## Communications to the Editor

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# 2-CARBOXY-1-HYDROXY-4-NAPHTHYLMETHYLDIMETHYLSULFONIUM CHLORIDE AS A NEW FLUORESCENT KOSHLAND REAGENT

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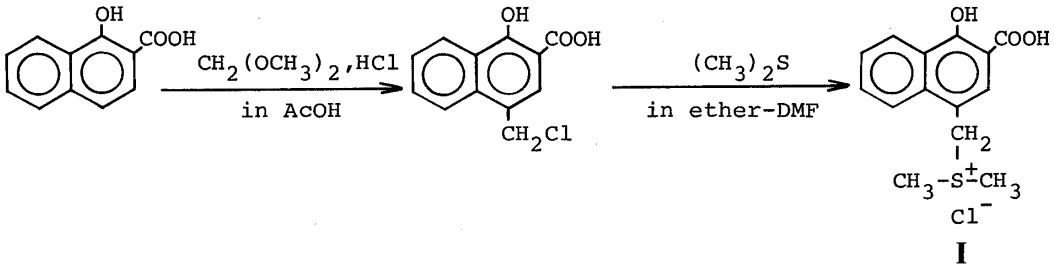
A fluorescent Koshland reagent, 2-carboxy-1-hydroxy-4-naphthylmethyldimethylsulfonium chloride (I), was newly synthesized. I rapidly reacts with tryptophan under mild condition to give a fluorescent adduct ($\lambda_{ex}$= 255 nm, $\lambda_{em}$=416 nm). I was applicable to the modification of bovine serum albumin molecule and the calculation of the number of its tryptophan residues.

KEYWORDS——2-carboxy-1-hydroxy-4-naphthylmethyldimethylsulfonium chloride; Koshland reagent; tryptophan; amino acid; bovine serum albumin; high performance liquid chromatography; labelling reagent

A series of Koshland reagents, the representative of which is 2-hydroxy-5-nitrobenzyl bromide (Koshland I), have been used for specific reagents for tryptophan(Trp) residues and have been applied to the modification of enzymes containing Trp such as α-chymotrypsin[1] or to the quantitative determination of Trp residues in protein.[2]

In addition to Koshland I, 2-methoxy-5-nitrobenzyl bromide (Koshland II),[3] 2-bromoacetamide-4-nitrophenol (Koshland III)[4] and dimethyl(2-hydroxy-5-nitrobenzyl)-sulfonium bromide[5] as water-soluble Koshland I have been developed. These reagents are chromophoric reagents and the absorption of nitrophenol has been used for the measurement. In order to raise the sensitivity, we have developed a new fluorescent Koshland reagent, 2-carboxy-1-hydroxy-4-naphthylmethyldimethylsulfonium chloride (I).

The synthetic route of I is shown in Chart 1. Crude crystals were recrystalli-




I

Chart 1

zed with methanol-ether. Although thus prepared I contained trace amounts of 2-carboxy-1-hydroxy-4-methoxymethylnaphthalene which was produced from I and methanol, the presence of this hydrolysate did not disturb the reaction of I with Trp.

The reaction of I with Trp was so rapid that it was completed within two minutes.[6] The produced Trp-adduct was fractionated by high performance liquid chromatography (HPLC) to measure its fluorescence spectrum. As shown in Fig. 1, the Trp-adduct had the fluorescence maximum at 416 nm (exciting at 255 nm) and its excitation and emission spectra closely resembled those of the hydrolysate of I (III), indicating that the fluorescence of the Trp-adduct is based on the naphthalene skeleton of I. Since the Koshland I reacts with Trp at position 3 of indole ring to give an indolenine derivative,[7] it might be considered that I would also react with Trp to give indolenine, II (Chart 2).

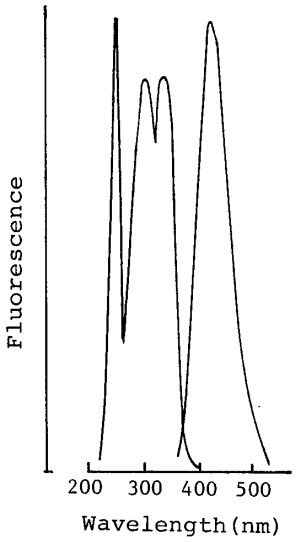



Fig. 1

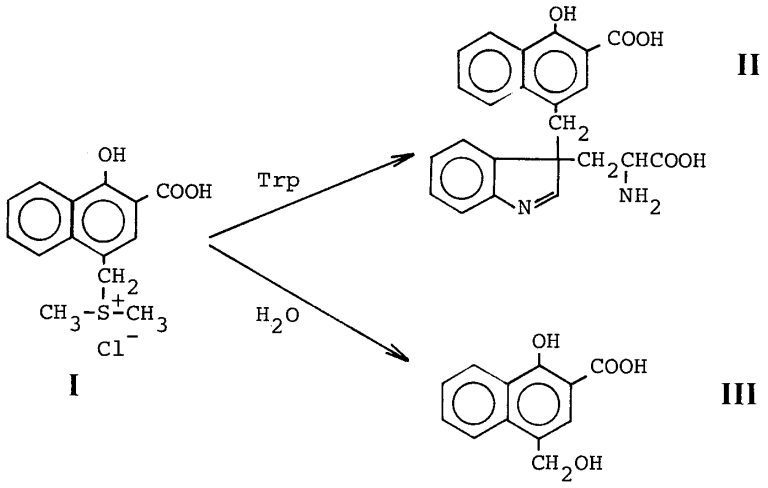



Chart 2

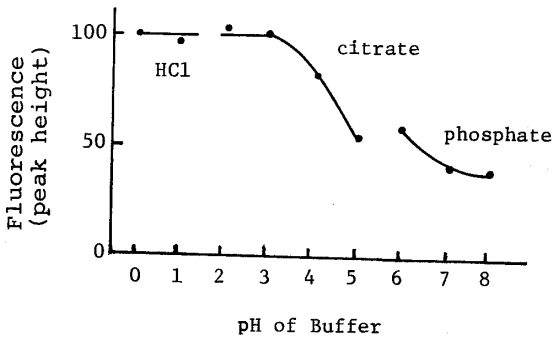



Fig. 2. Effect of pH on the Reaction
Buffers used; HCl solution for pH 0 and pH 1, 0.1M citrate buffer for between pH 2 and pH 5, 0.1M phosphate buffer for between pH 6 and pH 8.

When Trp reacted with I at various pHs and the reaction mixture was analysed by HPLC with fluorescence detection,[8] a constant peak height was given between pH 0 and pH 3. In contrast, the peak height began to decrease with increasing pH above pH 3. This may be caused by the hydrolysis of the reagent (Fig. 2).

In acidic solution, amino acids other than Trp did not react with I except cysteine. However, the reactivity of cysteine, namely the SH group, was poor compared with that of Trp. On the contrary, in neutral or alkaline solution,

histidine, methionine, serine, threonine, tyrosine and hydroxyproline also reacted with I .

An attempt was made to apply I to estimate the number of Trp residues in the bovine serum albumin (BSA) molecule. One millilitter of 0.5 mM BSA ($5\times10^{-7}$ mol) in 0.1M phosphate buffer (pH 7.0) and 4 ml of 1.25 mM N-ethylmaleimde in the same buffer containing 5M guanidine hydrochloride were mixed and allowed to stand at room temperature for 20 min. To this solution was added 15 mg of I ($5\times10^{-5}$ mol) suspended in 0.7 ml of glacial acetic acid.(The final pH of the reaction mixture was 2.5). Then the mixture was loaded on a Sephadex G-25 column and modified BSA fractions were collected. The number of Trp residues was calculated by the fluorimetric determination of the modified BSA assuming that it would have the same fluorescence property as Trp-adduct. The number of Trp residues obtained was 2.2, which was in good agreement with the literature of 2.[9]

In summary, the fluorescent Koshland reagent, I , proved to have the same reactivity as chromophoric Koshland reagents hitherto known. Furthermore, I gave fluorescent Trp-adduct ($\lambda_{ex}$=255 nm, $\lambda_{em}$=416 nm), which enable sensitive detection of Trp[10] and Trp containing peptides and proteins. Since I has the naphthalene skeleton, it can be used as a fluorescent reagent for microenvironmental analysis around a Trp residue in protein. The application of I as a precolumn reagent for peptides containing Trp is now in progress.

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6) The reaction was carried out as follows; 15 mg of I was weighed into a test tube and was added with 1.0 ml of 10 mM Trp solution in 0.1M citrate(pH 3.0)-$CH_3CN$ (1:1, v/v) with vigorous shaking.
7) T. F. Spande, M. Wilchek and B. Witkop, J. Am. Chem. Soc., 90, 3256 (1968); G. M. Louden and D. E. Koshland, Jr., J. Biol. Chem., 245, 2247 (1970).
8) Chromatographic conditions: column, TSK GEL LS-410 AK (300×4 mm I.D.); eluent, 20 mM acetate(pH 4.0)-$CH_3CN$ (3:1, v/v); flow rate, 0.62 ml/min. Fluorescence detection was performed with a Shimadzu FLD-1 fluorescence detector (excitation: 254 nm, emission>330 nm).
9) M. O. Dayhoff, "Atlas of Protein Sequence and Structure", Vol. 5, National Biomed. Research Foundation. Washington D. C. (1972).
10) Five picomoles of Trp were detectable under the condition described in the note 8).

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