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Crowned-coumarin as a New Fluorescence Derivatization Reagent for Carboxylic Acids

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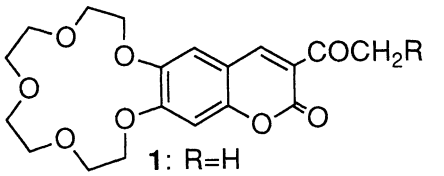
3-Bromoacetyl-6,7-[2',3'-(1',4',7',10',13'-pentaoxacyclopentadeca)-2'-ene]-coumarin (**2**) was prepared as a new fluorescence derivatization reagent for carboxylic acids in high-performance liquid chromatographic analysis. The derivatization of lauric acid with **2** was readily accomplished under a quite mild condition to yield a highly fluorescent lauric acid derivative. The reaction was accelarated by combining crown moiety as a catalytic site to coumarin fluorophore.

The determination of carboxylic acids in biological samples is significant for the elucidation of various physiological functions in the body. For the purpose, a number of fluorogenic compounds have been developed as fluorescence derivatization reagents for carboxylic acids in high-performance liquid chromatographic (HPLC) analysis.[1] Among these reagents, coumarin derivatives are known to have excellent fluorogenic characters.[1a-e] We recently developed 3-bromoacetyl-6,7-methylenedioxycoumarin (BrAMDC) which readily reacts with fatty acids in the prescence of 18-crown-6 ether and potassium bicarbonate to give highly fluorescent fatty acid derivatives.[2]

In the present communication, this derivatization reaction was further extended to simplify and to accelarate the reaction by permitting the reagent itself to hold a certain function. Thus, a crowned-coumarin (**2**) which combined benzo-15-crown-5 (B15Cr5) moiety as a catalytic site to coumarin fluorophore was designed and prepared as a new fluorescence derivatization reagent for carboxylic acids.



1: R=H
2: R=Br
3: R=OCO$(CH_2)_{10}CH_3$

Reagent **2** was prepared from a known compound, 4'-hydroxybenzo-15-crown-5,[3] as follows. The Gattermann formylation of starting material in dry ether gave 4'-formyl-5'-hydroxybenzo-15-crown-5 in 73% yield.[4] This compound was obtained as pasty product, so that it was used fot the next step without further purification. Cyclization of the compound was accomplished by refluxing with ethyl acetoacetate and piperidine in ethanol[5] to give **1** almost quantitatively (94% yield), followed by bromination with tetrabutylammonium tribromide in dichloromethane according to the method of Kajigaeshi *et al.*[6] gave the desired **2** as yellow needles in 85% yield. The structures of these compounds were confirmed by ^{1}H-NMR and mass spectral data.[7]

Figure 1 shows the fluorescence spectral responses for **1** in methanol on addition of alkali metal acetates. The fluorescence intensity of **1** was successively decreased in order of $Li^+ < Cs^+ < Rb^+ < K^+ < Na^+$ along with the blue shifts of fluorescence maxima, indicating the complexation of the crown site of **1** with metal ions. The stability constants (log *K*s) for complexation with K^+ and Na^+ were 2.64 and 2.15, respectively, which were almost comparable with the values of 2.8 and 3.05 for B15Cr5.[8] From these results, **1** would likely have a catalytic function such as 18-crown-6 ether in BrAMDC derivatization system.

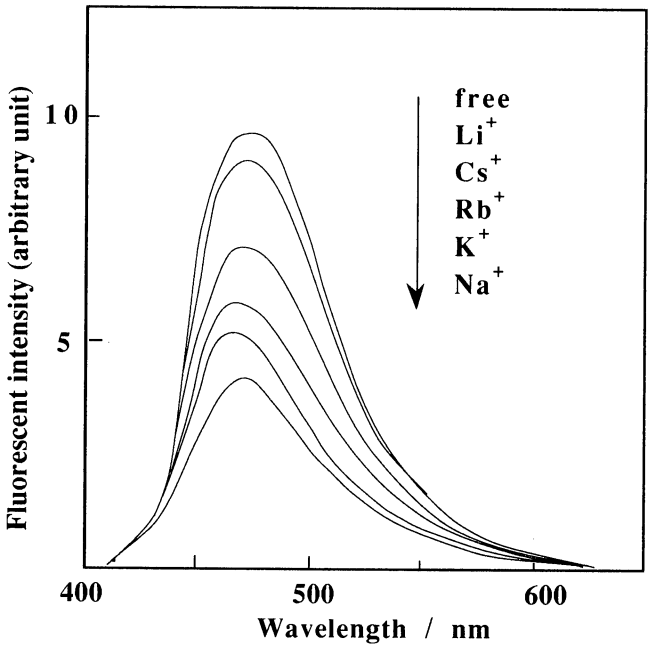


Fig. 1. Fluorescence spectra of **1** in the absence and the prescence of alkali metal acetates.
[**1**]=5.0x10^{-6} mol dm^{-3}, [alkali metal acetate]=2.5x10^{-3} mol dm^{-3}

Thus, the derivatization reaction of a test compound, lauric acid, with **2** was attempted in acetonitrile at 30°C in the presence of the bases ($KHCO_3$ and $NaHCO_3$) alone and monitored by a reversed phase HPLC equipped with a fluorescence detector (λex=392 nm, λem=482 nm) using methanol as a mobile phase. The reaction kinetics of **2** with large excess of lauric acid are shown in Fig. 2 together with the results on BrAMDC in the presence of B15Cr5 ether and bases for the comparison. Interestingly, the derivatization of lauric acid were definitely accelarated by the presence of $KHCO_3$, but not $NaHCO_3$. This finding is matched with the facts that B15Cr5 forms 2:1 complex with K^+ in sandwich manner and 1:1 complex with Na^+ and its complexation ability

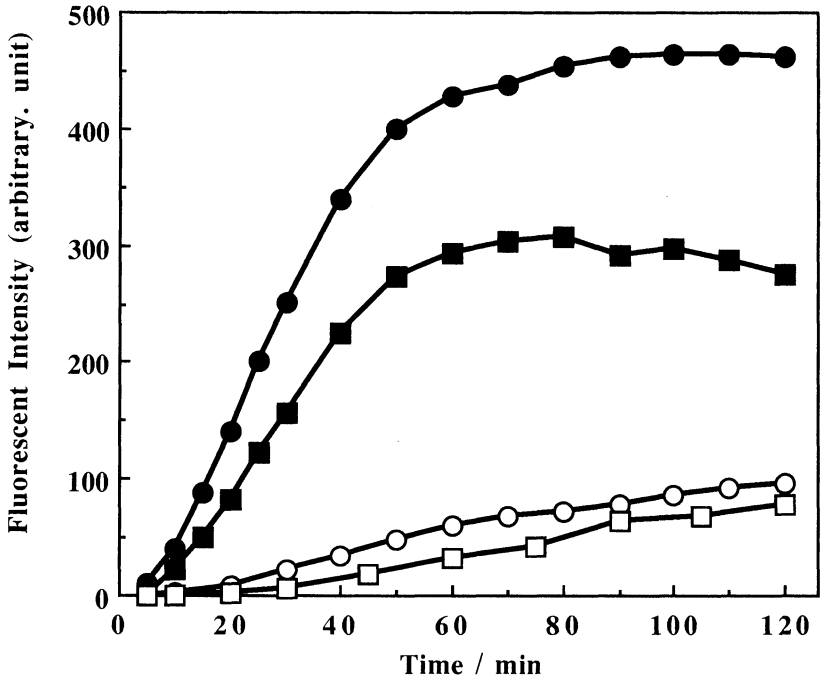


Fig. 2. Time course of the derivatization of lauric acid with **2** and BrAMDC in acetonitrile at 30 °C.
[**2** and BrAMDC]=2.5x10^{-5} mol dm^{-3}, [B15Cr5]=2.5x10^{-5} mol dm^{-3}, [lauric acid]=2.5x10^{-3} mol dm^{-3}, [base]=2 mg in a reaction solvent(2 ml).
●: **2** + $KHCO_3$; ■: BrAMDC + B15Cr5 + $KHCO_3$
○: **2** + $NaHCO_3$; □: BrAMDC + B15Cr5 + $NaHCO_3$

to K^+ in methanol are slightly greater than that to Na^+.[8] The fluorescence quantum yield (ϕ=0.82) of the derivatized laurate (**3**)[9] in methanol was very close to the value (ϕ=0.80) for AMDC-laurate.[2] Therfore, the reactivity between **2** and BrAMDC in the presence of $KHCO_3$ may be approximately comparable by their pseudo first-order rate constants (k_{obsd} / Ms^{-1}), 8.65x10^{-4} for **2** and 5.31x10^{-4} for BrAMDC, estimated from the lines in Fig. 2. This indicates that **2** apparently surpasses BrAMDC in the reactivity.

From these results, it was suggested that **2** complexes with K^+ in the same manner as that of B15Cr5 and that the derivatization reaction with **2** is consequently accelarated because of easy access of laurate anion and reagent molecule.

This is the first example applied a crowned-type reagent to the derivatization reaction for HPLC analysis. The limitted data may be afford a clue to develop such functionalized reagents for not only carboxylic acids but also other applications. Further works on the other crowned-reagents along this lines are currently under way.

References

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7) Spectral data and others are as follows. Formyl-compound: ^{1}H-NMR ($CDCl_3$) δ 3.73-4.15 (m, 16H, -OCH_2CH_2O-), 6.45 (s, 1H, ArH), 7.05 (s, 1H, ArH), 9.66 (s, 1H, CHO), 11.34 (s, 1H, OH); MS *m/z*=312(M^+). **1**: mp 194-196°C; ^{1}H-NMR ($CDCl_3$) δ 2.71 (s, 3H, $COCH_3$), 3.75-4.21 (m, 16H, -OCH_2CH_2O-), 6.80 (s, 1H, C_8-H), 6.97 (s, 1H, C_5-H), 8.46 (s, 1H, C_4-H); MS *m/z*=378 (M^+). **2**: mp 188-190°C; ^{1}H-NMR ($CDCl_3$) δ 3.75-4.23 (m, 16H, -OCH_2CH_2O-), 4.77 (s, 2H, $COCH_2Br$), 6.81 (s, 1H, C_5-H), 6.99 (s, 1H, C_8-H), 8.58 (s, 1H, C_4-H); MS *m/z*=456 (M^+), 458 (M^++2).

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9) Mp173-5 °C; ^{1}H-NMR ($CDCl_3$) δ 0.88 (t, 3H, -CH_3), 1.27-2.49 (m, 20H, -CH_2-), 3.75-4.22 (m, 16H, -OCH_2CH_2O-), 5.37 (s, 2H, C_3-$COCH_2O$-), 6.81 (s, 1H, C_5-H), 6.98 (s, 1H, C_8-H), 8.56 (s, 1H, C_4-H); MS *m/z*=576 (M^+).

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