tallized twice from 95% ethanol (3 ml. per 100 mg.); 43 mg., m.p. 202.5–204°.

Carbobenzoxy-L-histidylglycine hydrazide and carbobenzoxy-L-histidyl-L-leucine hydrazide were prepared in the same way except that the reactions were carried out for 24 hours at room temperature.

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Preparation and Properties of 2,4-Dinitrophenyl-L-amino Acids

BY KRISHNARAU R. RAO[1] AND HERBERT A. SOBER

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The crystalline 2,4-dinitrophenyl derivatives of the following L-amino acids have been prepared for the first time: valine, norvaline, isovaline, leucine, isoleucine, alloisoleucine, α-aminononylic acid, serine, γ-hydroxy-α-aminobutyric acid, ε-hydroxy-α-aminocaproic acid, threonine, allothreonine, S-benzylcysteine, hydroxyproline, aspartic acid, glutamine, α,γ-diaminobutyric acid, histidine, as well as those of DL-ethionine, DL-methionine, DL-glutamic acid, DL-pipecolic acid and γ-aminobutyric acid. The derivatives of L-glutamic and allohydroxy-L-proline were obtained as hygroscopic yellow solids. The purification of many of these compounds required anhydrous conditions. The molar rotations of one of the optical enantiomorphs of the above as well as those of alanine, α-aminobutyric acid, asparagine, arginine, ornithine, lysine, cystine, phenylalanine, tryptophan, tyrosine and proline, have been determined in *N* NaOH or 4% $NaHCO_3$ and in glacial acetic acid and vary from 2 to 40 times that of the parent amino acid. Ultraviolet absorption data and molar extinction values were obtained and the chromatographic behavior on paper in several solvent systems was examined.

The dinitrophenyl (DNP) derivatives of the amino acids glycine, DL-alanine, DL-histidine, DL-leucine and L-asparagine were first prepared by Abderhalden and Blumberg[2] using 1-chloro-2,4-dinitrobenzene which reacts with the free amino group. Sanger[3-5] found that 1-fluoro-2,4-dinitrobenzene (FDNB) was a more suitable reagent capable of reacting near neutrality at room temperature with the free amino groups of proteins and peptides. He evolved thereby an elegant method of identifying the N-terminal residues of proteins and peptides based on the stability of the DNP-amino acid to acidic or enzymatic hydrolysis.

Preliminary experiments indicated that the melting points of the DNP derivatives of optically active amino acids differed in many instances from those of the racemic form and that the molecular rotation of the optically active derivative was much larger than that of the parent amino acid. Except for asparagine,[2] arginine,[5] lysine,[6,7] ornithine,[4] cystine,[5,6] phenylalanine,[3] tryptophan,[4,5] proline,[5,6] tyrosine,[6] alanine[8] and α-aminobutyric acid,[8] all the amino acids whose crystalline DNP derivatives have hitherto been prepared were racemic.

Optical configuration has been correlated with biological activity and is also believed to be implicated in the effect of several natural antimetabolites. It seemed likely that the characterization of DNP derivatives of amino acid enantiomorphs would help in the determination of optical configuration as well as in the identification of amino acids present singly or in combination in natural compounds. Such an application was made recently by Alderton[8] while this work was in progress. He prepared the DNP derivatives of L-alanine and L-α-aminobutyric acid and determined their optical rotation in alkali in order to elucidate the optical configuration of the α-carbon in a sulfide amino acid in the antibiotic peptide, subtilin.

The availability of a large variety of pure amino acid enantiomorphs, prepared by the resolution procedure of Greenstein and collaborators (*cf.* Greenstein, Birnbaum and Otey[9] for details), made possible the preparation of a large number of DNP-L-amino acids. These derivatives were shown to be homogeneous by chromatography on paper in various solvents and were characterized with respect to optical rotation, ultraviolet absorption and melting point.

Experimental

L-Amino acids, obtained by resolution of the corresponding racemic amino acids and determined to be better than 99.9% optically pure,[9] were kindly donated by Dr. J. P. Greenstein and were employed for the preparation of the derivatives except for hydroxy-L-proline, L-cystine hydrochloride, DL-glutamic acid and DL-methionine,[10] L-asparagine[11] and DL-ethionine[12] which were commercial preparations recrystallized before use. DL-Pipecolic acid was kindly donated by Dr. Alton Meister and γ-aminobutyric acid by Dr. H. Steinman.

The coupling reaction was performed by shaking the amino acid with FDNB[13] in the presence of a slight excess of sodium bicarbonate for 2–5 hours in 50% ethanol at room temperature. Protection of DNP-glutamic acid and DNP-aspartic acid from light at all stages of their preparation as advocated by Mills[14] was helpful in increasing yields and inducing crystallization. After the reaction was completed, alcohol was removed at room temperature and the excess FDNB was extracted by shaking thrice with ether. Ether was removed and the aqueous solution was acidified with 6 *N* HCl till strongly acid and the solid or oil that separated was washed several times with small quantities of ice-cold water. Anhydrous conditions were found to be essential for crystallization in many cases although crystallization took place on standing in the cold in a few instances. Rep-

(1) Fellow of the Rockefeller Foundation; on leave from the Biochemistry Department, Andhra Medical College, Vizagapatam, India.

(2) E. Abderhalden and P. Blumberg, *Hoppe Seyler's Z.*, **65**, 318 (1910).

(3) F. Sanger, *Biochem. J.*, **39**, 507 (1945).

(4) F. Sanger, *ibid.*, **40**, 261 (1946).

(5) R. R. Porter and F. Sanger, *ibid.*, **42**, 287 (1948).

(6) F. C. Green and L. M. Kay, *Anal. Chem.*, **24**, 726 (1952).

(7) H. M. Rice and F. J. Sowden, *Can. J. Chem.*, **30**, 575 (1952).

(8) G. Alderton, THIS JOURNAL, **75**, 2391 (1953).

(9) J. P. Greenstein, S. W. Birnbaum and M. C. Otey, *J. Biol. Chem.*, **204**, 307 (1953).

(10) Nutritional Biochemicals Corporation, Cleveland, Ohio.

(11) Merck and Co., Inc., Rahway, New Jersey.

(12) Krishell Laboratories, Inc., Portland 2, Oregon.

(13) Preparations of FDNB obtained from Eastern Chemical Corp., Newark 2, N. J., L. Light and Co., Ltd., Bucks, England, and Eastman Kodak Co., Rochester 2, New York, were equally satisfactory.

(14) G. L. Mills, *Biochem. J.*, **50**, 707 (1952).

resentative details for the purification of the various derivatives are given below.

DNP-L-Valine.—The gummy material obtained on acidification was washed several times with small quantities of ice-cold water to remove HCl and excess water was drained away. The substance was now dissolved in a large volume of acetone, dried over anhydrous sodium sulfate, filtered and brought down to a small volume. Approximately an equal volume of benzene was added to the acetone solution and the DNP derivative was thrown out of solution with an excess of petroleum ether.[15] The material was dried in a stream of air, then dissolved in ether and precipitated with petroleum ether. This ether–petroleum ether procedure was repeated several times until the material crystallized in the cold. The DNP derivatives of the following L-amino acids were prepared in this manner; alanine, α-aminobutyric acid, norvaline, isovaline, threonine, allothreonine, leucine, isoleucine, alloisoleucine, α-aminononylic acid, hydroxyproline, phenylalanine, aspartic acid, cystine, S-benzylcysteine; also DL-glutamic acid, DL-proline, DL-ethionine, DL-methionine, DL-threonine, DL-α-aminobutyric acid, DL-valine, D-threonine and DL-pipecolic acid.

DNP-L-Glutamine.—On acidification, a solid separated out which was washed with a large volume of ice-cold water and recrystallized from aqueous methanol. The DNP derivatives of the following L-amino acids also separated out as solids on acidification: serine, γ-hydroxy-α-aminobutyric acid, ε-hydroxy-α-aminocaproic acid, tyrosine, tryptophan, arginine, histidine, α,γ-diaminobutyric acid, ornithine, lysine, γ-aminobutyric acid and asparagine. They were washed with water and recrystallized from the appropriate solvents listed in Table I.

DNP-L-Glutamic Acid.—The derivative prepared from L-glutamic acid failed to yield a crystalline derivative even after exhaustive attempts at purification. DNP-L-Glutamine was obtained easily in crystalline form and acid hydrolysis should yield DNP-L-glutamic acid. Accordingly, DNP-L-glutamine was left overnight with ten times the volume of 6 *N* HCl and heated over a steam-bath until all the material just went into solution. The solution was allowed to cool to room temperature and left in the cold, when a viscous yellow oil separated out which crystallized after standing in the cold for several weeks. This was washed with water to remove HCl and desiccated over P_2O_5 *in vacuo* to a yellow hygroscopic solid with the correct elementary analysis.

DNP-Allohydroxy-L-proline.—The oil that separated on acidification was washed with ice-cold water to remove HCl, taken up in acetone, dried over anhydrous sodium sulfate brought down to a small volume and precipitated from acetone solution with petroleum ether. After repeated precipitation with ether and petroleum ether, the viscous material was desiccated over P_2O_5 *in vacuo* to yield a yellow hygroscopic solid with the correct analysis.

Paper chromatography of these materials was done in a variety of solvent systems on Whatman No. 1, no. 4 and Schleicher and Schuell no. 598 on a grid consisting of 20 7-mm. strips in aquarium tanks as described by Block.[16] The solvent systems used were: the buffered propanol–ligroin, ethanol–benzyl alcohol and tertiary amyl alcohol systems as recommended by Blackburn and Lowther,[17] the "toluene" and phenol–isoamyl alcohol system of Biserte and Osteux,[18] and decalin–acetic acid as suggested by Li and Ash[19] and aqueous buffers (*p*H 6.0) as well as the usual systems used for amino acids (*cf.* Block, *et al.*[16]). Column chromatography was not attempted. Five to 15 micrograms (applied to the paper in 0.01–0.03 ml. acetone) were visualized directly or under ultraviolet light[20] as intense brown or purple areas as noted by Biserte and Osteux.[18]

Melting points are uncorrected and were determined on a Fisher–Jones melting point block. Ultraviolet absorption spectra of these substances were obtained in 1 cm. silica cuvettes with a Beckman DU quartz spectrophotometer, the solutions being diluted to contain approximately 10 micrograms per ml. of *N* NaOH or 4% $NaHCO_3$. Molar rotations, $[M]_D$, were calculated as specific rotations multiplied by the molecular weight and divided by 100. Specific rotations were obtained in a Hilger triple-field polarimeter, using a two-decimeter tube and adjusting the concentration (in *N* NaOH, 4% $NaHCO_3$ or glacial acetic acid) so that the angular rotation was always more than 0.2° and usually between 0.5–1.0°.

(15) Boiling point range, 30–75°.

(16) R. J. Block, R. LeStrange and G. Zweig, "Paper Chromatography, A Laboratory Manual," Academic Press, Inc., New York 10, N. Y., 1952.

(17) S. Blackburn and A. G. Lowther, *Biochem. J.*, **48**, 126 (1951).

(18) G. Biserte and R. Osteux, *Bull. soc. chim. biol.*, **33**, 60 (1951).

(19) C. H. Li and L. Ash, *J. Biol. Chem.*, **203**, 419 (1953).

(20) Hanovia Inspectolite Lamp (Hanovia Chemical and Manufacturing Co., Newark 5, N. J.) No. SC-5041 with a filtered (to pass 3600 Å.) EH-4 arc tube.

Results and Discussion

The DNP derivatives of L-norleucine, L-methionine and L-ethionine could not be obtained in solid form. The analytical data,[21] melting points and solvents used for purification of the derivatives are listed in Table I. Yields varied from 10–70% of the theoretical since the compounds were recrystallized until sharp, constant melting points and theoretical analytical values could be obtained. The purification of these derivatives appears to require in many cases anhydrous conditions which could possibly explain the failure of previous investigators to obtain the DNP derivatives of hydroxyproline and glutamic acid in crystalline form. The derivatives of the L-amino acids are frequently more difficult to isolate in crystalline form than those of the racemic amino acids as they yield more gummy products on acidification. This may well be due to the generally lower melting point of the derivatives of the L-amino acids.

Paper chromatography of these substances in a great number of solvents served only to indicate their homogeneity. The relative movement of the previously prepared compounds agreed with that reported by other authors.[17–19] The new compounds reported in this paper are not completely resolved with the above-mentioned systems from a complex mixture of these derivatives. Other systems will have to be devised to avoid erroneous identification.

The spectral absorption curves of DNP-L-valine, representing the usual type, DNP-L-proline representing the heterocyclic groups of amino acid derivatives, and of DNP-L-arginine, representing the basic amino acids are given in Fig. 1. Table I lists the molar extinction coefficients in *N* NaOH obtained at maximum absorption. Ultraviolet absorption spectra as reported by Sanger[22] for phenylalanine, tyrosine and lysine are in general agreement with those reported here. The absorption maxima in ultraviolet light is at 360–365 mμ for the following DNP-L-amino acids: alanine, α-aminobutyric acid, valine, norvaline, isovaline, leucine, isoleucine, alloisoleucine, α-aminononylic acid, asparagine, glutamine, serine, γ-hydroxy-α-aminobutyric acid, ε-hydroxy-α-aminocaproic acid, threonine, allothreonine, aspartic acid, glutamic acid, phenylalanine, tyrosine, tryptophan and histidine, as well as D-allo-L-isoleucine, DL-ethionine, DL-methionine, DL-glutamic acid, β-alanine and γ-aminobutyric acid. A shift to the shorter wave lengths at maxima was shown by DNP-glycine to 325–330 mμ, DNP-L-arginine to 345 mμ, DNP-S-benzylcysteine to 350 mμ and the di-DNP-L-

(21) We are indebted to R. J. Koegel and his staff for the analyses reported in this paper.

(22) F. Sanger, *Biochem. J.*, **45**, 563 (1949).

TABLE I

DNP-Amino acid	Theoretical C	H	N	Observed C	H	N[a]	Observed m.p., °C. (uncor.)	Reported M.p., °C.	Solvent[b]	$E_M \times 10^{-4}$ at max.
Glycine	39.8	2.9	17.4	40.2	2.9	17.5	203–204	205,[2] 193,[6] 206[7]	B	0.79
L-α-Alanine	42.3	3.5	16.5	42.5	3.8	16.5	177	178,[2,7] 173[6]	A	1.72
β-Alanine	42.3	3.5	16.5	42.8	3.9	16.9	145–146	121–125[7]	F	1.58
L-α-Aminobutyric acid	44.6	4.0	15.6	44.6	4.2	15.9	133		A	1.71
DL-α-Aminobutyric acid	44.6	4.0	15.6	44.5	4.1	15.6	143		A	1.80
γ-Aminobutyric acid	44.6	4.0	15.6	44.4	4.4	15.8	145–146			1.94
L-Norvaline	46.6	4.6	14.8	47.4	4.8	14.5	58–60		A	1.70
L-Valine	46.6	4.6	14.8	46.7	4.6	14.8	132		A	1.94
DL-Valine	46.6	4.6	14.8	46.4	4.7	14.8	184	185,[2,7] 183[6]	A	1.90
L-Isovaline	46.6	4.6	14.8	..	...	14.7	141		A	1.81
L-Leucine	48.5	5.1	14.1	48.7	5.3	14.1	94–95	DL 203,[2] 133,[6] L 126[7]	A	1.88
L-Isoleucine	48.5	5.1	14.1	48.5	5.1	14.0	113–114		A	1.78
DL-Isoleucine	48.5	5.1	14.1	48.4	5.2	14.3	174–175	166,[5] 168–172[6]	A	..
L-Allo-isoleucine	48.5	5.1	14.1	48.5	4.9	14.0	119		A	1.75
DL-Allo-isoleucine	48.5	5.1	14.1	48.4	5.2	14.4	135–136		A	..
D-Allo-L-isoleucine	48.5	5.1	14.1	48.4	5.4	14.4	146–147		A	1.71
L-α-Aminononylic acid	53.1	6.2	12.9	53.0	6.1	12.6	69–70		A	1.79
L-Serine	39.9	3.0	15.5	39.7	3.1	15.7	173–174		B	1.73[c]
DL-Serine	39.9	3.0	15.5	40.0	3.5	15.3	200–202	199,[5] 186–188,[6] 200[7]	B	
L-Threonine	42.1	3.9	14.5	42.3	4.0	14.5	145		A	1.72[c]
DL-Threonine	42.1	3.9	14.5	42.3	4.1	14.2	178	152,[5] 178[6]	A	
L-Allothreonine	42.1	3.9	14.5	42.5	4.1	14.5	152		A	1.70[c]
DL-Allothreonine	42.1	3.9	14.5	43.3	4.6	14.1	133–134		A	..
γ-Hydroxy-L-α-aminobutyric acid	42.1	3.9	14.5	43.6	3.4	15.4	164–165		C	1.69[c]
ε-Hydroxy-L-α-aminocaproic acid	46.0	4.8	13.1	45.9	4.7	13.1	141–142		C	2.26[c]
DL-Methionine	41.9	4.1	13.3	41.8	4.2	13.3	117–118	117,[5] 107–112[6]	A	1.55
DL-Ethionine	43.8	4.6	12.8	44.1	5.1	12.9	104–105		A	1.34
L-Cystine (di)	37.8	2.8	14.7	37.5	3.4	14.5	109	109,[5] 118–121[6]	A	2.75
S-Benzyl-L-cysteine	50.9	4.0	11.1	50.6	4.2	11.3	111		A	1.74[c]
L-Phenylalanine	54.4	3.9	12.7	54.1	3.9	12.8	189	DL 204–206,[6] L 186[3]	A	2.00
L-Tyrosine (O:N, di)	49.1	2.9	13.1	49.6	3.9	13.0	178–182(d)	92–98[6]	D	1.69[c]
L-Tryptophan	55.3	3.5	15.1	55.1	3.9	15.0	221 d.	175,[5] 196–198[6]	D	1.68[c]
L-Proline	47.0	3.9	14.9	47.0	3.9	14.9	138	137,[5] 137–138.5[6]	A	1.92
DL-Proline	47.0	3.9	14.9	47.2	4.0	15.2	181		A	1.87
Hydroxy-L-proline	44.4	3.7	14.1	44.8	3.9	13.8	174–175		A	1.87
Allohydroxy L-proline	44.4	3.7	14.1	44.6	3.7	13.7			A	1.73
DL-Pipecolic acid	48.8	4.4	14.2	48.9	4.5	14.6	138–139		A	1.58
L-Aspartic acid	40.1	3.0	14.0	40.7	3.2	14.2	186–187	DL 196,[5] 189,[6] 190[7]	A	1.82
L-Glutamic acid	42.2	3.5	13.4	42.6	4.4	13.8			A	..
DL-Glutamic acid	42.2	3.5	13.4	42.3	3.7	13.3	148–149	155–162[6]	A	1.74
L-Asparagine	40.3	3.4	18.8	40.4	3.5	19.3	180–182	191–192,[2] 185[7]	B	2.00[c]
L-Glutamine	42.3	3.8	18.0	42.4	3.9	18.2	189–191		B	2.07[c]
L-α,γ-Diaminobutyric acid (di)	42.7	3.1	18.7	42.5	3.8	18.8	120–122d		C	2.95[c]
L-Ornithine (di)	44.1	3.4	18.1	44.2	3.8	17.3	156–157		E	2.92[c]
L-Lysine (di)	45.2	3.7	17.6	44.4	3.8	16.8	170–172d	146,[5,7] 173–174.5[6]	D	2.86[c]
L-Histidine (di)	44.3	2.7	20.2	44.9	3.0	19.8	232–4	250,[2] 228[7]	C	2.15[c]
L-Arginine	42.4	4.7	24.7	42.4	4.5	24.6	260	252[5]	C	1.76[c]

[a] Determined by the modified Friederick method (G. E. Secor, M. C. Long, M. D. Kilpatrick and L. M. White, *J. Assoc. Offic Agr. Chemists*, **33**, 872 (1950)). [b] Solvents used for purification and recrystallization were as follows: A, ether–petroleum ether (b.p. 30–75°); B, aqueous methanol; C, aqueous acetone; D, acetone–ether; E, acetone–petroleum ether; F, aqueous ethanol. [c] Absorption spectra in 4% $NaHCO_3$ in which the compounds were difficultly soluble at the concentrations used. Many required warming.

derivatives of α,γ-diaminobutyric acid, ornithine, lysine and cystine to 355 mμ. A decided shift to the longer wave lengths at maxima was shown by the derivatives of proline, hydroxyproline and allohydroxyproline to 380–385 mμ and by DNP-DL-pipecolic acid to 390–395 mμ. It would be interesting to investigate the absorption maxima of DNP prolyl peptides and DNP peptides containing proline in the chain.

The optical rotation of most of the compounds could be obtained in *N* NaOH and in glacial acetic acid. However, the derivatives of the hydroxy amino acids, namely, serine, γ-hydroxy-α-aminobutyric acid, threonine and allothreonine, but not ε-hydroxy-α-aminocaproic acid, hydroxyproline and allohydroxyproline, darkened very considerably in *N* NaOH solution but did not do so in 4% $NaHCO_3$. With the other derivatives, however, there was little or no change in the color of the solution in *N* alkali and no variation in optical rotation with time, indicating that the compounds were stable in alkaline solution under these conditions (4 hr. at

24–26°). However, solutions of these compounds (10 μg./ml.) in 1 *N* NaOH or $NaHCO_3$ did show significant decreases in ultraviolet absorption at maxima when kept for five days at room temperature. The di-DNP derivatives of the dibasic amino acids and of tyrosine, as well as DNP-S-benzylcysteine, were too insoluble in the alkaline solutions and significant rotations could only be obtained in glacial acetic acid. Di-DNP-L-histidine was too insoluble in glacial acid and the rotation of this compound was obtained in a mixture of NaOH and $NaHCO_3$. The molar rotations and maximum

TABLE II

DNP-L-Amino acid	Mol. wt.	[M]D[a] in *N* NaOH	[M]D[a] in 4% $NaHCO_3$	[M]D[a] in Glacial acetic acid	Shift in [M]D[b]
α-Alanine	255	+367	.....	+39	+335
α-Aminobutyric acid	269	+266	+277	−23[h]	+223
Norvaline	283	+170	.....	−78	+129
Valine	283	+309	.....	−79	+236
Isovaline	283	+114	.....	.....	+88
Leucine	297	+177	+176	−135	+147
Isoleucine	297	+252	.....	−104	+188
Alloisoleucine	297	+260	.....	−119	+204
α-Aminononylic acid	339	−277	.....	−118	−335
Serine	271	.....	+341	−65	+325[e]
Threonine	285	.....	+305	−141	+341
Allothreonine	285	.....	+305	−84	+260
γ-Hydroxy-α-amino-butyric acid	285	.....	+75	−179	+61
ε-Hydroxy-α-amino-caproic acid	313	+119	.....	−134	+72
Cystine (di)	572	.....	−1487	−1833	−930[e]
S-Benzylcysteine	377	.....	.....	−669	−616
Phenylalanine	331	−310[c]	−261	−342	−298
Tyrosine (O:N, di)	513	.....	.....	−60	−42[e]
Tryptophan	370	−1291	.....	−672	−1222
Proline	281	−2172	.....	−1978	−2080
Hydroxyproline	297	−3852	.....	−3410	−3751
Allohydroxyproline	297	−2706	−1874	−1322	−2665
Aspartic acid	299	+275	.....	−20[h]	+241[e]
Glutamic acid	313	.....	−20[h]	−253	−67[e]
Asparagine	298	.....	+190	−100	+98[f]
Glutamine	312	−177	−172	−302	−157[g]
α,γ-Diaminobutyric acid (di)	450	.....	.....	−360	−398[e]
Ornithine (di)	464	.....	.....	−339	−377[e]
Lysine (di)	478	.....	.....	−127	−165[e]
Histidine (di)	487	−107[d]	.....	.....	−119[e]
Arginine	340	.....	.....	−121	−169

[a] Molar rotations calculated as specific rotation multiplied by the molecular weight and divided by 100; temp. 24–26°; two-decimeter tube employed. Concentrations except where indicated were 0.2–1.0%. [b] [M]D DNP-amino acid in alkali or $NaHCO_3$ minus [M]D amino acid in glacial acetic acid (J. P. Greenstein, S. W. Birnbaum and M. C. Otey, *J. Biol. Chem.*, **204**, 307 (1953)) unless noted otherwise. The signs were maintained throughout. Where no other value for the DNP amino acid is listed, the [M]D in glacial acetic acid was used. [c] Darkened slowly on standing in alkali. [d] In 4% $NaHCO_3$ with enough *N* NaOH added to dissolve; compound too insoluble in glacial acetic acid; angular rotation below 0.2 degree. [e] [M]D of amino acid was taken in 5 *N* HCl. [f] [M]D of asparagine in 3 *N* HCl. (A. Meister, H. A. Sober, S. V. Tice and P. E. Frazer, *J. Biol. Chem.*, **197**, 319 (1952).) [g] [M]D of glutamine in water (L. Levintow and A. Meister, THIS JOURNAL, **75**, 3039 (1953)). [h] 1.5% solutions.

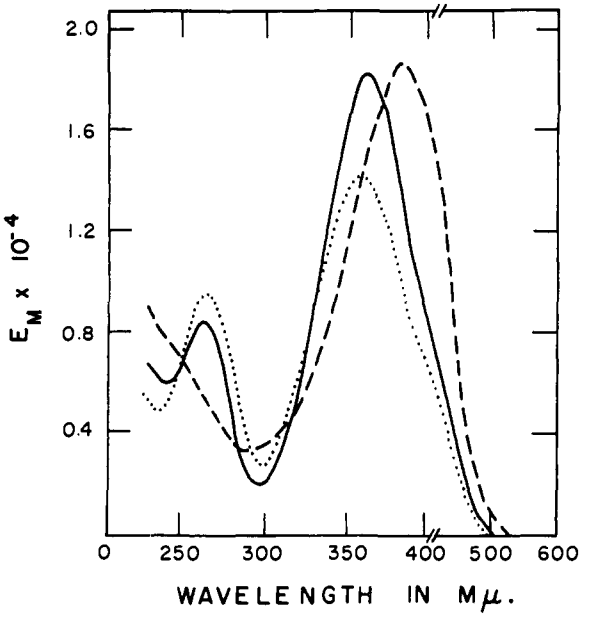

Fig. 1.—Absorption spectra of DNP-amino acids: solid line, DNP-L-valine, 16.3 μg./ml. *N* NaOH; dashed line, DNP-hydroxy-L-proline, 14.4 μg./ml. *N* NaOH; dotted line, DNP-L-arginine, 20.9 μg./ml. *N* NaOH.

shift in rotation obtainable in any of the three media as compared with that of the parent amino acid in glacial acetic acid[9] are listed in Table II.

The results listed in Table II show that the molar rotation of the DNP derivatives was in general higher, and in some cases very much higher than that of the corresponding amino acid, a finding that would permit the identification and optical characterization of a small quantity of an amino acid enantiomorph, as was done by Alderton.[8] All the derivatives except that of alanine showed negative rotation in glacial acetic acid. The shift of rotation due to the introduction of the DNP moiety into the amino acid is considerable, varying from 100–300 degrees for most of the compounds to 3752 degrees for hydroxyproline. As might be expected from the heterocyclic character of the prolines, the magnitude of rotation was very high. Other derivatives giving relatively high values are those of tryptophan, cystine, threonine, allothreonine, serine, valine, alanine and α-aminobutyric acid, making the use of the DNP derivatives of these compounds particularly suitable for the determination of optical configuration. As little as 1.4, 0.43, and 0.12 mg./ml. in *N* NaOH would produce an angular rotation in a two-decimeter tube of 0.3 degrees when the DNP derivatives of valine, tryptophan and hydroxyproline, respectively, are used.

BETHESDA 14, MD.