with oxygen-free water at the centrifuge. The supernatant solution, combined with the washings, was lyophilized immediately to a colorless residue which was taken up in a small amount of 95% alcohol, and stored under nitrogen at 0–5° for precipitation. An aqueous solution of the solid was concentrated in a desiccator over phosphoric oxide to low bulk, left overnight in a desiccator containing sodium hydroxide and a dish of alcohol and evacuated to 300 mm. pressure. The moist crystalline prisms were collected two days later with the aid of a little alcohol, weight 72 mg., m.p. 164–166°, $[\alpha]^{28}D$ +12.4° (20.2 mg. in 1.2 ml. of water). The reported values for γ-L-glutamyl-L-cysteine are m.p. 167°, $[\alpha]D$ +13.6° (110 mg. in 10 ml. of water).[5]

Treatment of XIII with Mercuric Chloride at Elevated Temperature.—A solution of 1.20 g. (3.78 millimoles) of L-4-carboxy-3-γ-L-glutamyl-2,2-dimethylthiazolidine monohydrate (XIII) in 30 ml. of water was heated on a steam-bath and 2.5 g. (9.3 millimoles) of mercuric chloride dissolved in 20 ml. of hot water was added. The mixture was maintained at 60–70° for 15 min. During this time, the mercuric mercaptide that had precipitated at the beginning dissolved slowly, and solid mercaptide separated on cooling. After storage at room temperature for 14 hours, the heating process was repeated in order to effect decomposition and the mixture was allowed to cool gradually. The mercuric mercaptide was collected, washed thoroughly with small amount of water and treated with hydrogen sulfide by the regular procedure. The hydrochloride thus obtained was found to be cysteine hydrochloride, weight 0.24 g. (40%). A mixed melting point with an authentic sample of cysteine hydrochloride gave no depression (m.p. 178–179.5°).

Anal. Calcd. for $C_3H_8NO_2SCl$: N, 8.90. Found: N, 9.10.

L-4-Carbomethoxy-2,2-dimethylthiazolidine Hydrochloride (XV).—The ester was prepared from the corresponding thiazolidinecarboxylic acid following the procedure for the esterification of 4-carboxy-2,2,5,5-tetramethylthiazolidine.[25] A mixture of 15.0 g. (0.069 mole) of 4-carboxy-2,2-dimethylthiazolidine hydrochloride in 300 ml. of absolute methanol, cooled in a water-bath, was saturated with anhydrous hydrogen chloride. The solution was stored at room temperature for 8 hours and at 0–5° for 14 hours. Removal of solvent under reduced pressure gave a crystalline residue which was digested twice with 100-ml. portions of anhydrous acetone for 20 min. The acetone mixture was concentrated at reduced pressure and the residue was recrystallized from 25 ml. of methanol and 40 ml. of acetone, yielding 8.5 g. (60%) of fine needles, m.p. 159–159.5°. A mixed melting point with the starting material (m.p. 161–164°) gave a depression of 20°.

Anal. Calcd. for $C_7H_{13}NO_2S \cdot HCl$: C, 39.72; H, 6.67; N, 6.62. Found: C, 40.01; H, 6.67; N, 6.64.

(25) H. T. Clarke, J. R. Johnson and R. Robinson, Editors, "The Chemistry of Penicillin," Princeton University Press, Princeton, N. J., 1949, p. 960.

L-4-Carbomethoxy-2,2-dimethyl-3-(phthaloylglycyl)-thiazolidine (XVI).—A solution of 2.48 g. (0.012 mole) of phthaloylglycine and 1.68 ml. (0.012 mole) of triethylamine in 10 ml. of methylene chloride was cooled to −8° and treated with 1.30 g. (0.012 mole) of ethyl chloroformate. The mixture was stirred at −8° and a pre-cooled solution of 2.54 g. (0.012 mole) of L-3-carbomethoxy-2,2-dimethylthiazolidine hydrochloride (XV) and 1.68 ml. (0.012 mole) of triethylamine in 50 ml. of methylene chloride was added. After 20 min. of stirring at −8° and 4 hours at room temperature, the solution was diluted with 100 ml. of chloroform. The reaction mixture was washed thoroughly with 5% hydrochloric acid, 5% sodium bicarbonate and water, dried, and concentrated at reduced pressure. The residue (3.8 g., 86%, m.p. 220–223°) was crystallized from chloroform–methanol yielding 1.80 g. (41%) of small needles, m.p. 225–226° dec. An analytical sample was prepared by two recrystallizations from chloroform–methanol; m.p. 227–228° dec., $[\alpha]^{30}D$ +11.4° (7.9 mg. in 1.8 ml. of 1:1 methanol-chloroform).

Anal. Calcd. for $C_{17}H_{18}N_2O_5S$: C, 56.34; H, 5.01; N, 7.73. Found: C, 56.28; H, 4.84; N, 7.80.

L-4-Carboxy-2,2-dimethyl-3-(phthaloyl-L-phenylalanyl)-thiazolidine (XVII).—A solution of 1.95 g. (6.7 millimoles) of phthaloyl-L-phenylalanine and 0.927 ml. (6.7 millimoles) of triethylamine in 18 ml. of 1:1 methylene chloride–dioxane mixture was cooled to −8° and 0.725 g. (6.7 millimoles) of ethyl chloroformate was added. The mixture was stirred at −8° for 12 min. during which time a large amount of triethylamine hydrochloride precipitated. A second pre-cooled solution of 1.07 g. (6.7 millimoles) of 4-carboxy-2,2-dimethylthiazolidine in 50 ml. of dioxane was then added. Stirring was continued at −8° for 20 min. and at room temperature for 6 hours.

The reaction mixture was concentrated at reduced pressure and the residue was dissolved in 150 ml. of chloroform. The chloroform solution was washed thoroughly with 5% hydrochloric acid and water and the acid fraction was extracted from this solution using 5% sodium bicarbonate. The bicarbonate extracts were combined, washed well with ethyl acetate, acidified to *p*H 3 and re-extracted with ethyl acetate. The ethyl acetate extracts, after thorough washing with water, were dried over magnesium sulfate and concentrated at reduced pressure to a viscous oil which solidified readily on scratching. Recrystallization from aqueous ethanol gave 0.20 g. of small needles, m.p. 193–195°. A second crop of 0.48 g. (m.p. 190–193°) could be obtained by concentration of the mother liquor to 10 ml. and inoculation of the cold solution with a seed crystal. The combined yield was 0.68 g. (24%). A sample was recrystallized from aqueous ethanol for analysis; m.p. 196–197°, $[\alpha]^{30}D$ −280° (26.0 mg. in 1.3 ml. of methanol).

Anal. Calcd. for $C_{23}H_{22}N_2O_5$: C, 63.00; H, 5.06; N, 6.39. Found: C, 63.16; H, 5.15; N, 6.35.

CAMBRIDGE 39, MASS.

[CONTRIBUTION FROM THE PASADENA FOUNDATION FOR MEDICAL RESEARCH]

Synthesis of the Optically Active Tripeptides of Valine

BY S. SHANKMAN AND Y. SCHVO

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With the use of N,N′-dicyclohexylcarbodiimide as a condensing agent, all eight optically pure tripeptides of D-valine and L-valine have been prepared. A high degree of optical purity was demonstrated by microbiological assay.

The presence of D-amino acid residues in antibiotics such as the gramicidins,[1,2] tyrocidine,[3] the penicillins,[4] bacitracin[5] and aerosporin[6] has aroused interest in the inhibitory potentialities of peptides

(1) F. Lipmann, R. D. Hotchkiss and R. J. Dubos, *J. Biol. Chem.*, **141**, 163 (1941).

(2) R. L. M. Synge, *Biochem. J.*, **39**, 363 (1945).

(3) S. W. Fox, M. Fling and G. N. Bollenback, *J. Biol. Chem.*, **155**, 465 (1944).

(4) Committee on Medical Research, O.S.R.D., Washington, and the Medical Research Council, London, *Science*, **102**, 627 (1945).

(5) G. T. Barry, J. D. Gregory and L. C. Craig, *J. Biol. Chem.*, **175**, 485 (1948).

(6) T. S. G. Jones, *Biochem. J.*, **42**, lii (1948).

containing D-amino acids.[7-9] This occurrence of the D-amino acids and especially D-valine[1,2] makes of particular interest the synthesis of optically active peptides containing the D-isomer of the essential amino acid valine for possible use as antimetabolites.

This report concerns the preparation of isomeric di- and tripeptides and derivatives of optically active valine. A total of 36 compounds, dipeptides and their derivatives were prepared and characterized. These were all optically active. Twenty-eight of these were not previously reported.

High yields of peptides reported by Sheehan and Hess[10] using N,N′-dicyclohexylcarbodiimide (subsequently abbreviated DCC) as the coupling agent, led us to choose this procedure in our preliminary studies with L-leucine which were made to obtain yield data. The coupling of phthaloyl-L-leucine with L-leucine methyl ester with DCC in methylene chloride proceeded with an 80% yield of pure phthaloyl-L-leucyl-L-leucine methyl ester. No side product could be detected. The yields were 83% of crude phthaloyl tripeptide methyl ester and 86% of crude phthaloyl tetrapeptide methyl ester obtained from coupling phthaloylleucylleucine and phthaloyl tripeptide, respectively, with leucine methyl ester. No significant racemization occurred in any of these reactions as indicated by the microbiological assay, 95% or more, of L-leucine in the hydrolyzed peptides and derivatives.

When valine was substituted for leucine, phthaloyl-L-valyl-N,N′-dicyclohexylurea was isolated in 18–20% yield as a side product, which both decreased the yield and complicated the isolation of the desired product. Khorana[11] obtained other acylureas with several other N-substituted amino acids and glycine methyl ester in the presence of DCC.

The desired phthaloyl-L-valyl-L-valine methyl ester could not be crystallized in the presence of valylurea. The valylurea did not undergo change in acid hydrolysis, so it was possible to hydrolyze the mixture of phthaloyl dipeptide methyl ester and valylurea and obtain the phthaloyl dipeptide by extraction. This compound was purified by passage through a silicic acid column.

The acid hydrolysis[12] of phthaloylvalylvaline methyl ester proceeded with some production of the free peptide hydrochloride in 8–16% yield of starting material. The identity of this peptide was established by comparison with valylvaline hydrochloride prepared *via* hydrazinolysis[12] of phthaloylvalylvaline.

In coupling phthaloylvalylvaline with valine methyl ester, no side product could be isolated and characterized; however, the crude phthaloyl tripeptide methyl ester was of low purity as indicated by wide melting range.

After acid hydrolysis of phthaloylvalylvalylvaline methyl ester, yields of 37% of phthaloylvalylvalylvaline were encountered. The cause of the low yield was not pursued further. Instead, the trivaline peptides were obtained by first removal of the phthaloyl group using hydrazine hydrate,[13,14] and then saponifying the resulting ester. This route proved to be superior to the acid hydrolysis route both in yield and purity. No racemization occurred in any of these procedures as indicated by microbiological assay for L-valine in the compounds containing only D-valine. Additional confirmatory evidence was obtained from the assay of L-valine in other compounds prepared (97–104% of theory). Two derivatives of D-valyl-D-valyl-L-valine assayed beyond this limit (Table III).

The DCC coupling should be of considerable value in preparing pure stereoisomers of peptides without fractional crystallization as may be needed in the acid chloride method.[8]

Nitrogens were determined by semi-micro Kjeldahl. Optical rotations were done with the Keston polarimetric attachment (standard model D) to the Beckman DU spectrophotometer. Neutralization equivalents were determined by the method of Ellenbogen and Brand[15] using phenosafranine–*m*-cresol purple mixed indicator. Ascending paper chromatography was performed by the method of Williams and Kirby.[16] Solvent systems were 10:8:1 of water:1-butanol:glacial acetic acid.

Microbiological assays for L-valine content were performed with *L. arabinosus*[17] using a modified medium similar to that used for glutamic acid by Dunn, *et al.*[18]

Prior to assay, the samples were hydrolyzed 10 hours with 4 *N* hydrochloric acid in sealed tubes in the autoclave at 20 pounds, or 60 hours where low initial assay data indicated incomplete hydrolysis. The pronounced acid stability of the valyl–valine bond[8] was indicated by the 60-hour hydrolysis period required to obtain theoretical L-valine content of D-valyl-L-valine. Noteworthy in the study of peptide bond strength is the use of D_1L_2 or L_1D_2 type peptides where 1 and 2 are any optically active amino acids, with microbiological assay of L-forms only. Yields and characterizations are presented in Tables I–IV.

Experimental

The best commercial L- and D-valines were analyzed for L-valine content by microbiological assay. The L-valine was 97% or better. The D-valine had less than 0.1% L-valine.

Phthaloyl-L-valine.—The procedure of Sheehan, *et al.*,[12] for the preparation of phthaloylamino acids was used. The starting material was L-valine. For analysis, a sample was recrystallized 5 times from ether–hexane (Table I).

Phthaloyl-D-valine was prepared in an identical manner from D-valine (Table I). Subsequently the purification of the crude material from fusion was greatly improved using adsorption chromatography. Six grams of crude product was dissolved in 100 ml. of 1:1 chloroform:carbon tetra-

(7) M. Fling, F. N. Minard and S. W. Fox, THIS JOURNAL, **69**, 2466 (1947).

(8) J. W. Hinman, E. L. Caron and H. N. Christensen, *ibid.*, **72**, 1620 (1950).

(9) F. N. Minard and S. W. Fox, *ibid.*, **71**, 1160 (1949).

(10) J. C. Sheehan and G. P. Hess, *ibid.*, **77**, 1067 (1955).

(11) Phthaloyl amino acid N.N′-dicyclohexylureas were reported by H. G. Khorana, *Chemistry & Industry*, 1087 (1955), and J. C. Sheehan, M. Goodman and G. P. Hess, THIS JOURNAL, **78**, 1367 (1956).

(12) J. C. Sheehan, D. W. Chapman and R. W. Roth, *ibid.*, **74**, 3822 (1952).

(13) The general procedure for room temperature removal of the phthaloyl group was used (ref. 14).

(14) J. C. Sheehan and W. L. Richardson, THIS JOURNAL, **76**, 6329 (1954).

(15) E. Ellenbogen and E. Brand, *Anal. Chem.*, **27**, 2007 (1955).

(16) R. J. Williams and H. Kirby, *Science*, **107**, 481 (1948).

(17) S. Shankman, *J. Biol. Chem.*, **150**, 305 (1943).

(18) M. S. Dunn, M. N. Camien, L. B. Rockland, S. Shankman and S. C. Goldberg, *ibid.*, **155**, 591 (1945).

TABLE I

VALINE DERIVATIVES

Compound	M.p.,[a] °C.	Nitrogen, % Calcd.	Found	Neut. equiv. Calcd.	Found	L-Valine, % Calcd.	MBA[c]	Rotations °C.	$[\alpha]_D$	Concn.	Solv.	Yield, %
Phthaloyl-L-valine[b]	115–116.5	5.67	5.51	247.5	247.5	47.4	46.6	27	−61.0°	3.29	$CHCl_3$	72 pure
Phthaloyl-D-valine[b]	115–117	5.67	5.55	247.5	246.9	0.0	0.4	27	+60.7	3.08	$CHCl_3$	78 pure
L-Valine methyl ester HCl	160–162.5	8.36	8.48	...	...	70.0	70.0	25	+14.8	3.50	Water	94 crude
D-Valine methyl ester HCl	.....	8.36	8.35	...	...	0.0	0.2–1.0	25	−14.1	3.50	Water	98 crude

[a] Not corrected. [b] Made by Fling, Minard and Fox[7]: for L- $[\alpha]^{27}D$ −68.5 ± 1.0° in absolute ethanol; for D- $[\alpha]^{27}D$ +69.0 ± 0.9° in absolute ethanol. [c] Microbiological assay.[17,18]

chloride containing 3 drops of 0.1% alc. methyl red indicator. Nitrogen was used to force the solution down a silicic acid column 150 × 46 mm., previously wet with 1:1 chloroform:carbon tetrachloride. A red band was adsorbed on top of the column. The column was washed with 100 ml. of chloroform then eluted with 1:5 acetone:chloroform. The first red band was collected (150 ml.). The effluent was evaporated to dryness *in vacuo* and the residue was crystallized from ethyl acetate:hexane yielding 5.2 g. (87% recovery) of colorless plates (Table I).

Valine Methyl Ester Isomers.—The optically active methyl ester hydrochlorides were prepared from L- and D-valine by the method of Synge[19] using methanolic hydrogen chloride (Table I). The free esters were obtained according to the procedure of Fischer.[20]

Phthaloylvalylvaline Isomeric Dipeptides. Phthaloyl-valylvaline Methyl Ester Isomers. Phthaloyl-L-valyl-L-valine Methyl Ester.—To a solution of 15.8 g. (62.8 mmoles) of phthaloyl-L-valine and 8.23 g. (62.8 mmoles) of L-valine methyl ester in 210 ml. of purified methylene chloride[21] was added 13.7 g. (66.5 mmoles) of crystalline DCC, upon which immediate precipitation of the insoluble urea occurred. After four hours at room temperature 2 ml. of glacial acetic acid was added to decompose excess DCC. After cooling to 0°, the insoluble urea was removed by filtration (12.2 g., 82%). The methylene chloride filtrate was washed with two 25-ml. portions of *N* hydrochloric acid, two 25-ml. portions of 5% potassium carbonate solution and one 25-ml. portion of water. Following drying over sodium sulfate, the solvent was removed *in vacuo* yielding an oil. Attempted crystallization of the oil from ether:hexane yielded 1.5 g. of phthaloyl-L-valyl-N,N′-dicyclohexylurea,[11] colorless needles, m.p. 170–172°. Calcd. for $C_{26}H_{35}N_3O_5$: N, 9.27. Found: N, 9.18. The desired product (phthaloyl-L-valyl-L-valine methyl ester) could not be crystallized from various solvent systems which were employed. The other three isomers (LD, DL and DD) of phthaloylvalylvaline methyl ester were also prepared using the appropriate starting materials.

Phthaloyl-L-valyl-L-valine.—The oil was hydrolyzed[12] without further purification. To the viscous oil were added 330 ml. of acetone, 210 ml. of water and 112 ml. of concentrated hydrochloric acid,[22] the homogeneous solution was refluxed for three hours, and the acid solvent was removed *in vacuo*. The residue was flushed with ethyl acetate several times *in vacuo*, upon which crystalline L-valyl-L-valine hydrochloride[10] separated, and was collected by filtration (1.80 g.). The ethyl acetate filtrate was extracted with 160 ml. of 8% potassium carbonate solution, which was then acidified to congo red with concentrated hydrochloric acid. The oil which separated was extracted into 250 ml. of methylene chloride. Following drying over sodium sulfate the methylene chloride was removed *in vacuo;* 12.4 g. of colorless amorphous material resulted. The ethyl acetate phase from the carbonate extraction was washed with a 10-ml. portion of water and evaporated to dryness. The residue was rehydrolyzed using half amounts of material; 2.4 g. of colorless amorphous product was obtained using the above isolation procedure. The combined crops of phthaloyl-L-valyl-L-valine were chromatographed and recrystallized as described for phthaloyl-D-valine. The solution applied to the column was 2:1 chloroform:carbon tetrachloride. The eluting solvent was 1:10 acetone:chloroform; 86% recovery of colorless needles, m.p. 140–142°, was isolated. Additional crystallization from ethyl acetate:hexane yielded analytically pure product, m.p. 141–142.5°. A total of 8.5 g. (39% yield based on phthaloyl-L-valine) of crystalline phthaloyl-L-valyl-L-valine was obtained. The remaining three isomers of phthaloylvalylvaline (LD, DL and DD) were synthesized and purified as above using the appropriate starting materials.

Valylvaline Hydrochloride Isomers. L-Valyl-L-valine Hydrochloride.—The ethyl acetate-insoluble product (1.8 g.) from the acid hydrolysis of phthaloyl-L-valyl-L-valine methyl ester was dissolved in 50 ml. of water. The solution was washed with three 15-ml. portions of ether, filtered, and evaporated to dryness *in vacuo*. The solid residue was twice crystallized from methanol:acetone:isopropyl ether to give 1.0 g. of colorless needles. Analytical data conform to the dipeptide hydrochloride. The corresponding LD- and DL-dipeptide hydrochlorides were isolated from the hydrolysis of the appropriate isomer of phthaloylvalylvaline methyl ester.

For structure confirmation of the above peptides, D-valyl-D-valine hydrochloride was prepared by cleaving the phthaloyl group[12] from phthaloyl-D-valyl-D-valine. The resulting D-valyl-D-valine was dissolved in 0.5 *N* hydrochloric acid, filtered and evaporated to dryness. The dipeptide hydrochloride was recrystallized 2 times from methanol:isopropyl ether. L-Valyl-D-valine hydrochloride was also prepared by both methods (Table II). Each of the four isomeric dipeptides yielded a single ninhydrin spot, R_f 0.58 to 0.61, on ascending paper chromatography at 2–5% concentrations.

Hinman, *et al.*,[8] prepared the four isomeric dipeptide hydrochlorides of valylvaline from the corresponding carbobenzoxy derivatives and found R_f values from 0.84 to 0.88. D-Valyl-L-valine was prepared previously by Fischer and Scheibler,[23] L-valyl-L-valine previously by Abderhalden and Vlassopoulos.[24]

Phthaloylvalylvalylvaline Methyl Ester Isomers. Phthaloyl-L-valyl-L-valyl-D-valine Methyl Ester.—0.460 g. (1.33 mmoles) of phthaloyl-L-valyl-L-valine was dissolved in 4 ml. of purified methylene chloride solution containing 0.349 g. (2.66 mmoles) of D-valine methyl ester. Upon addition of 2.74 g. (1.33 mmoles) of crystalline DCC immediate crystallization of the insoluble urea took place. After 5 hours at room temperature and storage overnight at 0°, the insoluble urea was removed by filtration (0.265 g., 89%). The methylene chloride filtrate was washed with two 25-ml. portions of *N* hydrochloric acid, two 25-ml. portions of 5% potassium carbonate solution and finally with water. Following drying over sodium sulfate the solution was concentrated. Upon addition of hexane, 0.477 g. of colorless needles was obtained, m.p. 165–175°. After two additional crystallizations from acetone:water, followed by 4 hours drying at 80°, 1 mm. over P_2O_5, there was obtained 0.40 g. of colorless needles, analytically pure. The seven remaining isomers were obtained in the same manner using the proper phthaloyl di-peptide and valine methyl ester (Table III). Second crops of crystals were of such low purity that further purification was not warranted.

Valylvalylvaline Methyl Ester Hydrochloride Isomers. D-Valyl-D-valyl-D-valine Methyl Ester Hydrochloride.—The procedure of Sheehan and Richardson[13] for the removal of

(19) R. L. M. Synge, *Biochem. J.*, **42**, 99 (1948).

(20) E. Fischer, "Untersuchungen über Aminosaüren, Polypeptide und Proteine," Julius Springer, Berlin, 1906, Vol. I, p. 173.

(21) Boiling range 0.5°.

(22) Pronounced stability of the dipeptide to acid hydrolysis has been demonstrated.

(23) E. Fischer and H. Scheibler, *Ann.*, **363**, 136 (1908).

(24) E. Abderhalden and V. Vlassopoulos, *Fermentforschung*, **10**, 365 (1929).

TABLE II

VALYLVALINE ISOMERS AND PHTHALOYL DERIVATIVES

Compound, valine	M.p.,[a] °C.	Nitrogen, % Found	Neut. equiv. Found	L-Valine Calcd.	L-Valine MBA[b]	Rotations T, °C.	Rotations [α]D	Rotations Concn.	Rotations Solvent
Phthaloyl-									
L-valyl-L-	141–142.5	8.13	351.0	67.4	65.5	27	+59.3°	2.77	$CHCl_3$
L-valyl-D-	137–139	8.10	352.0	33.7	33.4	25	+48.4	2.74	$CHCl_3$
D-valyl-L-	137.5–139.5	7.94	349.0	33.7	33.6	25	−49.6	3.23	$CHCl_3$
D-valyl-D-	141–142.5	8.02	349.0	0.0	2.4[f]	25	−59.2	2.40	$CHCl_3$
Calcd. for above		8.09	346.4						
L-Valyl-L- HCl[c]		11.13	129.7			25	+13.6	2.89	Water
				92.6	90.2				
L-Valyl-L- HCL[d]						25	+12.6	4.32	Water
L-Valyl-D- HCL[c]		10.96	127.6			24	+51.1	2.72	Water
				46.3	46.7				
L-Valyl-D HCL[d]		11.32	128.5			24	+49.7	2.42	Water
D-Valyl-L- HCL[c]		11.04	125.0	46.3	46.4	24	−51.7	3.29	Water
D-Valyl-D- HCl[d]		11.15	128.5	0.0	0.6–3.0[g]	25	−12.8	5.24	Water
Calcd. for above		11.09	126.3						

[a] Not corrected. [b] Microbiological assay.[17,18] [c] By acid hydrolysis. [d] By hydrazinolysis. [e] All dipeptide HCl isomers decomposed with charring at 250–300°. [f] Not corrected for racemization during 60-hour hydrolysis. [g] The L-valine found varied with hydrolysis period. 2.4% of L-valine was formed from D-valine in a 65-hour hydrolysis period.

TABLE III

VALYLVALYLVALINE DERIVATIVES

Compound Phthaloyl methyl ester	M.p.,[a] °C.	Nitrogen, % Found	L-Valine, % Calcd.	L-Valine, % MBA[b,e]	Rotations T, °C.	Rotations [α]D	Rotations Concn.	Rotations Solv.	Yield, %
L-L-L[c]	163.5–164.5	9.09	76.2	73.8	30	+21.6°	2.62	$CHCl_3$	63 pure
L-L-D	176–177	9.21	50.8	49.2	28	− 9.8	2.92	$CHCl_3$	66 pure
L-D-L	156.5–157.5	9.19	50.8	49.8	26	+64.2	2.66	$CHCl_3$	88 crude
L-D-D	158–159	9.19	25.4	26.0	25	+53.8	2.56	$CHCl_3$	59 pure
D-D-D	163–164.5	9.17	0.0	0.3	28	−21.7	2.76	$CHCl_3$	65 pure
D-D-L	176–177	9.21	25.4	27.2	28	+ 9.7	2.57	$CHCl_3$	82 crude
D-L-L	158–159	9.14	50.8	50.5	25	−53.0	2.45	$CHCl_3$	60 pure
D-L-D	156.5–157.5	9.09	25.4	25.2	26	−63.2	2.62	$CHCl_3$	89 crude
Calcd. for above		9.15							
Methyl ester HCl									
L-I-L[d]	225–228 d.	11.3	96.0	94.7	22	+48.8	2.12	Water	55 pure
L-L-D	220–224 d.	11.6	64.0	63.4	21	+16.3	2.02	Water	55 pure
D-D-D	226–228 d.	11.4	0.0	0.7	22	−51.2	2.31	Water	77 pure
D-D-L	218–222 d.	11.2	32.0	33.8					71 crude
Calcd. for above		11.5							

[a] Not corrected. [b] Microbiological assay.[16,17] [c] L-L-L represents L-valyl-L-valyl-L-valine, etc. [d] LDL, LDD, DLL and DLD isomers were oils. [e] Hydrolyzed 65 hours at 120°. No corrections for racemization on hydrolysis.

TABLE IV

ISOMERIC VALYLVALYLVALINES

Compound	Nitrogen, % Found	Neut. equiv.	L-Valine, % Calcd.	L-Valine, % MBA[b,d]	R_f	Rotations T, °C.	Rotations [α]D	Concn. in N HCl
L-L-L[a]	13.3	318	111.3	108.0	0.67	21	−41.8	2.69
L-L-D	13.3	316	74.2	77.6	.70	33	+ 2.4[c]	1.66
L-D-L	13.1	315	74.2	75.6		33	+39.9	3.87
L-D-D	13.1	313	37.1	36.5	.73	33	+73.6	3.30
D-D-D	13.3	319	0.0	0.9	.65	21	+41.3	2.82
D-D-L	13.3	314	37.1	36.7		33	− 0.4[c]	1.77
D-L-L	13.4	321	74.2	72.2	.74	33	−71.9	3.64
D-L-D		322	37.1	36.2	.72			
Calcd. for above	13.3	315						

[a] L-L-L represents L-valyl-L-valyl-L-valine, etc. [b] Microbiological assay.[17,18] [c] Approximate only due to instrument limitation. [d] Hydrolyzed 65 hours at 120°. No corrections for racemization on hydrolysis.

the phthaloyl group was employed. 1.71 g. (3.73 mmoles) of phthaloyl-D-valyl-D-valyl-D-valine methyl ester was suspended in 35 ml. of methanol. The suspension was brought to reflux, a clear solution resulted, 3.73 g. (7.46 mmoles) of hydrazine hydrate was added, and the solution was stored 19 hours at 29°. The solvent was removed *in vacuo*, and the residue kept 19 hours at 1 mm. to remove unreacted hydrazine hydrate. To the dry residue there was added 10 ml. of 0.5 *N* hydrochloric acid, the suspension was kept in an ice-bath for two hours, and the insoluble phthalhydrazide was

removed by filtration. The filtrate was concentrated *in vacuo* to about 3 ml., cooled in an ice-bath, and filtered. The filtrate was evaporated to dryness *in vacuo*. To the oily residue was added 20 ml. of acetone, followed by 60 ml. of ether, and the solution was stored 8 hours at 0°. The product was collected by filtration. There was obtained 1.36 g. of colorless needles, which, upon two crystallizations from methanol:acetone:isopropyl ether, yielded 1.06 g. of analytically pure material.

Four of the eight isomers (LDL, DLD, LDD, DLL) failed to crystallize from acetone or from acetone:ether solution; instead semi-solid products were obtained (Table III).

Valylvalylvaline Tripeptide Isomers. D-Valyl-D-valyl-D-valine.—0.500 g. (1.36 mmoles) of D-valyl-D-valyl-D-valine methyl ester hydrochloride was suspended in 11 ml. of 0.5 *N* potassium hydroxide, stored 75 minutes at 37°, chilled, and washed with three 2-ml. portions of ethyl acetate. The *p*H of the clear aqueous solution was adjusted to 5.0 with dilute acetic acid and the resulting solution was concentrated *in vacuo* to about 2 ml., 25 ml. of ethanol was added and the crystallizing solution was stored overnight at 0°. The crystalline product was collected by filtration, and recrystallized from 5 ml. of water and 30 ml. of ethanol; 0.371 g. (76% yield) of fine colorless plates was isolated. Flame photometry revealed less than 0.1% potassium. The product was dried to constant weight at 1 mm. at 100°, over P_2O_5, then exposed to room humidity until constant weight was reached. The tripeptides were hygroscopic and moistures were determined each time samples were weighed for characterization. The tripeptide isomers LLL, DDL and LLD were obtained in the same manner.

The four tripeptide methyl ester hydrochlorides which failed to crystallize were saponified as above and yielded crystalline peptides (Table IV).

PASADENA 4, CALIF.

[CONTRIBUTION FROM THE DEPARTMENT OF CHEMISTRY, CARNEGIE INSTITUTE OF TECHNOLOGY]

A New Synthesis of *dl*-Vasicine and a Methoxy Analog[1]

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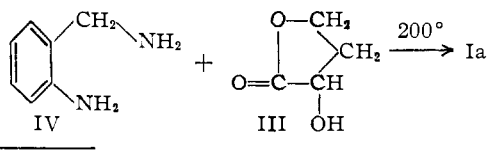
dl-Vasicine has been prepared from *o*-nitrotoluene by a reaction sequence in which *o*-nitrobenzylamine hydrochloride ethyl β-(*o*-nitrobenzylamino)-propionate hydrochloride, 1-(*o*-nitrobenzyl)-4-carbethoxy-2,3-dioxopyrrolidine, 1-(*o*-nitrobenzyl)-2,3-dioxopyrrolidine and 1-(*o*-nitrobenzyl)-3-hydroxy-2-oxopyrrolidine were the intermediates isolated and purified. A strictly parallel series of reactions, with only very slight modification of the details of procedure, served for the preparation of a new vasicine analog, *dl*-3-hydroxy-6-methoxypeg-9-ene ("*dl*-6-methoxyvasicine"), from 3-methyl-4-nitroanisole.

Work on the chemistry of vasicine (Ia) by several groups of workers[3] culminated in the synthesis of the alkaloid by Späth, Kuffner and Platzer[4] in 1935. The first synthesis depended on the preparation of methyl α-hydroxy-γ-aminobutyrate, which was treated with *o*-nitrobenzyl chloride to produce a low yield (*ca.* 7%) of 1-(*o*-nitrobenzyl)-3-hydroxy-2-oxopyrrolidine (IIa). Compound IIa, when treated with stannous chloride in hydrochloric acid, underwent reduction of the nitro group and spontaneous cyclization with dehydration to give a 35% yield of *dl*-vasicine. Later (1936) Späth and Platzer[5] described a more convenient procedure whereby α-hydroxybutyrolactone (III) was prepared from butyrolactone and heated to a high temperature with *o*-aminobenzylamine (IV) to give *dl*-vasicine, with a yield in the final step of 20%.



There have been indications in the literature[6] of interesting physiological effects, such as bronchodilator activity, produced by vasicine, and for this reason we have been prompted to undertake the synthesis of closely related analogs of vasicine in the hope of obtaining compounds of increased activity. In preparation for this effort we have investigated a new route to vasicine which proceeds through the final intermediate IIa of the first synthesis by Späth, Kuffner and Platzer,[4] but utilizes a different synthesis for this key compound. It was desired to achieve a preparative route which might be expected to be reliable when used on a relatively large scale and applicable without extensive modification when applied to the preparation of vasicine analogs It was also important that the synthetic sequence serve as an adequate structure proof for the products. These requirements led us to seek to avoid those cyclization methods used in the earlier syntheses which had produced low yields, necessitated the use of complex procedures for the isolation of the product, or utilized pairs of reactants whose manner of reacting might be considered uncertain.

The reaction sequence adopted is shown in Chart I. If vasicine analogs substituted in the aromatic ring were to be obtained by this route, a reasonably convenient method for the preparation of ring-substituted *o*-nitrobenzylamines (V) was needed. Chart II outlines a sequence which may prove to be applicable to the preparation of a number of such compounds. It was tested initially in the preparation of *o*-nitrobenzylamine itself (Va). *o*-Nitrotoluene was brominated with N-bromosuccinimide

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(2) Institute Fellow in Organic Chemistry, 1956–1957. This paper is abstracted from a thesis submitted by Joseph Casanova, Jr., in partial fulfillment of the requirements for the degree of Doctor of Philosophy at the Carnegie Institute of Technology, May, 1957.

(3) Reviews of this work include those (a) by H. T. Oppenshaw (R. H. F. Manske and H. L. Holmes, "The Alkaloids," Vol. III, Academic Press, Inc., New York, N. Y., 1953, pp. 101–111); (b) by T. A. Henry, "The Plant Alkaloids," 3rd ed., P. Blakiston's Sons and Co., Inc., Philadelphia, Pa., 1939, pp. 544–548; and (c) by E. Späth, *Monatsh.*, **72**, 115 (1938).

(4) E. Späth, F. Kuffner and H. Platzer, *Ber.*, **68**, 699 (1935).

(5) E. Späth and N. Platzer, *ibid.*, **69**, 255 (1936).

(6) (a) D. Hooper, *Pharm. J.*, [3] **18**, 841 (1888); (b) R. M. Chopra and S. Ghosh, *Indian Med. Gas.*, **60**, 354 (1925); *C. A.*, **19**, 3323 (1925).