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Chromophoric Labeling of Amino Acids with 4-Dimethylaminoazobenzene-4′-sulfonyl Chloride

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4-Dimethylaminoazobenzene-4′-sulfonyl chloride (DABS-Cl,dabsylchloride) was synthesized by reaction of sodium 4-dimethylaminoazobenzene-4′-sulfonate with phosphorus pentachloride. DABS-Cl formed deep red, plate-shaped crystals and was stable at room temperature. DABS-Cl reacted readily with all amino acids to form dabsyl amino acids. The dabsyl amino acids (10^{-11}–10^{-10} mol) were visualized on thin layer plates and found to be photo-stable. DABS-Cl was shown to be a new chromophoric labeling reagent for amino acids, peptides, and proteins, and because of its stability and sensitivity, the reagent is useful for qualitative and quantitative analyses.

Since Sanger used 2,4-dinitrofluorobenzene for the identification of *N*-terminal amino acids (*1*), many reagents such as dinitrofluoroaniline (*2*), chlorodinitropyridine (*3*), phenylthiocyanate (*4*), 4-(*N,N*-dimethylamino)-1-naphthylisothiocyanate (*5*), 2-*p*-isothiocyanophenyl-3-phenylindone (*6*), and dansyl chloride (*7, 8*) have been developed for the same purpose. Most of these reagents were designed on the basis of their fluorescence emission and ultraviolet absorption properties. Although the electronic absorptions of some nitro derivatives in the visible region are detectable, the sensitivity is not sufficient to allow estimation of amino acids in nanomolar quantities.

The purpose of this study was to find a new, sensitive, chromophoric labeling reagent for amino acids. The new compound, 4-dimethylaminoazobenzene-4′-sulfonyl chloride (DABS-Cl, dabsyl chloride) (Figure 1, *B*) was found to be an active end group reagent for amino acids, peptides, and proteins. The dabsyl derivatives of amino acids even on scales as low as 10^{-11}–10^{-10} mol were stable and could be visualized on thin layer plates. The synthesis of DABS-Cl, reaction of it with amino acids, peptides, and proteins, and TLC analyses of these dabsyl derivatives are described in this report.

EXPERIMENTAL

Materials. Crystalline pancreatic insulin, dansyl chloride, 2,4-dinitrofluorobenzene, and L-amino acids kit (No. LAA-21, Sigma) containing alanine, arginine HCl, asparagine, aspartic acid, cysteine, cystine, glutamic acid, glutamine, glycine, histidine HCl, hydroxyproline, isoleucine, leucine, lysine HCl, methionine, phenylalanine, proline, serine, threonine, tryptophan, tyrosine, and valine were purchased from Sigma Chemical Co., St. Louis, Mo. Glycylglycine was the product of Nutritional Biochemical Corporation, Cleveland, Ohio. Silica gel plates (Chromagram Sheet 6061) were obtained from Eastman Organic Chemicals, Rochester, N.Y. Wakogel C-200 (a preparation of silica gel) was purchased from Wako Chemical Co., Japan.

Synthesis of 4-Dimethylaminoazobenzene-4′-sulfonyl Chloride (DABS-Cl). Sodium 4-dimethylaminoazobenzene-4′-sulfonate (DABS-ONa) was prepared by coupling diazotized sulfanilic acid with dimethylaniline (*9*). DABS-ONa (6 g) was ground in a mortar with 9 g of phosphorus pentachloride for 8 minutes. The reaction mixture was poured onto a mixture of cracked ice and water (500 ml); insoluble DABS-Cl was collected by suction filtration and washed three times with water (200 ml). The product was dried and extracted with acetone (300 ml) in a Soxhlet apparatus. After concentration, the acetone extract gave shiny, deep red crystals of DABS-Cl; yield, 20%. The compound turned black on slow heating (2 °C/min) and had no definite melting point; however, the same compound melted sharply at 186–188 °C on rapid heating (20 °C/min).

Anal. Calcd for $C_{14}H_{14}N_3O_2ClS$: C, 51.91; H, 4.32; N, 12.97; Cl, 10.97; S, 9.91. Found: C, 51.91; H, 4.42; N, 13.13; Cl, 10.96; S, 10.06.

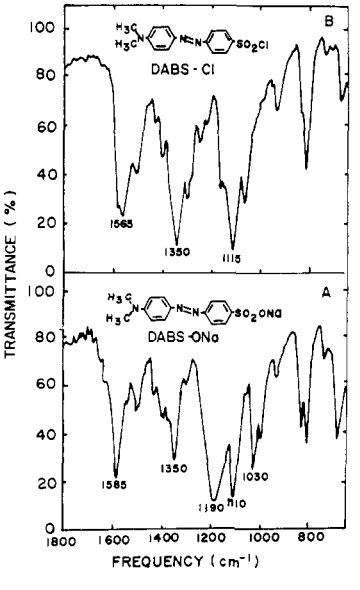

Figure 1. Infrared spectra of DABS-Cl and DABS-ONa prepared as KBr disks

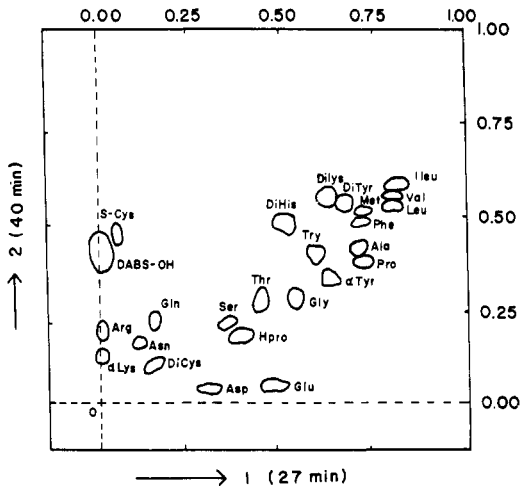

Figure 2. Bidimensional thin layer chromatography of dabsyl amino acids

A mixture of 22 dabsyl amino acids (0.2 nmol each) was applied successively on the silica gel plate at the origin and ascending TLC was performed in a closed chamber by using solvent 1 for development in direction 1 (27 min) and solvent 2 for development in direction 2 (40 min)

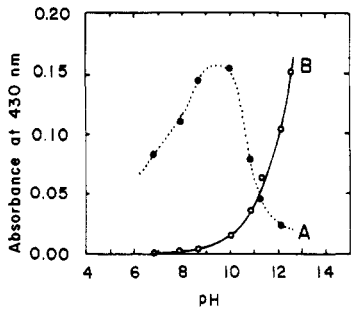

Figure 3. Reaction of DABS-Cl with serine at various pH values

Dabsylation of serine was carried out in 0.1*M* phosphate buffer (pH 6, 7, and 8), in carbonate–bicarbonate buffer (pH 9, 10, and 11), and in carbonate-NaOH (pH 12 and 13). The other conditions are described in the text. Curves *A* and *B* indicated the formation of *N*-dabsyl serine and *O*-dabsyl serine, respectively

The infrared spectra of DABS-Cl and DABS-ONa (1 mg in 200 mg KBr) were recorded on a Perkin-Elmer IR Spectrophotometer Model 700 and are given in Figure 1. Haszeldine and Kidd have assigned the strong absorptions in the ranges 1190–1170 and 1064–1040 cm^{-1} to sulfonic acids and their salts (*10*). DABS-ONa showed three strong absorption bands at 1190, 1110, and 1030 cm^{-1} (Figure 1, *A*); the first and third bands disappeared and a new band at 1115 cm^{-1} was detected when DABS-ONa was converted to DABS-Cl (Figure 1, *B*).

The electronic absorption maxima and molar absorptivities of DABS-Cl (acetone) and DABS-ONa (ethanol) were 465, 4.0×10^4 and 420, 3.4×10^4, respectively and those in acidic medium (ethanol:HCOOH, 2:1, v/v) were 496, 7.5×10^4 and 515, 6.2×10^4, respectively.

Thin Layer Chromatography (TLC) (Figure 2). Silica gel plates (Chromagram 6061, 20 cm × 20 cm) were cut into 10 cm × 10 cm, 5 cm × 5 cm, or 2.5 cm × 5 cm strips and used for ascending TLC in a closed glass chamber. The following solvent systems were employed: solvent 1, benzene–pyridine–acetic acid (80:20:5, v/v/v); solvent 2, toluene–2-chloroethanol–25% ammonia water (100:80:6.7, v/v/v); and solvent 3, *tert*-butanol–ethyl acetate–acetic acid (6:3:1, v/v/v).

Dabsylation of Amino Acids with DABS-Cl. Since DABS-Cl (dabsyl chloride) has the same functional group as dansyl chloride, conditions of dabsylation described here were mostly similar to that of dansylation (*11*). The best conditions for dabsylation were the following: 600 nmol of each amino acid (22 amino acids were studied) was dissolved in 0.3 ml of sodium carbonate–sodium bicarbonate buffer (0.27 ml of 0.1*M* $NaHCO_3$ plus 0.03 ml of 0.1*M* Na_2CO_3, pH 8.9). To this amino acid solution, 0.3 ml of DABS-Cl solution (3.3 mg/ml, 10 μmol/ml) in acetone was added. The mixture was tightly stoppered with a glass stopper and allowed to react at 70 °C in a water bath with constant shaking for 6 minutes. After the precipitate redissolved and its color turned from red-orange to orange, the reaction mixture was ready for TLC. The volume of reaction mixture could be reduced to 20 μl by using only 20 nmol of each amino acid. Higher pH values and lower dabsyl chloride to amino acid ratios gave rise to multiple derivatives of several amino acids (Figure 3 and Table I).

For bidimensional TLC, about 0.2 nmol (0.2 μl) of each reaction mixture was applied successively at the origin of a 10 cm × 10 cm silica gel plate. To ensure good separation of dabsyl amino acids, the diameter of the original spot should not exceed 2–3 mm. Solvents 1 and 2 were used for the first and second development, respectively. After chromatography, the plates were dried with a hot air stream and the dabsyl amino acids detected as yellow spots. Greater sensitivity was achieved by exposing the plate to HCl vapor in a closed chamber for a few seconds. The dabsyl amino acids were easily observed as bright red spots.

For synthesis of dabsyl amino acids on a large scale, 5 ml of each amino acid solution (30–40 μmol/ml in 0.1*M* $NaHCO_3$) was mixed with 5 ml of DABS-Cl in acetone (10 μmol/ml). The mixture was allowed to react at 70 °C in a water bath for 10 minutes and the acetone was partially evaporated by a stream of hot air. The residual mixture was then applied on a column (20 cm × 6 cm) packed with Wakogel C-200. Separation of dabsyl amino acid from DABS-ONa was achieved by using solvent 1 as eluting agent, with which dabsyl amino acid was easily eluted while DABS-ONa stayed on the column.

Table I. Reaction of DABS-Cl with Polyfunctional Amino Acids

		Dabsyl amino acids		
			R_f[b]	
Amino acid	System[a]	Product	Solvent 1	Solvent 2
Histidine	A	Bis-dabsyl	0.48	0.49
	B	Bis-dabsyl (minor)	0.48	0.49
		Mono-dabsyl (major)	0.00	0.14
		Mono-dabsyl (major)	0.00	0.24
Lysine	A	Bis-dabsyl	0.62	0.56
	B	Bis-dabsyl (minor)	0.62	0.56
		Mono-dabsyl (major)	0.00	0.14
		Mono-dabsyl (minor)	0.00	0.57
Tyrosine	A	Bis-dabsyl	0.67	0.54
	B	Bis-dabsyl (minor)	0.67	0.54
		N-monodabsyl (major)	0.58	0.33
		O-monodabsyl (major)	0.00	0.26
Cysteine	A	Bis-dabsyl	0.13	0.09
	B	Bis-dabsyl (minor)	0.13	0.09
		S-dabsyl (major)	0.06	0.45

[a] System A: The molar ratio of DABS-Cl to amino acid is 5:1. System B: The molar ratio of DABS-Cl to amino acid is 1:1. [b] The composition of solvents 1 and 2 is described in the text.

Dabsylation of Peptide and Protein with DABS-Cl. Glycylglycine or insulin (0.3 μmol) was dissolved in 0.5 ml of carbonate–bicarbonate buffer, pH 8.9. To this solution, 0.5 ml of DABS-Cl (10 μmol/ml) in acetone was added. The mixture was heated at 70 °C with constant shaking for 6 minutes and then evaporated to dryness in vacuo. The residue was dissolved in 1.5 ml of 5.7*N* HCl and transferred to a thick-walled glass tube which was then sealed and heated at 105 °C for 3 hr. After hydrolysis, the hydrolysates were subjected to TLC analysis.

Quantitative Determination of Amino Acids. Under the conditions described above most amino acids reacted completely with DABS-Cl when the molar ratio of DABS-Cl to amino acid was greater than 2:1. Taking advantage of this characteristic, the following procedure was developed for quantitative determination of amino acids. After dabsylation of the amino acids as described, the volume of the reaction mixture was restored with acetone, aliquots (16 μl) of the solution were then applied zonally to a silica gel plate and developed with solvent 1 to separate dabsyl amino acids from DABS-OH. Zones containing dabsyl amino acids were removed and extracted with 2 ml of solvent 1 or 3. Absorbances were mea-

Table II. Physicochemical Properties of DABS-Amino Acids

Amino acid	Color[a]	Melting point °C	Electronic absorption peaks (nm) and molar absorptivities ($\epsilon \times 10^{-4}$)		
			Solvent 1	Acetone	0.1*M* $NaHCO_3$
Ala	R-O	180 (dec)	430 (2.22)	434 (1.55)	474 (1.73)
Asp	R-O	... (dec)	428 (2.43)	428 (1.89)	472 (3.51)
DiLys	R-O	147 (dec)	430 (6.18)	430 (6.03)	473 (1.14)
Glu	R-O	178 (dec)	432 (3.21)	436 (1.71)	472 (2.12)
Gln	R-O	196 (dec)	433 (2.51)	436 (4.04)	476 (4.35)
Gly	R	177 (dec)	430 (1.86)	436 (2.19)	474 (1.95)
HPro	R	180 (dec)	436 (2.72)	436 (2.99)	475 (2.72)
Ileu	R-O	188 (dec)	432 (3.14)	437 (3.99)	470 (1.80)
Leu	R-O	185 (dec)	432 (2.36)	437 (2.59)	472 (2.72)
Met	O	... (dec)	435 (2.23)	436 (1.96)	472 (1.01)
N-Tyr	O	210 (dec)	434 (2.97)	435 (2.79)	470 (2.21)
Pro	R-O	165 (dec)	435 (2.67)	440 (4.23)	476 (1.63)
Phe	Y-O	189 (dec)	432 (2.18)	436 (2.09)	473 (1.70)
Ser	O	266 (dec)	430 (2.76)	436 (4.56)	471 (2.16)
Thr	R-O	216 (dec)	430 (3.05)	436 (3.12)	472 (2.94)
Trp	O	150 (dec)	430 (2.36)	436 (2.63)	472 (1.81)
Val	O	190 (dec)	433 (4.83)	436 (3.15)	469 (2.36)

[a] R, red; O, orange; R-O, red-orange; Y-O, yellow-orange.

sured at 430 nm in a Beckman-DK-2A Spectrophotometer. Amounts of amino acids were calculated from standard curves of corresponding amino acids.

RESULTS AND DISCUSSION

Identification of Dabsyl Amino Acids. Dabsyl derivatives of 22 amino acids were prepared and separated on silica gel plates by bidimensional TLC. The results are shown in Figure 2. Nearly all dabsyl amino acids were separated. Only derivatives of leucine, isoleucine and valine, methionine and phenylalanine, and alanine and proline gave trouble by overlapping to some extent. The relative positions of these overlapped amino acids could be recognized by the heterogenous color intensity within the overlapped spots.

The microcrystals of 17 DABS-amino acids were prepared as described in the Experimental Section. The physicochemical properties of these derivatives, including the colors, melting points, electronic absorption maxima, and molar absorptivities are given in Table II. The presence of sulfonamide groups (–SO_2–NH–) in these DABS-amino acids were revealed by their infrared absorptions around 1140–1130 cm^{-1} and 1370–1350 cm^{-1} (Table III), since several authors have reported that sulfonamides absorb at 1178–1159 cm^{-1} and 1370–1333 cm^{-1} (*12*).

Stabilities of DABS-Amino Acids. Aliquots of DABS-amino acid solutions were separated on silica gel plates before and after hydrolysis or exposure to light, the DABS-amino acid zones were scraped off and extracted with solvent 1. The absorbances were read at 430 nm to estimate the recovery percentages. Data in Table IV demonstrate that most DABS-amino acids were quite photostable in $NaHCO_3$ buffer or on silica gel plates. Some DABS-amino acids were moderately stable to acid hydrolysis at 105 °C, whereas DABS-trp was extremely labile.

Dansyl chloride and 2,4-dinitrofluorobenzene have been widely used for amino acid labeling. However, the photodecomposition of DNP-amino acids and the fading process of dansyl amino acids seemed to hamper the quantitative determination of these derivatives on silica gel plates. Pollara and von Korff found that some DNP-amino acids were decarboxylated to the corresponding DNP-alkylamines under the influence of light (*13*). Furthermore, Russel observed that most DNP-amino acids were decomposed to 4-nitro-2-nitrosoaniline in $NaHCO_3$ solution under intense illumination (*14*). Seiler reported that on freshly dried thin layer plates one hour after the chromatographic separation, about 0.1n*M* of a dansyl amino acid could be recorded; and on plates dried over a 24-hr period an amount of only 1–10n*M* could be observed, even though the plates were stored in a dark desiccator (*15*). To improve the reproducibility, some defined conditions for drying the dansyl amino acids on silica gel plates should be followed (*16*). All of these reflect the instability of these two widely used reagents for the micro quantitative analysis of amino acids on the thin layer plates. DABS-Cl is able to overcome some aspects of these problems.

Identification of N-Terminal Amino Acids of Glycylglycine and Insulin. Glycylglycine and insulin were dabsylated and hydrolyzed as described in the Experimental section. TLC of hydrolyzates from glycylglycine demonstrated the presence of dabsyl glycine while bidimensional TLC of hydrolyzates from dabsyl insulin revealed the presence of dabsyl glycine and dabsyl phenylalanine. Aliquots applied to the plate were approximately 5×10^{-10} mol (assuming all the insulin molecules had been dabsylated and hydrolyzed). Identification of the two *N*-terminal amino acids of insulin, glycine and phenylalanine, was easily achieved.

In the *N*-terminal amino acid determination of insulin, the entire procedure was performed in a thick-walled glass tube and TLC apparatus. There was no need of any other operation, precipitation, or extraction. The reagents used were DABS-Cl, carbonate buffer, and hydrochloric acid. Even under these simple conditions, two *N*-terminal amino acids of insulin were easily identified in a few hours when only 5×10^{-10} mol of hydrolyzed dabsyl insulin was used. However, the –N=N– bond could be partially cleaved if the dabsylated protein was hydrolyzed in acid at 105 °C for 20 hr. Furthermore, the –N=N– bond might be chemically cleaved if the hydrolyzing mixture contained reducing substance. The poor resistance of –N=N– bond to acid hydrolysis (Table IV) will somewhat reduce the sensitivity of DABS-Cl in *N*-terminal amino acid analysis.

Quantitative Determination of Amino Acids. When a given amino acid (glycine, alanine, hydroxyproline, glutamic acid, phenylalanine, or serine) at various concentrations reacted with DABS-Cl under the conditions described, the dabsyl amino acids formed were isolated by TLC and estimated at 430 nm. A linear relationship was obtained for all amino acids studied.

Table III. Infrared Spectra of DABS-Amino Acids

Amino acid	Positions and intensities of absorption maxima (cm^{-1})[a]						
	$=SO_2-N=$	Others					
Ala	1130s	675m,	745w,	815s,	835s,	875w,	960m,
	1350s	1080s,	1220w,	1290s,	1405w,	1505w,	1587s.
Asp	1140s	685s	820s,	850s,	970w,	1090s,	1225w,
	1370s	1310m,	1510w,	1595s,	1700w		
DiLys	1135s	680s,	745w,	820s,	840s,	940m,	1085s,
	1155s	1220w,	1250w,	1305s,	1405m,	1505m,	1595s,
	1360s	1700w.					
Glu	1140s	680s,	752w,	820s,	850m,	900m,	975s,
	1160s	1087s,	1225w,	1250m,	1325s,	1405s,	1480w
	1365s	1520w,	1540w,	1600s,	1675s.		
Gln	1140s	690s,	745m,	820s,	845m,	940w,	980m,
	1160s	1087s,	1225m,	1250w,	1330m,	1410m,	1440w,
	1360s	1510w,	1595s,	1630s.			
Gly	1140s	695s,	750m,	825m	840m,	855s,	945m,
	1160s	1090s,	1220s,	1255w,	1325s,	1415m,	1515m,
	1367s	1600s,	1718s.				
HPro	1135s	685s,	740w,	820s,	845m,	940w,	1020m,
	1155s	1095m,	1225w,	1325s,	1405w,	1505w,	1435w,
	1360s	1595s,	1715m.				
Ileu	1140s	690s,	745m,	815s,	840m,	890w,	940m,
	1160s	1055w,	1085s,	1210w,	1245w,	1325m,	1415m,
	1360s	1505m,	1595s,	1700m.			
Leu	1135s	685s,	745m,	820s,	845m,	905m,	940m,
	1165s	1085s,	1225w,	1250w,	1325m,	1410w,	1510m,
	1360s	1595s,	1680w.				
Met	1140s	685s,	745w,	820s,	840m,	870w,	940w,
	1160s	975m,	1000m,	1090s,	1220w,	1250w,	1300s,
	1367s	1400s,	1590s,	1660w.			
N-Tyr	1140s	690s,	745w,	825s,	840s,	940s,	1080s,
	1160s	1105m,	1225s,	1250m,	1335m,	1415m,	1440m,
	1360s	1505s,	1600s,	1720s.			
Pro	1130s	680s,	745w,	820s,	845m,	940w,	1000m,
	1160s	1090m,	1195m,	1225m,	1330s,	1405m,	1435w,
	1360s	1510w,	1595s,	1720w.			
Phe	1135s	685s,	745s,	820s,	850s,	895s,	960s,
	1155s	1090s,	1225w,	1240w,	1295s,	1435m,	1510w,
	1370s	1600s,	1685w.				
Ser	1140s	690s,	745w,	820s,	845s,	890w,	945w,
	1160s	970m,	1020s,	1060m,	1087s,	1115m,	1200m,
	1360s	1220m,	1250w,	1320s,	1390m,	1410m,	1510m,
		1600s,	1720s.				
Thr	1140s	690s,	750w,	820s,	850m,	910m,	940m,
	1160s	1020m,	1092s,	1225w,	1250w,	1310m,	1330m,
	1360s	1410m,	1505w,	1600s,	1700w,	1718w,	
Trp	1135s	680s,	740s,	820s,	840s,	940s,	1005w,
	1160s	1060m,	1087s,	1215m,	1240w,	1305m,	1325m,
	1355s	1410m,	1505m,	1595s,	1700m.		
Val	1135s	680s,	750m,	820s,	845s,	887m,	940m,
	1160s	1040w,	1060w,	1085s,	1220w,	1250w,	1320s,
	1360s	1410m,	1435w,	1505m,	1595s,	1700m.	

[a] s, strong; m, medium; w, weak.

When an amino acid mixture containing alanine, glycine, and hydroxyproline in various ratios reacted with DABS-Cl under the conditions described, the various dabsyl amino acids formed were separated by TLC and determined spectrophotometrically at 430 nm. The results are summarized in Table V.

Table IV. Stabilities of DABS-Amino Acids

Amino acid	Recovery, % A	B	C	D
Ala	95	91	80	43
Asp	100	85	...	...
Gln	92	71	...	...
Glu	100	95	73	30
Gly	100	98	75	39
HPro	100	92	68	46
Ileu	100	92	85	...
Met	97	89	...	...
Phe	95	86	83	73
Pro	96	84	90	60
Ser	...	...	75	45
Thr	100	90	78	61
Trp	100	94	0	0
Tyr	97	92	75	61
Val	95	89	...	...
GlyTyr	98	90	...	...
DABS-ONa	...	...	82	...

[a] A: 8 hr, exposed to tungsten lamp (110 V, 60 W) in $NaHCO_3$ buffer. B: 8 hr, exposed to tungsten lamp (110 V, 60 W) on silica gel plate. C: 2-hr hydrolysis, 105 °C, 5.7*N* HCl. D: 5-hr hydrolysis, 105 °C, 5.7*N* HCl.

Table V. Quantitative Determination of Amino Acids by Dabsylation

Mixture[a]	Amino acid	Quantity (μmol/ml) Added	Found[b]
A	Alanine	1.00	1.01
	Glycine	2.00	1.94
	Hydroxyproline	3.00	3.06
B	Alanine	3.00	2.94
	Glycine	1.00	1.12
	Hydroxyproline	2.00	2.14
C	Alanine	2.00	2.10
	Glycine	3.00	3.00
	Hydroxyproline	1.00	1.10

[a] Mixture A, B, or C (0.5 ml) was mixed with 0.5 ml of DABS-Cl (10 μmol/ml) in acetone. After dabsylation, the dabsyl alanine, glycine, and hydroxyproline were separated and estimated. [b] The amounts of amino acids were calculated from the absorbances at 430 nm with reference to the standard curves constructed.

The use of DABS-Cl for quantitative determination of amino acids was quite simple and satisfactory. A linear relationship between concentration and absorbance was observed for most amino acids studied; therefore, it appears possible to develop a colorimetric procedure to determine amino acid composition of proteins.

Effect of pH on Reaction of DABS-Cl with Serine. Serine reacted with DABS-Cl to form *N*-dabsyl serine, *O*-dabsyl serine, and *N,O*-bis-dabsyl serine. As illustrated in Figure 3, formation of *N*-dabsyl serine reached an optimal plateau between pH 9 and 10, and that of *O*-dabsyl derivatives was predominant at pH 12 or higher. *N,O*-bis-dabsyl serine was detected around pH 11; none of the product appeared below pH 10 or above pH 12. The reaction was then carried out routinely at pH's lower than 9.

Reaction of DABS-Cl with Polyfunctional Amino Acids. DABS-Cl was found to bind covalently to free amino, phenol, imidazole, and sulfhydryl groups under the described conditions. Therefore, the polyfunctional amino acids such as histidine, lysine, tyrosine, and cysteine were selected for further study; results are tabulated in Table I. When the ratio of DABS-Cl to amino acid was 5:1, all the polyfunctional amino acids gave only bis-dabsyl derivatives. If the ratio was kept at 1:1, the amino acids gave both mono- and bis-dabsyl derivatives. Most of these dabsyl derivatives were identified by TLC as mentioned in Table I. However, mono-dabsyl derivatives of lysine and histidine were not definitely identified. Further characterization of these derivatives is in progress.

The major directions for improvement of the technique in amino acid detection have been aimed at both increasing sensitivity and simplifying operations. DABS-Cl has been provided to meet these challenges. The sensitivity of DABS-Cl is about 100 times greater than that of 2,4-dinitrofluorobenzene and almost approaches that of dansyl chloride. Furthermore, the intensely chromophoric dabsyl group permits the detection of amino acids as colored spots right on the TLC plates and does not require the examination of the plates under ultraviolet light, a tedious operation indispensable in the use of dansyl chloride. The stability of the dabsyl derivatives to illumination in bicarbonate buffer and on silica gel plates have suggested DABS-Cl to be a promising reagent for quantitative analysis of amino acids.

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