

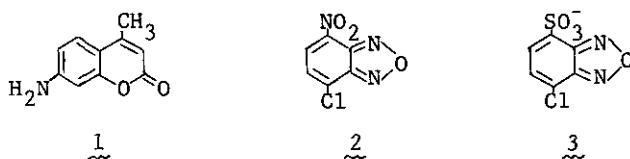
A NOVEL FLUOROGENIC SUBSTRATE FOR CHYMOTRYPSIN
— 4-AMINO-7-NITROBENZO-2-OXA-1,3-DIAZOLE DERIVATIVES —

Eisuke Sato, Makiko Miyakawa and Yuichi Kanaoka

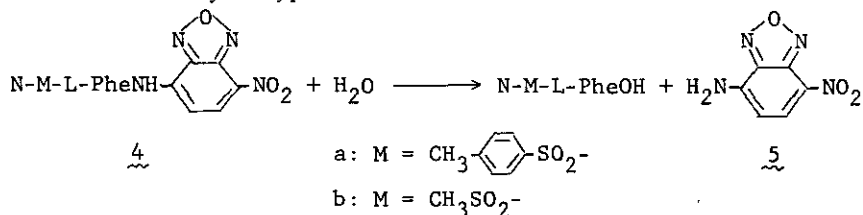
Faculty of Pharmaceutical Sciences, Hokkaido University

Sapporo, 060 Japan

In the course of exploring a sensitive fluorometric assay for amidase activity of hydrolytic enzymes¹⁾ such as leucine aminopeptidase²⁾, trypsin and so on, we have been noticed usefulness of 7-amino-4-methylcoumarin(1) and its amide derivatives as a useful fluorogenic substrates.



Recently, 4-chloro-7-nitro or 7-sulfo-2-oxa-1,3-diazole(2 or 3) have been used as a fluorogenic probe in biological research³⁾. A benzo-2-oxa-1,3-diazole system is expected to be a promising candidate as a fluorogenic amine. We describe the preparation of 7-(N-tosyl or N-mesyl-L-phenylalanyl)amino-4-nitrobenzo-2-oxa-1,3-diazole(4) and its ability to serve as a sensitive fluorogenic substrate for chymotrypsin.



Kinetic Parameters

substrate	K _m (M)	K _{cat} (s ⁻¹)	K _{cat} /K _m (s ⁻¹ M ⁻¹)
4a	1.92×10 ⁻⁵	4.13	2.15×10 ⁵
4b	3.13×10 ⁻⁴	3.69	1.18×10 ⁴

References

- 1) Y.Kanaoka, Ang.Chem.Int.Ed., 16, 137 (1977).
- 2) Y.Kanaoka et al., Chem.Pharm.Bull., 25, 3126 (1977).
- 3) J.L.Andrews et al., Arch.Biochem.Biophys., 214, 384 (1982).