(1/300 of $PGF_{2\alpha}$) and III did not possess the PG-like activity. This fact was shown that the hydroxy group of 15-position of PGs was also very important for such bicyclo[4.3.0]nonane derivatives.

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N-(3-Fluoranthyl)maleimide (FAM): A Medium Lifetime Fluorescent Thiol Reagent[1)]

N-(3-Fluoranthyl)maleimide (FAM) is a fluorescent probe for thiols with a medium lifetime (~20 nsec), which can be used for studies of time-dependent processes of biopolymers.

Fluorescent probes have been effectively employed to map the microenvironments of biological macromolecules such as proteins and polynucleotides and to clock the dynamics of intramolecular interactions.[2)] The general idea behind a fluorescent probe is that fluorescence emission, sensitive to changes in microenvironments, will display distinct fluorescent properties which will characterize uniquely each environment. A number of different aspects of the fluorescence emission include spectral distribution of emission, the degree of polarization, and the time dependence of emission or polarized components of emission. In a series of systematic study we have developed several fluorescent thiol reagents of maleimide-type which satisfy the above requirements to varying degrees: *e.g.*, N-(*p*-(2-benzimidazolyl)phenyl)-maleimide (BIPM)[1,3)] is a reagent for microdetermination; N-(1-anilinonaphthyl-4)maleimide (ANM)[4)] is a hydrophobic probe; N-(7-dimethylamino-4-methylcoumarinyl)maleimide (DACM)[5)] has emission maximum at a longer wave region. Those reagents have proved useful

1) Fluorescent Thiol Reagents. XI. For Part X, see: T. Sekine, K.A. Kato, K. Takamori, M. Machida, and Y. Kanaoka, *Biochim. Biophys. Acta*, **354**, 139 (1974).
2) For example, see: *a*) G. Weber and F.W.J. Teale, "The Proteins," ed. by H. Neurath, Academic Press, New York, Vol. 3, 1965, p. 445; *b*) L. Brand and B. Witholt, "Methods in Enzymology," Academic Press, New York, Vol. 11, 1967, p. 776; *c*) C.R. Cantor and T. Tao, "Procedures in Nucleic Acid Research," Vol. 2, ed. by G.L. Cantoni and D.R. Davis, Harper & Row, Publishers, New York, 1971, p. 31; *d*) L. Brand and J.R. Gohlke, *Ann. Rev. Biochem.*, **41**, 843 (1972).
3) *a*) Y. Kanaoka, M. Machida, K. Ando, and T. Sekine, *Biochem. Biophys. Acta*, **207**, 269 (1970), and papers cited therein; *b*) T. Sekine, T. Ohyashiki, M. Machida, and Y. Kanaoka, *ibid.*, **351**, 205 (1974); *c*) K. Kimura, A. Watanabe, M. Machida, and Y. Kanaoka, *Biochem. Biophys. Res. Comm.*, **43**, 882 (1971); *d*) T. Sekine, K. Ando, M. Machida, and Y. Kanaoka, *Anal. Biochem.*, **48**, 557 (1972); *e*) M. Machida, T. Sekine, and Y. Kanaoka, *Chem. Pharm. Bull.* (Tokyo), **22**, 2642 (1974).
4) *a*) Y. Kanaoka, M. Machida, M.I. Machida, and T. Sekine, *Biochim. Biophys. Acta.*, **317**, 563 (1973); *b*) T. Ohyashiki, T. Sekine, and Y. Kanaoka, *ibid.*, **420**, 27 (1976).
5) *a*) M. Machida, N. Ushijima, M.I. Machida, and Y. Kanaoka, *Chem. Pharm. Bull.* (Tokyo), **23**, 1385 (1975); *b*) T. Sekine, *Seikagaku*, **47**, 441 (1975).

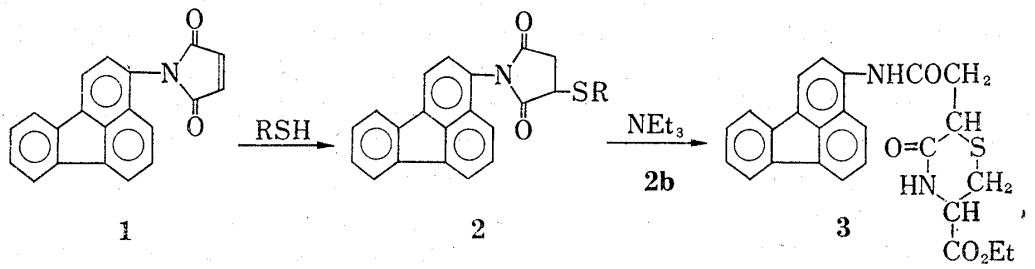
in the studies of various biological systems[1,3-5] particularly because they are fluorescent only when reacted with thiols obeying the empirical rule we had proposed.[3a] However, the time-dependent problems of fluorescence has remained largely unsolved. In this communication we wish to present data on the synthesis[6] and the properties of a new thiol reagent **1** which has been found after screening search of many aromatic compounds[7] and can be used for these problems since the reaction products show medium lifetime of the fluorescence.

3-Fluoranthylamine was converted into the maleamic acid (mp 198—200°) by reaction with maleic anhydride followed by cyclization with acetic anhydride to give N-(3-fluoranthyl)-maleimide (FAM) **1**: Pale yellow needles from benzene, mp 185—186°. N-(Fluoranthyl)-succinimide **4** was also prepared as a fluorescent model compound of the reaction products of **1** with thiols: Yellow needles from EtOAc, mp 257—258°. In agreement with the rule,[3a] FAM **1** had no fluorescence, whereas **4** showed fluorescent spectra as listed in Table I. On treatment with N-acetyl-L-cysteine **1** immediately reacted to give a fluorescent product **2a** whose spectra are nearly superimposable with those of **4**. The reaction product **2b** of **1** with L-cysteine ethyl ester was transformed by treatment with triethylamine into the rearranged product **3** (mp 227—229°),[3a,4a] thus establishing facile reaction of **1** with a cysteine derivative (Chart 1). When egg albumin in aqueous solution was allowed to react with **1** (0.1M phosphate buffer, pH 7.0, 25°) as in a previous paper,[3e] 0.3 mole of **1** was introduced to the thiol group(s) of the protein (UV spectra and amino acid analysis).

Table I. Fluorescent Properties[a] of **2**, **4**

Compound	**2a**	**2c**	**4**
Fluorescence maxima (nm) (ex. 362 nm)	462	462	460
Quantum yield	0.25	0.16	

a) measured in 0.1 M phosphate buffer, pH 7.0



1 2 3

a: RSH=N-acetyl-L-cysteine
b: RSH=L-cysteine ethyl ester
c: RSH=egg albumin

Chart 1

While the fluorescence lifetimes of the reaction products of thiols with the usual reagents such as BIPM, ANM, and DACM range less than 10 nsec, being too short for most biological studies, the excited-state lifetimes of the adducts of **1** with thiol compounds were around 20 nsec as determined by a phase shift method.[7] FAM **1** is thus a fluorescent probe for thiols

6) All new compounds gave satisfactory elemental analysis and their structures were supported by spectral (ultraviolet (UV), infrared (IR), nuclear magnetic resonance (NMR), mass) data.
7) T. Sekine, T. Ohyashiki, K. Yamamoto, H. Suzuki, T. Takahashi, Y. Takahashi, M. Machida, and Y. Kanaoka, "in preparation."

with medium[8] excited-state lifetimes which provides a novel entry to direct information about the time dependence of the processes including rotational motions and conformational changes of biopolymers. The use of FAM as a dynamic probe for various biological systems is currently under investigation.

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8) Based on our empirical rule, Weltman, *et al.* synthesized N-(3-pyrene)maleimide as a "long" lifetime probe whose adducts with proteins had lifetimes around 100 nsec; J.K. Weltman, R.P. Szars, A.R. Flockelton, R.M. Dowben, J.R. Bunting and R.E. Cathon, *J. Biol. Chem.*, **248**, 3173 (1973).
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Chemische Untersuchungen der Inhaltsstoffe von *Xanthium canadense* Mill.

Aus den Wurzeln von *Xanthium canadense* Mill. wurden zwei neue Sesquiterpen-Lactone vom Eremophilantyp isoliert, deren Strukturen mittels chemischer und spektroskopischer Methode als Eremophil-1(10),11(13)-dien-12,8β-olid (V, Xanthanodien) und 11R-Eremophil-1(10)-en-12,8β-olid (VIII, Xanthanen) identifiziert. Ausserdem wurden Isoalantolacton, Taraxerol und Taraxerolmonoacetat nachgewiesen.

Die Gattung *Xanthium* (Fam. Compositae, Tribus Heliantheae) ist bereits gut auf die Inhaltsstoffe untersucht worden und die bisherigen Ergebnisse zeigen, dass diese Gattung im Hinblick auf die Inhaltsstoffe nicht sehr einheitlich ist.[1] Bei *X. strumarium* L. geben es verschiedene chemoveränderliche Glieder. Einige[2] enthalten Xanthinin (I), die anderen[1,3] Xanthumin (II), die weiteren[1] die beiden und die sonstigen[1] Xanthinin zusammen mit Xanthanol (III) und Isoxanthanol (IV). Nach Minato und Horibe soll die in Japan heimische Art, *X. strumarium* L. (jap. Name: Onamomi) nur Xanthumin enthalten.[3] Wir haben jetzt eine in Nordamerika einheimische Art *X. canadense* Mill. (jap. Name: O-onamomi), die ebenfalls in Japan weit verbreitet ist, untersucht und zwei neue Sesquiterpen-Lactone isoliert.

Die Wurzeln (Sammelzeit: Oktober, 1974; Fundort: Komae, Tokyo) wurden mit Methanol extrahiert und der Extrakt im Vak. eingeengt. Dieser wurde im Wasser suspendiert und mit Benzol ausgezogen. Die Benzol-Schicht wurde mit Benzol mit zunehmendem $CHCl_3$-Zusatz

1) T.E. Winters, T.A. Geissman, und D. Safir, *J. Org. Chem.*, **34**, 158 (1969).
2) T.A. Geissman, P. Deuel, E.K. Bonde, und F.A. Adicott, *J. Am. Chem. Soc.*, **76**, 685 (1954).
3) H. Minato und I. Horibe, *J. Chem. Soc.*, **1965**, 7009.