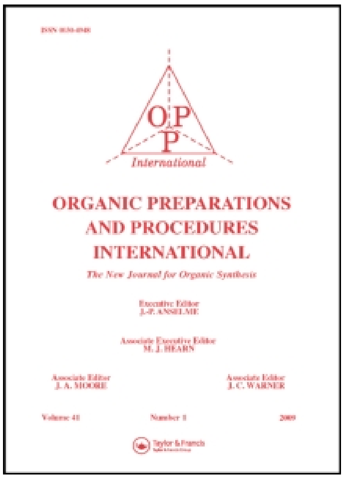

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DANSYL AMINO ACIDS

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DANSYL AMINO ACIDS

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The determination of the peptide structure has hitherto been done with a number of reagents, analogous to dinitrofluorobenzene used by Sanger.[1] Nitro derivatives of benzene, pyridine, pyrimidine or indone have been used as colour giving compounds.[2]

A reagent most widely used now is dansyl chloride (1-dimethylamino-naphthalene-5-sulfonyl chloride)[3] which reacts with free amino groups of peptides or proteins. After hydrolysis, the fluorescent dansyl derivatives of N-terminal and/or basic amino acids are detected by chromatography. The fundamental advantages of this procedure are its high sensitivity[4] (10^{-11} mole) and the great resistance of dansyl amino acids to acid hydrolysis. Dansyl amino acid formation in dilute aqueous solutions has been described by different authors.[5] These solutions were used without separation as chromatographic standards. However, no preparative method for obtaining these compounds is documented in the literature,[6] and their physical data have not been described.[7]

We report the experimental details of the preparation of dansyl amino acid and an improved procedure for obtaining dansyl chloride. Amino acids were acylated with this reagent in homogenous water-acetone mixture with additions of sodium bicarbonate. For glutamic and aspartic acid derivatives, sodium carbonate must be used. ε-Dansyl lysine was obtained by the copper complex procedure. Yields, physical constants and typical IR, NMR and analytical data are summarized in Tables I, II and III.

Since dansyl amino acids do not readily crystallize, their cyclohexylammonium salts were also prepared. All compounds are chromatographically pure, except dansyl methionine which retains faint traces of sulfoxide. Chromatography gave in several systems R_f values as described in the literature.[5,9] The chiroptical properties of dansyl amino acids and their utilization for determining the configuration of N-terminal amino acids will be the subject of a forthcoming paper.[10]

EXPERIMENTAL

<u>1-Dimethylaminonaphtalene-5-sulfonyl chloride.</u> 1-dimethylaminonaphtalene-5-sulfonic acid (8 g, 32 mmole) was ground in a mortar with (11.2 g, 54 mmole) PCl_5 and the resulting melt was poured on cold water. The yellow crude solid was separated and dried in a dessicator over $CaCl_2$. The dry powder was extracted with dry ether, the solvent was evaporated to dryness and the residue was recrystallized from petroleum ether to yield 5,2 g (60%) DnsCl[11] as orange crystals, mp. 69°, lit.[12] mp. 69°. The ether insoluble material was extracted with sodium hydroxide solution. Upon acidification of this extract, 2.8 g (35%) of unchanged sulfonic

acid was recovered.

Preparation of Dansyl Amino Acids

A/ Standard Procedure. Two mmoles of amino acid in 6 ml saturated sodium bicarbonate was treated with 2 mmoles of DnsCl in 15 ml of acetone and 8 ml water. The homogenous solution was set aside at room temperature overnight. The acetone was evaporated under reduced pressure and the residue was neutralized with hydrochloric acid and extracted with ether. The etheral extract was dried and evaporated to dryness. The crude product was crystallized from the appropiate solvent (see Table I) or converted into cyclohexylamonium salt (see Table II).

B/ Procedure for Amino Dicarboxylic Acids. To 2.5 mmoles of amino acid solution in 14 ml of 1 N aqueous sodium carbonate, was added 2 mmoles of DnsCl in 20 ml acetone and 8 ml of water. The isolation was as in the standard procedure except that the extraction was performed by ethyl acetate.

C/ Special Procedure for N^{ε}-Dansyl-L-lysine. A solution of 440 mg of L-lysine in 20 ml water was refluxed with 800 mg cupric carbonate for 2 hours. The mixture was filtered while hot and the solid washed with hot water. The deep-blue filtrate was concentrated to 18 ml and 420 mg sodium carbonate and a solution of 540 mg of DnsCl in 20 ml of acetone were added. The mixture was left standing overnight. The acetone was evaporated and the blue copper complex was collected. The crude complex was suspended in 5 ml of boiling

TABLE I

1-Dimethylaminonaphthalene-5-sulfonyl Amino Acids

Compound	Method	Yield %	Mp. (°C)	N% Calcd.	N% Found
Dns-Gly[11]	A	78[a]	156-7[b]	9,12	8,90
Dns-DL-Ala	A	72[c]	75-82	8,69	8,76
Dns-DL-Val	A	76[c]	122-6	8,01	8,05
Dns-DL-Phe	A	66[c]	144-150	7,04	7,12
Dns-L-Phe	A	77[d]	166-8	7,04	7,26
Dns-DL-Asp	B	65[e]	100[f]	7,63	7,30
Dns-L-Glu	B	67[d]	165-7	7,37	7,77
Dns-L-Asn	B	53[d]	185-7	11,49	11,62
Dns-L-Gln	B	60[d]	109-112[f]	11,05	10,87
ε-Dns-L-Lys	C	45[e]	220[f]	11,07	11,16

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a) From H_2O b) Hartley[8] reports mp. 158°. c) From Et_2O + petroleum ether d) From AcOEt e) From AcOEt + $CHCl_3$ f) Mp. with dec.

water and a stream of H_2S was passed for 15 min. The precipitated copper sulphide was filtered and washed with hot water. The filtrate was acidified by conc. hydrochloric acid and boiled for a few minutes with charcoal and then neutralized by addition of a 1N sodium hydroxide solution. The precipitated product was collected and purified by crystallization from a mixture of $CHCl_3$ and ethyl acetate. The yield of ε-dansyl-L-lysine was 360 mg, mp. 220° (dec.).

D/ Cyclohexylamine salts of Dansyl Amino Acids. The crude dansyl amino acid (method A) was dissolved in dry ether and an equimolar quantity of etheral cyclohexylamine was added. The precipitated residue was filtred, washed with ether and crystallized using appropriate solvents (see Table II).

TABLE II

Cyclohexylamine Salts of 1-Dimethylaminonaphthalene-5-sulfonyl Amino Acids

	Yield %	Mp. (°C)	Formula	N%	
				Calcd.	Found
Dns-DL-Ala	60[a]	155-6	$C_{21}H_{31}N_3O_4S$	9,98	10,01
Dns-L-Leu	65[b]	167-170	$C_{24}H_{37}N_3O_4S$	9,05	9,20
Dns-DL-Ile	51[b]	154-6	$C_{24}H_{37}N_3O_4S$	9,05	8,63
Dns-DL-Pro	87[b]	181-4[e]	$C_{23}H_{33}N_3O_4S$	9,40	9,54
Dns-L-Pro	87[b]	196-9[e]	$C_{23}H_{33}N_3O_4S$	9,40	9,78
Dns-L-Hypro	82[b]	192-4[e]	$C_{23}H_{33}N_3O_5S$	9,05	9,20
Dns-DL-Met	56[c]	112-5	$C_{23}H_{35}N_3O_4S_2$	8,74	8,52
Dns-DL-Ser	62[d]	220-222[e]	$C_{21}H_{31}N_3O_5S$	9,58	9,59

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a) From MeOH+Et_2O + petroleum ether

b) From EtOH + Et_2O

c) From EtOH + petroleum ether

d) From MeOH + H_2O

e) With decomposition.

TABLE III

Typical Spectral Data for Dansyl Amino Acids

NMR (δ, in $(CD_3)_2CO$)

	ArH	NH	COCH	$C_\alpha H$	$N(CH_3)_2$	
Dns-Gly	8,6-7,1 (6H)	6,9 (br,1H)	4,14 (br,1H)	3,7 (s,2H)	2,8 (s,6H)	
Dns-DL-Asp	8,6-7,1 (6H)	6,9 (br,1H)	4,8 (br,2H)	4,2 (m,1H)	2,8 (s,6H)	2,65 (d,2H)
Dns-DL-Phe	8,6-7,1 (6H)	6,9 (br, 1H+5H,ArH)	4,6 (br,1H)	4,05 (m,1H)	2,8 (s,6H)	2,85 (m,2H)
Dns-DL-Ala[a]	8,6-7,1 (6H)	5,9 (br,1H)	8,6 (br,1H)	3,85 (m,1H)	2,8 (s,6H)	1,15 (d,3H)

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a) In $CDCl_3$.

Infrared (cm^{-1}; KBr pellets)

Dns-Gly	3290, 1705, 1600, 1510, 1420, 1350, 1155, 805, 680, 635, 580
Dns-DL-Asp	3290, 1730, 1600, 1520, 1410, 1335, 1150, 800, 675, 630, 580
Dns-L-Phe	3310, 1725, 1600, 1470, 1410, 1350, 1155, 805, 710, 625, 575
Dns-DL-Ala	3290, 1730, 1580, 1470, 1410, 1335, 1155, 800, 720, 630, 575

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   Several firms offer kits with dansyl amino acids, probably prepared based on unpublished results.

   Dns-Gly was described [8] as "dye labelled Gly" in 38% yield.

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