanol to give the amine (**6**) [59%,[5] δ: 0.98 (3H, t, J=7), 1.65 (2H, d-d, J=6, 4.5), 1.90 (2H, q, J=7), 2.02 (1H, s), 3.15 (2H, m), 3.83 (4H, m), 4.84 (1H, t, J=4.5), 5.33 (1H, m)]. Condensation of **6** with β-indolylacetyl chloride in methylene chloride afforded the amide (**7**) quantitatively, an acidic hydrolysis of which gave the aldehyde (**8**). On oxidation with silver(I) oxide the aldehyde (**8**) was converted smoothly to the carboxylic acid (**9**) in 67% yield from **7**. Cyclization of **9** to the dioxocleavamine (**10**) [36%, mp 199—200°, *m/e*: 308 (M^+), ν: 3470, 1650, δ: 1.02 (3H, t, J=7), 1.98 (2H, q, J=7), 3.39 (1H,

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