thiouracil and the ribosyl chloride (IV) in the presence of two equivalents of mercuric cyanide also afforded the nucleoside (V) in a slightly lower yield. Trace of the N-3 riboside[13] was detected.

6-Methyl-2-thiouracil (VIII) was next treated with the ribosyl chloride (IV) at the presence of one equivalent of mercuric cyanide and after the similar work-up a blocked nucleoside was obtained as a syrup in a 34% yield: *Anal.* Calcd. for $C_{31}H_{26}O_8N_2S$: C, 63.48; H, 4.47; N, 4.78; S, 5.46. Found: C, 63.30; H, 4.40; N, 4.58; S, 5.24. Debenzoylation afforded a crystal (IX), mp 208—210° (decomp.): *Anal.* Calcd. for $C_{10}H_{14}O_5N_2S$: C, 43.80; H, 5.15; N, 10.22; S, 11.67. Found: C, 43.79; H, 5.10; N, 10.17; S, 11.58. NMR (d-DMSO, ppm from TMS): 2.09(6-CH_3), 5.72(5-H), 6.95(1′-H, $J_{1',2'}$=3 cps). The UV spectra of IX ($\lambda_{max}^{H_2O}$, mμ 215, 275(shoulder), 296; $\lambda_{max}^{OH^-}$, mμ 213, 262, 321) resembled to those of 3-alkyl-2-thiouracils rather than 1-alkyl-2-thiouracils.[10] Therefore it was concluded that the ribosylation had occurred on N-3 of VIII to afford 6-methyl-3-(β-D-ribofuranosyl)-2-thiouracil (IX). The steric hindrance of 6-methyl group should be the main reflection of this result.

The present method of ribosylation and alkylation should have wider application to various N-heterocyclic bases possessing potential sulfhydryl group, which with the usual alkylation procedure, would give S-alkylated derivative. Further studies on the scope of this reaction are presently being undertaken.

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New Fluorescence Edman Reagent for Microanalysis of Peptides

Stepwise degradation from an N terminal by Edman[1,2] with phenylisothiocyanate is widely utilized for sequential analysis of peptides and proteines. As the sensitivity based on the ultraviolet (UV) absorption of phenylthiohydantoin ring at 250 mμ is not enough to practical use for microanalysis of peptides, some fluorescence reagents have been used to increase the sensitivity, however, fluorescein isothiocyanate[3,4] produced scarcely soluble thiohydantoin derivatives, and Edman-DNS method[5,6] is indirect and tedious.

We have developed a new Edman reagent, 4-(N,N-dimethylamino)-1-naphthyl isothiocyanate (DNTC), which is capable of stepwise degradation of minute quantities of peptides

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producing fluorescenct DNTC–amino acids. This reagent was synthesized by the route shown in Chart 1, pale yellowish needles, mp 67° (*Anal.* Calcd. for $C_{13}H_{12}N_2S$: C, 68.62; H, 5.49; N, 12.24. Found: C, 68.38; H, 5.31; N, 12.27; S, 14.05).

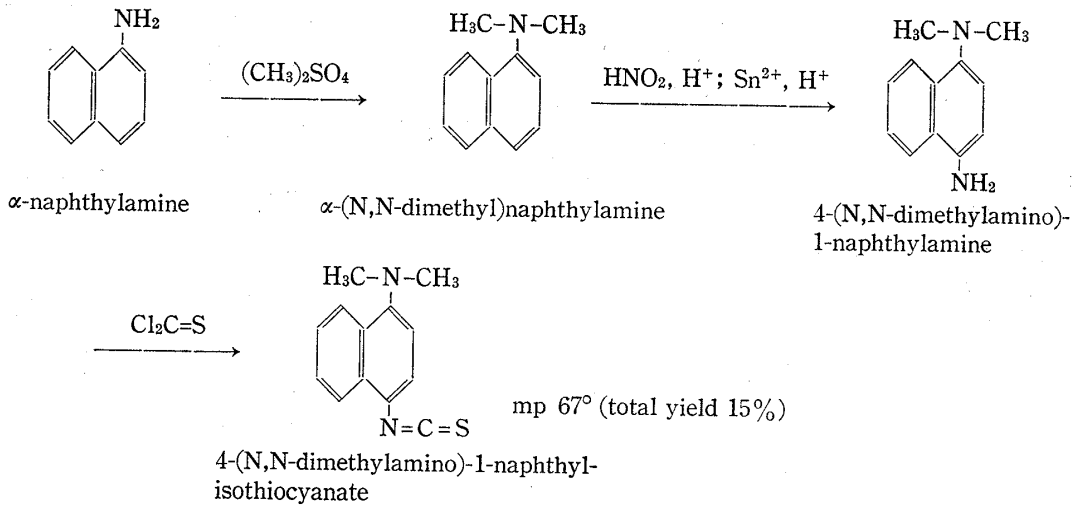

Chart 1. Route of Synthesis of 4-(N,N-dimethylamino)-1-naphthylisothiocyanate

When 10^{-7} mole of amino acid was dissolved in 0.2 ml of 0.1 M triethylamine–acetic acid (TEA–AcOH) buffer (pH 10.0) and was added with 2×10^{-6} mole of the reagent in 0.2 ml of dioxane at 40°, in 15 hr, all of the amino acids were quantitatively converted to the DNTC derivatives as shown in Table I. This experimental condition was also applied to oligopeptides such as Gly-dipeptides and tripeptide.

Table I. Rate of Coupling Reaction with Amino Acid

Amino acid	%	Amino acid	%	Amino acid	%
Lys	93.5	Glu	100	Met	100
His	100	Pro	100	Ileu	98.7
Arg	100	Gly	96.8	Leu	97.7
Asp	100	Ala	98.4	Tyr	98.9
Thr	93.2	Cys	100	Phe	97.7
Ser	99.5	Val	100		

The reaction rate was caluculated from the quantity of remaining amino cid determined with ninhydrin.

Table II. Rate of Ring forming Reaction of Gly-Dipeptide

Dipeptide	%	Dipeptide	%	Dipeptide	%
Gly-Pro	84	Gly-Met	74	Gly-Tyr	72
Gly-Ala	85	Gly-Ileu	82		
Gly-Val	80	Gly-Leu	77		

The reaction rate was calculated from the quantity of liberated C-terminal amino acid determined with ninhydrin.

After evaporation under N_2 stream or *in vacuo*, excess of reagent was extracted with *n*-hexane and DNTC–peptide was treated with 0.1 ml of 50% trifluoroacetic acid solution in benzene at 40°, protecting from the moisture. The formation of thiazolinone ring accomplishes within 15 min, as shown in Table II.

The thioazlinone ring made from the first amino acid residue is opened easily with TEA–CO_2 solution (pH 9.0) at room temperature and turned to DNTC–amino acid, which had fluorescence maximum at 450 mμ (exciting 340 mμ). Above mentioned condition was applied to Leu–Gly–Gly. In the first round, 75% production of Gly-Gly and in the second, 100% of Gly were determined with ninhydrin. DNTC–Leu, DNTC–Gly, DNTC–Leu–Gly–Gly and DNTC–Gly–Gly produced were identified on TLC plate, detection limit was about 10^{-10} mole and the fluorescence was stable in a dark place for a few days. The method is much more sensitive (about 100 times) than phenylisothiocyanate method, and has another merit that DNTC–peptide produced is also fluorescent and easily purified by thin-layer chromatography. Details of this experiment will be reported in the near future.

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Detection of Catecholamines after N-Dansylation on the Surface of Alumina

Catecholamines (CA), *i.e.* dopamine, norepinephrine and epinephrine are biologically important amines and determined by fluorometry (THI method and ED method) and gas chromatography. The THI method[1,2] is most popular, however the separate determination of norepinephrine and epinephrine is difficult on account of the similarity of those fluorofores. The ED method[3] is cumbersome because it requires the separation of each amines before the reaction. The gas chromatographic separation and detection have been examined by trimethylsilyl, acetyl, trifluoroacetyl and heptafluorobutyryl derivatives and Kawai, *et al.* succeeded in the determination of CA in urine and tumor by gas chromatography.[4] The method is sensitive and selective for CA, however it is difficult to treat many samples simultaneously in the case of clinical use. Now we investigated dansylation of CA with 1-dimethylaminonaphthalene-5-sulfonyl chloride (DNS–Cl) and detection of those fluorescent derivatives on a thin layer chromatogram.

Seiler, *et al.*[5] and Creveling, *et al.*[6] have studied dansylation of CA in the presence of sodium bicarbonate to gain some fluorescent products which are probably the mixture of N-mono-DNS, N,O-di-DNS and N,O,O-tri-DNS derivatives. To overcome this difficulty complete dansylation was examined by Diliberto, Jr., *et al.*[7] On the contrary, we have attained N-mono-dansylation of CA by protecting the pyrocatechol with alumina.

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