[CONTRIBUTION FROM THE CHEMICAL LABORATORY OF IOWA STATE COLLEGE]

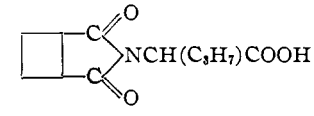
Studies on Antipodes. VI.[1] The Synthesis of Some Derivatives of Enantiomorphs of Valine[2]

BY FREDERICK N. MINARD[3] AND SIDNEY W. FOX

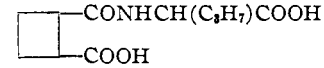
The possible significance of the D-amino acid component in antibiotics has been previously considered.[1a,4] Partly as the result of the finding of inhibition of bacterial growth by D-amino acids[1a,b,d] fundamental relationships involving D-amino acids, proteases, growth, and antibiotics have been suggested. Initial attempts to prepare model antibiotics in this laboratory were based on the knowledge surrounding gramicidin and tyrocidine.[1a] Since that time the penicillins have been shown to be D-amino acid derivatives[5] and the D-amino acid residue has been found to be one critical structural feature in this type.[6] Evidence now exists for the presence of D-amino acid residues in other antibiotics besides gramicidin (D-leucine and D-valine), tyrocidine (D-phenylalanine), and the penicillins (D-penicillamine). These include gramicidin S (D-phenylalanine),[7] bacitracin[8] (D-phenylalanine), and aerosporin[9] (D-leucine and D-α,γ-diaminobutyric acid). The D-amino acid residue thus appears to be a unit of widespread significance in the comparative biochemistry of antibiotics. The value of the D-amino acid unit may depend upon its contribution to inhibitory action, to non-metabolizability, to cyclizability of the whole molecule,[10] or upon some other function.[11]

All of the above antibiotics are peptides or peptide derivatives. Large natural peptide molecules subjected to treatment resulting in inversion of L-residues have given products which have so far not been found to possess antibacterial activity.[1e] Treatment of "racemized casein" aimed at bringing about cyclization of this material as for the diketopiperazines[12,13] has given products which possess relatively weak antibacterial activity not, however, found in the peptides prior to such treatment.[14]

The natural profusion of antibiotics of the D-amino acid derivative class increases interest in chemical attempts to synthesize D-amino acid derivatives with antibacterial activity. Among other working hypotheses for the synthetic approach to the antibiotic problem is the inclusion of strained structures in compounds with groups that may function to attract enzymes. To this end some cyclobutane[15] derivatives (Types I and II) have been prepared and are recorded in this article (Table I). These derivatives contain peptide and carboxyl groups, although not in a relationship such as is found in penicillin or in peptidase substrates.[16] The amino acid residues are L-valine, D-valine, and DL-valine. The *o*-carboxybenzoyl derivatives of the valine isomers (III) are also reported.



I



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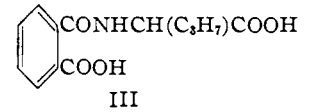

III

The phthaloyl-DL-valine reported in Table I has been recorded earlier by Billman and Harting.[17] The product described here is included for comparative purposes, since it was purified in a different manner. This compound formed a hydrate when crystallized from aqueous solutions. In the case of the corresponding cyclobutane compounds, the optical isomers yielded hydrates from aqueous solution, whereas the racemate did not.

The opening of the ring in the phthaloyl derivatives and the corresponding ring in the cyclobutane analog proceeded very rapidly. Mere warming of the cyclic imides with dilute alkali served to hydrolyze the cyclic imides without appreciable rupture of the remaining peptide linkage.

(1) Earlier papers in this series: (a) Fox, Fling and Bollenback, *J. Biol. Chem.*, **155**, 465 (1944); (b) Fling and Fox, *ibid.*, **160**, 329 (1945); (c) Fling, Minard and Fox, THIS JOURNAL, **69**, 2466 (1947); (d) Kobayashi, Fling and Fox, *J. Biol. Chem.*, **174**, 391 (1948); (e) Fox, Kobayashi, Melvin and Minard, THIS JOURNAL, **70**, 2404 (1948).

(2) Journal Paper No. J-1562 of the Iowa Agricultural Experiment Station, Project 980.

(3) Upjohn Company Fellow, 1946–1949.

(4) Fox, *Can. Med. Assoc. J.*, **56**, 76 (1947).

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

(6) du Vigneaud, Carpenter, Holley, Livermore and Rachele, *Science*, **104**, 431 (1946).

(7) Synge, *Biochem. J.*, **39**, 363 (1945).

(8) Barry, Gregory and Craig, *J. Biol. Chem.*, **175**, 485 (1948).

(9) Jones, *Biochem. J.*, **42**, lii (1948).

(10) Harris and Work, *Nature*, **161**, 804 (1948).

(11) How the D-amino acid content of antibiotics compares with the content of the same unit in the proteins of the parent organisms cannot be accurately evaluated since there are insufficient data of the latter type.

(12) Fling, Ph.D. thesis, Iowa State College, 1946.

(13) Fruton, THIS JOURNAL, **70**, 1280 (1948).

(14) Fox and Kobayashi, manuscript in preparation.

(15) Kilpatrick and Spitzer, *J. Chem. Phys.*, **14**, 463 (1946).

(16) Greenstein, in Schmidt, "Chemistry of the Amino Acids and Proteins," 2nd ed., Charles C. Thomas, Springfield, Illinois, 1944, pp. 267 and 1080.

(17) Billman and Harting, THIS JOURNAL, **70**, 1475 (1948).

TABLE I

DERIVATIVES OF ISOMERS OF VALINE

	Empirical formula	M. p. °C. (uncor.)	Nitrogen, % Calcd.	Found		Neut. equiv. Calcd.	Found	[α]D in abs. EtOH (3% solution) °C.	Rotation
Acyl derivatives of DL-valine									
N,N-Phthaloyl	$C_{13}H_{13}O_4N$	102–103	5.67	5.67		247	245		
N,N-Phthaloyl, monohydrate[a]	$C_{13}H_{15}O_5N$	80–81½	5.28	5.28	5.35	265	262		
N-(*o*-Carboxybenzoyl)	$C_{13}H_{15}O_5N$	171½–172	5.28	5.27	5.27	133	132		
N,N-(Cyclobutane-1,2-dicarbonyl)	$C_{11}H_{15}O_4N$	102–103½	6.22	6.20	6.18	225	223		
N-(2-Carboxycyclobutanecarbonyl)	$C_{11}H_{17}O_5N$	178–179	5.76	5.73	5.73	122	124		
Acyl derivatives of L-valine									
N-(*o*-Carboxybenzoyl)	$C_{13}H_{15}O_5N$	154–155	5.28	5.23		133	136	24	−15.9 ± 0.5°
N,N-(Cyclobutane-1,2-dicarbonyl), monohydrate[b]	$C_{11}H_{17}O_5N$	92–98	5.76	5.70	5.73	243	246	25	−76.1 ± 2.8°
N-(2-Carboxycyclobutanecarbonyl)	$C_{11}H_{17}O_5N$	168–169	5.76	5.81	5.73	122	123	26	− 7.2 ± 0.4°
Acyl derivatives of D-valine									
N-(*o*-Carboxybenzoyl)	$C_{13}H_{15}O_5N$	153–154	5.28	5.26	5.28	133	135	27	+16.2 ± 0.4°
N,N-(Cyclobutane-1,2-dicarbonyl), monohydrate[b]	$C_{11}H_{17}O_5N$	92–98	5.76	5.81	5.85	243	246	26	+77.5 ± 3.6°
N-(2-Carboxycyclobutanecarbonyl)	$C_{11}H_{17}O_5N$	168–169	5.76	5.71	5.70	122	123	27	+ 7.4 ± 0.3°

[a] By recrystallization of the anhydrous imide from ethanol–water. [b] The anhydrous peptide was hygroscopic and was not obtained.

Preliminary tests of the above compounds on *Lactobacillus arabinosus* and *Escherichia coli* showed neither high antibacterial activity nor antipodal specificity.[18] Activity was found at dilutions of 0.8 mg./ml., but not at half this concentration.

Experimental

Preparation of Imides.—The valine isomer was fused with either phthalic anhydride as previously described[1c,17] or with cyclobutane-1,2-*cis*-dicarboxylic anhydride[19] in equimolar ratio. The phthaloyl-DL-valine was recrystallized from carbon tetrachloride and the cyclobutanedicarbonyl valines were recrystallized from water.

Hydrolysis of Imides.—Compounds of Types II and III were prepared as follows: Five grams of the imide was titrated in a 125-ml. Erlenmeyer flask with 2 *N* sodium hydroxide solution to a phenolphthalein end-point. An equal amount of dilute alkali, plus 0.5 ml. excess, was added. The flask was placed in a water-bath (previously at the boiling-point) until the inside temperature reached 70°, and then quickly cooled in an ice-bath. The solution was acidified with concentrated hydrochloric acid solution, and an excess added to precipitate the organic acid. After one hour the product was filtered and dried.

The *o*-carboxybenzoyl derivatives were purified by solution of the dried material in acetone, filtration from undissolved sodium chloride, and addition of carbon tetrachloride to incipient precipitation. The peptides came out slowly and after several such purifications appeared as well-formed crystals.

The carboxycyclobutane derivatives were freed from inorganic matter by solution in anhydrous ethanol. After filtration, several ml. of dry ethyl acetate was added to prevent layering, and then enough hexane to cause incipient precipitation.

Yields were 50–75% for type I compounds, 40–50% for type II, and 60–75% for type III.

(18) Kobayashi and Fox, unpublished experiments.

(19) Buchman, Reims, Skei and Schlatter, THIS JOURNAL, **64**, 2696 (1942).

Summary

The N-(*o*-carboxybenzoyl), N,N-(cyclobutane-1,2-dicarbonyl), and N-(2-carboxycyclobutanecarbonyl) derivatives of L-, D-, and DL-valine have been prepared and characterized.

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