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N-(7-Dimethylamino-4-methyl-coumarinyl)maleimides (DACM): Novel Fluorescent Thiol Reagents[1]

Dimethylaminocoumarine (1) was proposed as one of candidate fluorogenic groups of choice. Thus N-(7-dimethylamino-4-methyl-coumarinyl)maleimides (4) are shown to be preferable fluorescent thiol reagents.

Certain N-substituted maleimides, nonfluorescent by themselves, react selectively with thiol compounds to form highly fluorescent addition products.[2] This "empirical rule" provides the basis for the use of these maleimides as a novel type of fluorescent thiol reagent. For example, BIPM[3] was the first practical reagent which can be used for microanalysis of small molecular thiol compounds,[4] for estimation of the states of thiols in proteins[5] and for other various studies of biological systems.[6] Further, N-(1-anilinonaphthyl-4)maleimide (ANM) was proposed as a fluorescent hydrophobic probe directed to thiol groups in protein.[7]

One common requirement for such a probe is that the fluorogenic group should have an emission maximum distinct from that of the aromatic residues of protein. In this respect BIPM leaves much to be desired since the emission of BIPM (excitation 320 nm, emission max. 360 nm, in 0.1 M phosphate buffer, pH 7.0)[1] can indeed be distinct from, but fairly close to those of proteins (tryptophan residue, emission max. *ca.* 350 nm). Therefore one of the purposes of our systematic studies on search for fluorogenic groups[8] was to find those which have fluorescent maxima in a significantly longer wave-length region than those of the "intrinsic" fluorescence of protein. In the present communication we describe fluorescent thiol reagents which meet such a criterion.

Nitration of 7-dimethylamino-4-methyl-coumarin (**1**) with nitric acid (d=1.42) in acetic acid forms three positional isomers of **2** (**a**, **b**, **c**: 31, 26, 5%), which were isolated through silica-gel chromatography. **2** were hydrogenated with Pd-carbon as catalyst to give the amines **3**, which were converted into the maleimides **4** by way of the maleamic acids as usual.[2,3,9] The corresponding succinimides **5** which lack the double bond were also prepared as the fluorescent models of the reaction products of **4** with thiols.[2,3]

N-(7-Dimethylamino-4-methyl-3-coumarinyl)maleimide (DACM-3; **4a**)[9]: Yellow needles from EtOAc, mp 218—219.5°; UV_{max}^{EtOH} nm (ε): 244 (16500), 377 (25700). N-(7-Dimethylamino-4-methyl-6-coumarinyl)maleimide (DACM-6; **4b**): Orange yellow needles from EtOH, mp 222—223.5°; UV_{max}^{EtOH} nm (ε): 242 (15900), 350 (17700). N-(7-Dimethyl-amino-4-methyl-3-coumarinyl)succinimide (**5a**): Colorless needles from EtOH, mp 262—265°, UV_{max}^{EtOH} nm (ε): 244 (15900), 379 (26000). N-(7-Dimethylamino-4-methyl-6-coumarinyl)succinimide (**5b**): Colorless needles from EtOAc, mp 220—221°; UV_{max}^{EtOH} nm (ε): 242 (14500), 350 (17500). Both

1) Fluorescent Thiol Reagents. IX. For Part VIII see: M. Machida, T. Sekine, and Y. Kanaoka, *Chem. Pharm. Bull.* (Tokyo), **22**, 2642 (1974).
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7) Y. Kanaoka, M. Machida, M.I. Machida, and T. Sekine, *Biochim. Biophys. Acta*, **317**, 563 (1973).
8) *a*) M. Machida, M. Bando, Y. Migita, and Y. Kanaoka, "in preparation."
9) All new compounds gave satisfactory elemental analyses and their structures were supported by spectral ultraviolet (UV), infrared (IR), nuclear magnetic resonance (NMR), Mass data.

4a and **4b** had no fluorescence, whereas **5a** and **5b** showed fluorescent spectra the characteristics of which are summarized in Table I. On treatment with N-acetyl-L-cysteine, **4a** and **4b** immediately reacted to give fluorescent products whose spectra are superimposable with those of **5a** and **5b**, respectively. The second-order rate constant for the reaction of **4a** with N-acetyl-L-cysteine as measured by the fluorometry was 1.5×10^3 $mol^{-1}sec^{-1}$ (0.1M phosphate buffer, pH 7.0; 25°). When egg albumin in aqueous solution was allowed to react with **4a** (0.1M phosphate buffer, pH 7.0), as described in the previous paper,[1] 0.6—0.7 mole of **4a** was introduced to the thiol group(s) of the protein.

TABLE I. Fluorescence Properties of **5a** and **5b**

Compound	**5a**		**5b**	
Solvent	EtOH	0.1M phosphate buffer, pH 7.0	EtOH	0.1M phosphate buffer, pH 7.0
Fluorescence maxima (nm) (ex. 390 nm)	457	477	447	447
Quantum yield	0.67	0.11	0.02	0.01>

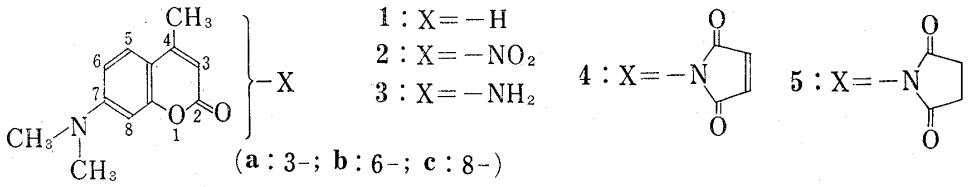

Chart 1

Dimethylaminocoumarine ring **1** seems to be one of candidate fluorogenic groups of choice. First, **1** is a bicyclic aromatic system, which is perhaps of the smallest size of rings that can exhibit sufficiently strong fluorescence,[8] and in addition, has some polar character due to presence of three heteroatoms. As a result **1** has improved water-solubility, which is one of essential properties to reagents to be employed for biological systems, in comparison with other general fluorogenic compounds. Second, **1** can be selectively excited in the presence of the intrinsic chromophores in natural biological systems because its absorption bands occur at longer wave-lengths. As a representative example, nonfluorescent **4a** reacts rapidly with a thiol compound to yield a product which emits intense fluorescence that can well be separated from that of protein. Thus N-(7-dimethylamino-4-methyl-coumarinyl)maleimides (DACM) are preferable fluorescent thiol reagents of maleimide-type. Application of DACM in various biochemical studies will be reported in forthcoming papers.

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