stallized from H_2O and EtOH; yield 35 mg (84.2%), mp 240—244°, $[\alpha]_D^{25}$ −45.4° (c=1.1, H_2O). (Lit.[8] $[\alpha]_D^{25}$ −50.4° in H_2O, Lit.[9] $[\alpha]_D^{22}$ −46.8° in H_2O). Parallel experiment was performed using trifluoromethanesulphonic acid (1 ml) in trifluoroacetic acid (5 ml). Yield of H-Gly-Ala-OH was 82.0%.

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Ennancement of Fluorescence Intensity of Dansyl Amino Acids on Silica Gel Plate using Cycloheptaamylose[1]

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Cycloheptaamylose (C7A) was found to greatly enhance and stabilize the fluorescence intensity of dansyl (DNS) amino acids on silicagel layer and this finding was applied to the detection and determination of these compounds on thin-layer chromatogram. Procedure: After the development of DNS-amino acids, the thin-layer plate is sprayed with 10 ml of 1.8% C7A, dried over phosphorus pentoxide and the spots are detected under a mercury lamp or determined using a scanning fluorometer.

Above 10 fold increase in the fluorescence was observed. The fluorescence was stable for 90 min under exposure to air in the diffused light and for 24 hr over phosphorus pentoxide under reduced pressure. Limits of detection for 24 DNS-amino acids were in the range of 0.1 to 0.5 pmole/spot. Standard curves for DNS-amino acids were linear in the range of 5 to 30 pmole. Samples from 1 to 5 pmole could also be determined semi-quantitatively.

Dansylation[3] coupled with thin-layer chromatography (TLC) has extensively been used for the detection or determination of low quantities of amines,[4] amino acids,[5] peptides,[6] and proteins.[7] However, the fluorescence of dansyl (1-dimethylaminonaphthalene-5-sulfonyl, DNS) derivatives on silica gel layers fades rapidly during storage.[3,8] Consequently, great care has been required in drying the thin-layer plates for the removal of developing solvents under strictly defined conditions to assure reproducibility, and the sensitivity was not always satisfactory.[3] Although the enhancement and stabilization of the fluorescence intensity was attained by spraying triethanolamine,[9] this reagent was susepeptible to oxidation on exposure to air and light, and not applicable to alkali-sensitive compounds. Recently,

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fluorescamine[10] was proved to be effective for the detection of various amines and amino acids on thin-layer chromatogram.[11] However, the TLC of DNS derivatives has been well established and many informations on solvent systems, application to biological samples, *etc.* are readily available.[3-9] Moreover, DNS-amino acids are the key substances for the fluorescent labeling method or sequence analysis of N-terminal determination of peptides and proteins.[3-12] Improvement of thin-layer chromatographic procedure for DNS derivatives therefore appears to be of much practical value.

In the preceding paper,[13] we have reported on the enhancement of the fluorescence intensity of DNS-amino acids by cycloheptaamylose (C7A) in aqueous media. In the present study, C7A was proved to greatly enhance and stabilize the fluorescence of DNS-amino acids on silica gel layer in the dry state.

Materials and Methods

DNS-L-amino acids were purchased from Seikagaku Kogyo Co. Stock solutions of DNS-amino acids were prepared by firstly dissolving 10 μmol of the sample in 2 ml of methanol and then diluting to 5 ml with glass-distilled water. The stock solutions were further diluted as indicated in each experimental regend. Thin-layer plates (20×20 cm) were prepared in the usual manner from Silica gel G (Merck Co.). Solvents were redistilled before use.

Procedure——One microliter of DNS-amino acid solution is applied on a silicagel plate and the plate is developed using chloroform-triethylamine (10: 1) as a solvent. The plate is splayed with 10 ml of 1.8% aqueous solution of C7A immediately after the development and dried over phosphorus pentoxide for 30 min under reduced pressure. The spots are detected by irradiation at 365 nm using Super-Light Model LS-DI, Nikko Seiki Works Co. The fluorescence intensity is measured by scanning fluorometer Model SFR 021, Yamato Scientific Instruments Co., equipped with a 40 W high voltage mercury lamp.

Results and Discussion

Above 10 fold increase in the fluorescence of DNS-amino acids was observed by spraying the C7A solution. For example, the fluorescence intensity of DNS-glycine was enhanced 11.8 fold by spraying 1.8% C7A. On the other hand, the solutions of glucose, dextran, and starch of the same concentration caused only 1.6, 1.4 and 2.8 fold increase in the fluorescence of this DNS-amino acid, respectively. The hydrophobic cavity of C7A has been proved to play an important role in the enhancement of the fluorescence intensity of DNS-amino acids by forming inclusion compounds.[13,14] Figure 1 displays the stability of the fluorescence of DNS-leucine, DNS-arginine, and DNS-alanine, after spraying the plate with 1.8% C7A. The fluorescence was stable for 90 min at room temperature under exposure to air in the diffused light. The fluorescence was not affected by irradiation from the light source of the scanning fluorometer in the usual determinations. When the plate was stored over phosphorus pentoxide under reduced pressure in the dark, the fluorescence was stable for 24 hr. Limit of detection was as follows: 0.1 pmole for DNS-leucine, DNS-isoleucine, DNS-serine, DNS-glycine, DNS-phenylalanine, DNS-threonine, DNS-hydroxyproline, N-ε-DNS-lysine, O-DNS-tyrosine, and N,O-di-DNS-tyrosine; 0.5 pmole for DNS-alanine, DNS-proline, DNS-valine, DNS-arginine, DNS-methionine, DNS-tryptophan, DNS-glutamic acid, DNS-glutamine, DNS-aspartic acid, DNS-asparagine, DNS-cysteic acid, N,N′-di-DNS-cystine, N,N′-di-DNS-histidine, DNS-amide, and DNS-sulfonic acid.

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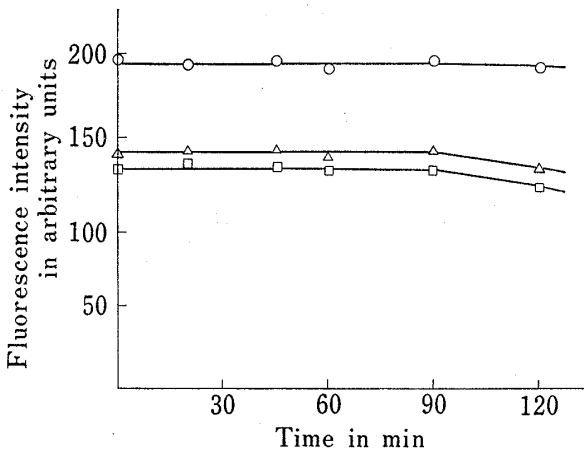

Fig. 1. Stability of the Fluorescence Intensity of DNS-Leu (○), DNS-Arg (△), and DNS-Ala (□), at Room Temperature under Exposure to Air in the Diffused Light

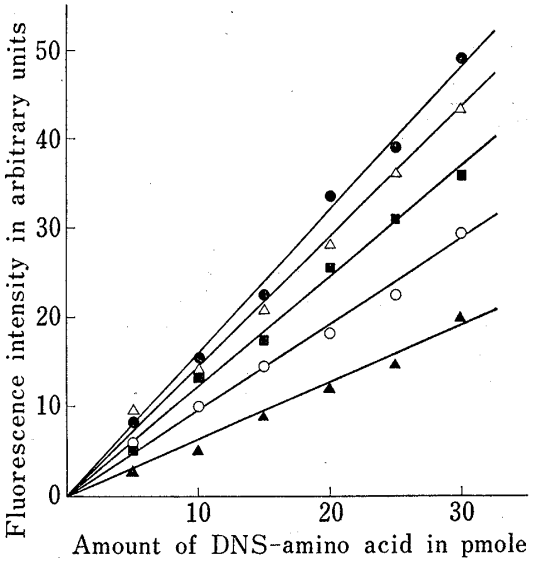

Fig. 2. Standard Curves for DNS-Ser (●), DNS-Glu (△), N-ε-DNS-Lys (■), DNS-Leu (○), and O-DNS-Tyr (▲)

Standard curves for DNS-amino acids were linear in the range of 5 to 30 pmole. Figure 2 illustrates the standard curves for DNS-serine, DNS-glutamic acid, N-ε-DNS-lysine, DNS-leucine, and DNS-tyrosine. DNS-glutamic acid showed intense fluorescence whereas this compound gave poorer value of detection limit than the other DNS derivatives exhibited in Fig. 2. This fact suggests that other factors than fluorescence intensity may affect the limit of detection for these compounds. Coefficients of variation were 18.5, 9.9, and 9.2% for 10, 20, and 30 pmole of DNS-leucine, respectively, and 14.8, 14.6, and 12.2% for 10, 20, and 30 pmole of DNS-phenylalanine, respectively. For calculation of the each coefficient, five runs were carried out on different plates. In the present study, samples from 1 to 5 pmole could also be determined semiquantitatively. For rapid detection and determination, the plate sprayed with C7A could be dried by heating it at 110° for 3 min, since C7A was far more stable than triethanolamine and the fluorescence of DNS-amino acid was little affected by heating in the presence of C7A.

Preliminary studies on the chromatography of DNS-amino acids on silica gel plates impregnated with 1.8% C7A indicated similar sensitivity as above. This procedure was especially suitable for two-dimentional development, since the spots were also detected in excellent sensitivity after the first development without spraying. Moreover, this procedure appeared to be more rapid and reproducible than the spray method. However, the impregnation of the layer with C7A was found to influence the *Rf* values. Details of the impregnation method, together with the *Rf* values, will be published elsewhere.