TABLE IX

EFFECT OF REDIGESTION WITH CHYMOTRYPSIN AND TRYPSIN

Fraction	Total amino acids	Labile amino acids	Ratio 2600/2800
Before digestion	125	18	0.88
Tubes 75–99			
After digestion	6.7	3.8	0.91
Tubes 70–120			
After digestion	1.6	1.6	1.57
Tubes 180–190			

Evidence of Polynucleotide Nature.—Although the peptidases were not inactivated by treatment with ribonuclease and no action of ribonuclease was detected by changes in optical properties or by release of titrable acidic groups, the material when treated with alkali had a marked "hyperchromic effect." A solution with an absorption of 0.33 at 2600 in 0.1 *N* NaOH when freshly prepared was found to have an absorption of 0.43 after 20 hr. at 37°. It is of interest that Magasanik and Chargaff[12] found a marked hyperchromic effect with polynucleotide "cores" rich in guanylic acid.

Amino Acid Composition.—Fraction IV was hydrolyzed with 6 *N* HCl for 24 hr. at 100° and the HCl was removed by repeated evaporation. A solution of the hydrolysate in water was subjected to two dimensional descending chromatography; the first dimension was with 1-butanol–water–acetic acid in the proportions of 4–5–1; the second dimension was with *n*-propanol–water in the proportions of 7–3. The amino acids were detected with a ninhydrin stain. Aspartic acid, glutamic acid, serine, isoleucine and leucine were identified; another component found somewhere near histidine was not identified. An aliquot of the hydrolysate containing 2 mg. of "leucine equivalents" was placed upon a column of Dowex 50 × 4 and the amino acids were eluted according to the procedures of Moore and Stein[13] with analysis of the eluates in terms of leucine equivalents by a photometric ninhydrin procedure.[10] The results are given in Table X; the amino acids found by paper chromatography were detected in the ion-exchange methods and, in addition, an unknown component was found. However, no basic amino acids were detected and the material behaving somewhat like histidine in paper chromatography must not be histidine. The results are not corrected for color yield or for destruction of amino acids during hydrolysis. Traces of glycine and alanine are found in the hydrolysates but it would appear these arise from the breakdown of the purines.

TABLE X

AMINO ACID CONTENT OF FRACTION IV

Amino acid	Effluent, ml.	Leucine equiv. (mg.)
Unknown	84–94	0.28
Aspartic acid	158–180	.35
Glutamic acid	252–276	.25
Serine	182–200	.27
Isoleucine	438–450	.33
Leucine	450–462	.31
		1.89

(12) B. Magasanik and E. Chargaff, *Biochem. et Biophys. Acta*, **7**, 396 (1951).

(13) S. Moore and W. H. Stein, *J. Biol. Chem.*, **211**, 893 (1954).

ATLANTA 22, GEORGIA

[CONTRIBUTION FROM PASADENA FOUNDATION FOR MEDICAL RESEARCH]

Peptide Studies. III. An Antibacterial Tripeptide of L- and D-Valine[1]

BY S. SHANKMAN, S. HIGA AND V. GOLD

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The relationship between antibacterial activity and configuration of five tripeptides of L- and D-valine has been investigated, employing *Streptobacterium plantarum, Leuconostoc citrovorum* parent and mutant, *Lactobacillus casei* and *Lactobacillus fermenti*. The tripeptide L-valyl-L-valyl-D-valine is shown to be antibacterial at 0.05 mg. per ml. for three cultures. The other peptides or derivatives were not antibacterial at 20 times this concentration.

Previous work on peptides with antibacterial activity has shown the presence of D-amino acids in such peptides[2] or has been concerned with large polymers of a single amino acid.[3] Work in this Laboratory has been directed to the systematic investigation of optical configuration and biologic activity of peptides.

The eight pure tripeptides of valine have been synthesized previously[4] and their utilization by lactic acid bacteria has been studied.[5] The present report is a study of five of these peptides and some lower molecular weight derivatives for the possible inhibition of bacterial growth. The compound L-valyl-L-valyl-D-valine has been found to be antibacterial. None of the other compounds tested had significant antibacterial properties.

(1) Supported in part by grant CY-3609 from the National Institutes of Health, National Cancer Institute.

(2) T. S. Work in Biochemical Society Symposia No. 1, Cambridge Univ. Press, 1948, p. 61.

(3) D. W. Watson and W. L. Bloom, *Proc. Soc. Exp. Biol. & Med.* **39**, 27 (1952).

(4) S. Shankman and Y. Schvo, THIS JOURNAL, **80**, 1164 (1958).

(5) S. Shankman, S. Higa, H. A. Florsheim, Y. Schvo and V. Gold, *Arch. Biochem. & Biophys.*, in press.

Valine was added to the medium employed for utilization studies.[5] The test period was 65–72 hr. unless otherwise noted. Other conditions have been described previously.[5] Compounds tested and antibacterial findings are given in Table I.

The data presented in Table I indicate that of the peptides tested, L-valyl-L-valyl-D-valine was much more effective as an antibacterial agent than any other compound. That such activity was due to the structure of inhibitor, and not to concentration effects, is demonstrated by the lack of toxicity of glycine or mixed amino acids. The slight antibacterial activity of phthalyl-D-valine and phthalyl-L-valine for *S. plantarum* has been observed previously.[6]

Neither L-valyl-L-valyl-D-valine nor L-valyl-L-valyl-L-valine inhibited *L. fermenti* under these test conditions. For other compounds listed, inhibitions for *L. fermenti* were approximately those of *S. plantarum*. The LLD tripeptide inhibited *L. casei* at the start of this work with a half-maximum concentration for inhibition of 0.25 mg./ml. Later studies

(6) F. N. Minard and S. W. Fox, THIS JOURNAL, **71**, 1160 (1949).

using turbidity measurements and a 28–36 hr. growth period gave much less inhibition. That *L. casei* strains show markedly different sensitivities to potassium acetate inhibition has been discussed by Camien and Dunn.[7] These workers have indicated that strain differences may arise in this organism on normal subculture. The LLL tripeptide did not inhibit *L. casei* significantly.

TABLE I

CONCENTRATION FOR HALF-MAXIMUM INHIBITION OF GROWTH

Compound	Mg. per ml. *S. plantarum*	Mg. per ml. *L. casei*	Mg. per ml. *L. citrovorum* mutant
L-Valyl-L-valyl-L-valine	N[a]	N	0.6
L-L-D[b]	N	See text	0.05
L-L-D (20 hr.)	0.02–0.05	See text	..
L-D-D	N	6	7
D-D-D	N	6	N
D-L-D	N	N	N
L-L	N	S[c]	2
L-D	N	N	N
D-L	N	N	3.5
Phthalyl-L-L	12	4	3
Phthalyl-L-D	8	4	8
Phthalyl-D-L	7	4	6
Phthalyl-D-D	8	2	6
Phthalyl-L[d]	6 ± 1	3 ± 1	3 ± 1
Phthalyl-D	4	2	3
L-Methyl ester·HCl	N	S	6
D-Methyl ester·HCl	9	7	8
L-Valine	N	N	2[d]
D-Valine	N	N	N
Glycine[e]	N	N	N
Amino acid mixture[f]	N	N	N

[a] N = *no* inhibition at 8 mg./ml. [b] L-L-D represents L-valyl-L-valyl-D-valine, etc. [c] S = *slight* inhibition at 8 mg./ml. [d] No inhibition was obtained in some trials. [e] Control sample. [f] Mixture of amino acids used in medium.

In growth experiments of 20 hr. and with dilute inocula, it was possible to obtain 50% inhibition of growth of *S. plantarum* at 0.02 to 0.05 mg./ml. of the LLD tripeptide. For *L. citrovorum* parent, after reducing the total amino acid concentration to 1/3 that used previously,[5] in 20 hr. turbidity experiments the LLD tripeptide gave 50% inhibition of growth at 0.02 to 0.05 mg./ml.

The highly specific optical configuration LLD of the five tripeptides tested is noteworthy. Whether this structure is general for several amino acids or is specific for valine is now under investigation in our Laboratory. These compounds are also being presently screened for possible anti-tumor activity.

PASADENA, CALIFORNIA

(7) M. N. Camien and M. S. Dunn, *Proc. Soc. Exp. Biol. & Med.*, **95**, 697 (1957).

[CONTRIBUTION FROM THE LABORATORY OF PHARMACEUTICAL CHEMISTRY, THE UNIVERSITY OF KANSAS SCHOOL OF PHARMACY]

The Amino- and Chloromethylation of Uracil[1]

BY J. H. BURCKHALTER, ROBERT J. SEIWALD AND HOMER C. SCARBOROUGH

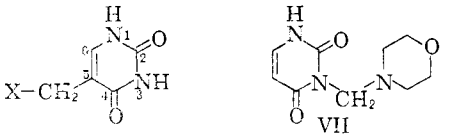
RECEIVED JULY 8, 1959

5-Morpholinomethyluracil (III) and 5-chloromethyluracil (V) have been synthesized, with results which are in disagreement with that of others. Both were converted through hydrogenolysis to thymine (I) as proof of structure. N-Chlorosuccinimide treatment of thymine (I), reported to give V, has been shown to yield 5-chloro-6-ethoxyhydrouracil (VIII).

Despite extensive investigations into the chemistry of the pyrimidines, particularly by Johnson and co-workers,[2] substitution reactions of uracil have not been thoroughly studied. In view of the occurrence of thymine (I), 5-methylcytosine and 5-hydroxymethylcytosine in nucleic acids,[3,4] it appears surprising that the 5-(substituted methyl)-uracils have received no more attention. A possible explanation is found in the instability imposed by the substitution of amino, chloro and hydroxyl groups at the 5-methyl of thymine (I).[5] Nevertheless, because of the importance of these substances as intermediates, or as such, in the search for antineoplastic and antiviral agents, we wished to investigate reactions which were expected to lead to desirable thymine derivatives (II–VI).[6]

	X
I	H
II	piperidino-
III	morpholino-

	X
IV	H_2N–
V	Cl^-
VI	HO



VII

The reaction of morpholine, formalin and uracil has been reported by Bombardieri and Taurins to yield 3-morpholinomethyluracil (VII).[7] This report was in disagreement with our unpublished studies which had suggested structure III instead of VII. Also, synthesis of a compound designated

(1) This study was supported in part through the General Research Fund, University of Kansas.

(2) T. B. Johnson in Gilman's "Organic Chemistry," John Wiley and Sons, Inc., New York, N. Y., 1938, Vol. II, Chapter 11; and R. H. Wiley, *ibid.*, 1953, Vol. IV, p. 864.

(3) A. Bendich in "The Nucleic Acids," Academic Press, Inc., New York, N. Y., 1955, Vol. I, p. 86.

(4) Also, 5-hydroxymethyluracil (VI) is said to be a product of the bacterial oxidation of thymine; R. D. Batt and D. D. Woods, *Proc. Biochem. Soc.* in *Biochem. J.*, **49**, lxx (1951).

(5) Reference 3, p. 92.

(6) In previous studies in this Laboratory, a number of 5-alkyl- and 5-aryluracils were synthesized: J. H. Burckhalter and H. C. Scarborough, *J. Am. Pharm. Assoc.*, **44**, 545 (1955).

(7) C. C. Bombardieri and A. Taurins, *Can. J. Chem.*, **33**, 923 (1955).