[CONTRIBUTION FROM THE CLAYTON FOUNDATION BIOCHEMICAL INSTITUTE AND THE DEPARTMENT OF CHEMISTRY, THE UNIVERSITY OF TEXAS]

Structural Specificity of Some Amino Acid Antagonists

BY JEROME EDELSON, CHARLES G. SKINNER, JOANNE M. RAVEL AND WILLIAM SHIVE

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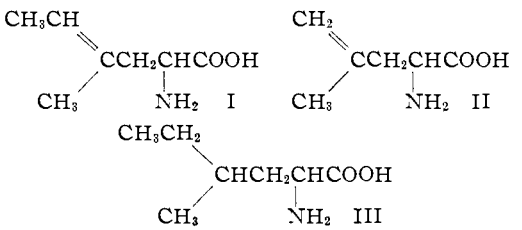
2-Amino-4-methyl-4-hexenoic acid (I), 2-amino-4-methylhexanoic acid and 2-amino-5-methylhexanoic acid were prepared by the usual acetamidomalonic or acetamidocyanoacetic ester procedures using the appropriate alkyl halide. The starting material for I was tiglaldehyde which has a *cis* configuration with respect to the methyl groups. The aldehyde was reduced with sodium borohydride to the alcohol and then converted to the corresponding bromide with phosphorus tribromide prior to reaction with the acetamido- derivative. Compound I, which has a planar 2-methyl-2-butenyl group in place of the planar benzyl group of phenylalanine, is an antagonist of phenylalanine for *Leuconostoc dextranicum* 8086. Since a lower homolog, methallylglycine, does not exert this effect, the additional terminal methyl group present in I must *sterically assist* the higher homolog in complexing with the enzyme at the site of phenylalanine interaction. The aliphatic planar group in I like the corresponding group of methallylglycine does not sterically prevent enzyme interaction at the site of leucine utilization as do larger planar alicylic groups such as the 1-cyclopentenylmethyl group. The corresponding saturated analog of I, 2-amino-4-methylhexanoic acid, is an antagonist of leucine but not of phenylalanine. 2-Amino-5-methylhexanoic acid did not possess any appreciable inhibitory properties.

In a series of studies on alicyclic amino acids, steric configurations and structural conformations have been found to have profound influences on the biological activity of structurally related analogs. For *Leuconostoc dextranicum* 8086, 1-cyclopentenealanine is a competitive antagonist of phenylalanine but not of leucine, while cyclopentanealanine is a competitive antagonist of leucine but not of phenylalanine.[1] The specificity of these amino acid analogs has been attributed to the relative position of the β-carbon of the alanine side chain to that of the adjacent ring carbons. When the ring carbons are in the same plane as the β-carbon of the alanine side chain (1-cyclopentenealanine) the analog would have a structure analogous to that of phenylalanine; while, a corresponding non-planar configuration (cyclopentanealanine) would be comparable to the structure of leucine in which the β-carbon is not in the same plane as the terminal isopropyl group of the natural amino acid.

The present investigation is concerned with a study of the minimal size of the grouping necessary to induce phenylalanine antagonism in β-substituted alanines which have the β-carbon in the same plane as the adjacent carbons of the substituent group. The geometric isomer of 2-amino-4-methyl-4-hexenoic acid (I) which has the two methyl groups in the *cis* position was accordingly synthesized for comparison with methallylglycine (II)[2] which had previously been reported to be a leucine antagonist for certain microörganisms.[3] Although methallylglycine (II) antagonized leucine but not phenylalanine in *L. dextranicum*, the higher homolog 2-amino-4-methyl-4-hexenoic acid (I) is a competitive antagonist of both phenylalanine and leucine, indicating that the size of the substituent group, as well as its relative position to the β-carbon, determines the type of biological activity exerted by an amino acid antagonist. In this respect, it is of interest to note that this is an example of a purely aliphatic compound (I) which possesses the enzyme-binding ability necessary to compete effectively with the aromatic amino acid, phenylalanine.



For the purpose of comparison, the corresponding saturated derivative of the phenylalanine antagonist I described above was prepared. This amino acid analog 2-amino-4-methylhexanoic acid (III) was previously predicted[4] to have the structure necessary for leucine antagonism, and such proved to be the case as herein demonstrated.

2-Amino-4-methyl-4-hexenoic acid (I) was prepared from tiglaldehyde which was reduced with sodium borohydride to the alcohol and subsequently converted to 2-bromomethyl-2-butene with phosphorus tribromide. The condensation of this halide with ethyl acetamidocyanoacetate produced the anticipated intermediate which then was converted to the desired amino acid by alkaline hydrolysis. Acid hydrolysis of the intermediate condensation product gave a lactone derivative, and less drastic alkaline hydrolysis formed 2-acetamido-4-methyl-4-hexenoic acid. 2-Methyl-1-bromobutane was treated with either ethyl acetamidocyanoacetate or ethyl acetamidomalonate to yield the corresponding condensation product. The acetamidomalonate intermediate was not isolated, but was converted directly *via* alkaline hydrolysis to 2-amino-4-methylhexanoic acid (III), whereas the acetamidocyanoacetate intermediate was easily recovered in crystalline form prior to hydrolysis to the amino acid derivative. 2-Amino-5-methylhexanoic acid was prepared in a similar fashion by treating 3-methyl-1-bromobutane with ethyl acetamidocyanoacetate, and the intermediate condensation product subsequently was converted to the amino acid by alkaline hydrolysis.

2-Amino-4-methyl-4-hexenoic acid (I), an aliphatic amino acid, inhibits the growth of *Leuconostoc dextranicum* 8086 at a level of about 250 γ/ml., and this inhibition is competitively reversed over a 20-fold range of inhibitor concentrations by the aro-

(1) P. R. Pal, C. G. Skinner, R. L. Dennis and W. Shive, THIS JOURNAL, **78**, 5116 (1956).

(2) N. F. Albertson, *ibid.*, **68**, 450 (1946).

(3) K. Dittmer, *Ann. N. Y. Acad. Sci.*, **52**, 1274 (1950).

(4) J. Edelson, C. G. Skinner, J. M. Ravel and W. Shive, *Arch. Biochem. Biophys.*, **80**, 416 (1959).

matic amino acid, phenylalanine (Table I-A). The planarity of the methylbutenyl group as a result of the double bond is sufficiently similar to the benzyl group in phenylalanine to allow the analog to compete for binding at the active enzyme site which complexes with the natural amino acid. The starting material for this synthesis was tiglaldehyde which has a *cis* configuration with respect to the methyl groups, and the resulting terminal 2-methyl-2-butene unit is thus sterically comparable to the 1, 2, 3, 6, and methylene carbons of the benzyl group in phenylalanine. There is evidence of a supplemental structural requirement other than

TABLE I

REVERSAL OF TOXICITY OF DL-2-AMINO-4-METHYL-4-HEXENOIC ACID IN *Leuconostoc dextranicum* 8086 BY PHENYLALANINE OR LEUCINE[a]

A. Phenylalanine

Inhibitor, γ/ml.	Supplement to basal media[b]: DL-phenylalanine, γ/ml. None	1	2	5	10	20
	Galvanometer readings[c]					
0	58	61	67	68	68	68
20	43					
50	27	63				
100	16	60				
250	6	19	50			
500		8	13	52	60	
1000			6	12	42	58
2000				6	10	30

B. Leucine

	Supplement to basal media[d]: DL-leucine, γ/ml. 5	10	20
0	66	68	69
100	64		
200	59		
500	52	61	
1000	25	56	62
2000		29	54

[a] Incubated at 30° for about 18 hr. [b] DL-Leucine, 40 γ/ml. [c] A measure of culture turbidity, distilled water reads 0, an opaque object 100. [d] DL-Phenylalanine and DL-tyrosine, 40 γ/ml. each.

planarity in this portion of the molecule since a corresponding unsaturated lower homolog, methallylglycine, is not an effective phenylalanine antagonist. The additional methyl group must sterically assist the higher homolog in complexing the enzyme at the site of phenylalanine interaction. Such an effect may be termed *steric assistance*, and in this example the mode of action could involve assistance either in stabilizing or in forming the enzyme–inhibitor complex. For example, the enzyme might have an area which sterically confines the methyl group in such a manner as to assist the functional groups of the analog to stabilize the complex; or, the group might sterically aid in directing the functional groups to their site of interaction with the enzyme. Differences in the biological activity of sterically similar analogs would thus be anticipated. A further increase in the size of the grouping corresponding to the phenyl group tends to promote slightly the effectiveness of the analog since both 1-cyclopentenealanine and 1-cyclohexenealanine are slightly more effective antagonists of phenylalanine than the 2-amino-4-methyl-4-hexenoic acid derivative.

Since methallylglycine has been reported to be an antagonist of leucine in several microörganisms,[3] its activity was compared with the 2-amino-4-methyl-4-hexenoic acid as a leucine antagonist. In the assay system which demonstrated that the latter compound (I) competitively inhibited the utilization of phenylalanine, the concentration of leucine was maintained at a sufficiently high level so that leucine antagonisms would not affect the results with phenylalanine. By supplementing the medium with high concentrations of phenylalanine and lowering the concentration of leucine as indicated in Table I-B, a growth inhibitory effect of 2-amino-4-methyl-4-hexenoic acid (I) could be demonstrated which was reversed in a competitive manner by leucine. As indicated in Table II, methallylglycine is slightly more effective than 2-amino-4-methyl-4-hexenoic acid in producing a growth inhibition which is reversed by leucine; however, only the latter compound has sufficient toxicity in the presence of high concentrations of leucine to allow a study of phenylalanine antagonism. Thus, while the introduction of the terminal methyl group of the higher homolog I permits phenylalanine antagonism by the antagonist, it also induces a steric hindrance which decreases the effectiveness of the compound as a leucine antagonist. A further increase in the size of the planar grouping attached at the β-carbon of alanine sterically prevents antagonism of leucine by the analog. 1-Cyclohexenealanine and 1-cyclopentenealanine do not show the dual antagonism exhibited by 2-amino-4-methyl-4-hexenoic acid, but are specific antagonists of phenylalanine alone.[1,5] It appears that some degree of steric freedom exists (with regard to the planarity of the terminal groups in inducing enzyme–analog interaction) with substituent groups containing up to about four carbon atoms in lieu of the isopropyl grouping of leucine; but, in contrast, larger substituent groupings must assume a steric configuration comparable to the plane of the terminal isopropyl group of leucine in order to interact at the enzyme binding site(s) of this natural amino acid.

TABLE II

RELATIVE TOXICITY OF DL-METHALLYLGLYCINE AND DL-2-AMINO-4-METHYL-4-HEXENOIC ACID UNDER LIMITING PHENYLALANINE AND LEUCINE CONDITIONS[a]

Concentration of inhibitor, γ/ml.	Supplement to basal media, γ/ml. DL-Phenylalanine, 40; DL-Tyrosine, 40; DL-Leucine, 5 MAG[b]	AMHA[c]	DL-Leucine, 40 MAG[b]	AMHA[c]	DL-Phenylalanine, 40; DL-Tyrosine, 40; DL-Leucine, 40 MAG[b]	AMHA[c]
	Galvanometer readings					
0	64	66	62	62	68	68
20		64		46		
50	44	59	54	30		
100	21	52	55	16		
250	6	25	53	7		
500			43		59	65

[a] *Leuconostoc dextranicum* 8086, incubated at 30° for about 18 hr. [b] Methallylglycine. [c] 2-Amino-4-methyl-4-hexenoic acid.

In order to indicate the specificity of inhibition induced by the planarity resulting from the carbon–

(5) J. Edelson, P. R. Pal, C. G. Skinner and W. Shive, THIS JOURNAL, **79**, 5209 (1957).

carbon double bond in 2-amino-4-methyl-4-hexenoic acid (I), the corresponding saturated amino acid analog 2-amino-4-methylhexanoic acid (ω-methylleucine) (III) also was prepared, and its biological properties were studied in both *L. dextranicum* and *Escherichia coli* 9723. The toxicity of this latter derivative was not affected by supplements of phenylalanine; however, it was competitively reversed by leucine over a wide range of inhibitor concentrations as indicated in Tables III and IV. In earlier reports, the structural similarity of 2- and 3-cyclohexenyl and cyclopentyl groups to a *sec*-butyl was demonstrated by substituting these groupings in lieu of the *sec*-butyl group of isoleucine with the resulting production of isoleucine antagonists.[5-7] Since the latter two substituent groupings have also been substituted in the β-position of alanine to produce leucine antagonists,[1,4] it was predicted that *sec*-butylalanine (ω-methylleucine) would also be a leucine antagonist.[4]

TABLE III

REVERSAL OF TOXICITY OF DL-2-AMINO-4-METHYLHEXANOIC ACID IN *Escherichia coli* 9723[a]

2-Amino-4-methylhexanoic acid, γ/ml.	Supplement, γ/ml. DL-leucine None	0.1	0.2	0.5	1.0	2.0	5.0	10	DL-Phenylalanine 6.0
	Galvanometer readings								
0	73	75	72	71	74	72	69	75	77
5	75								76
10	0	72							0
20	0	72	73						
50		1	50	73	71				
100			1	9	72	73	73		
250				0	13	67	69	75	
500					0	0	25	74	
1000							2	1	

[a] Incubated at 37° for about 16 hours.

TABLE IV

REVERSAL OF TOXICITY OF DL-2-AMINO-4-METHYLHEXANOIC ACID BY LEUCINE IN *Leuconostoc dextranicum* 8086[a]

2-Amino 4-methylhexanoic acid, γ/ml.	Supplement to basal media,[b] DL-leucine, γ/ml. 5	10	20	50
	Galvanometer readings			
0	69	68	66	60
25	56			
50	32	57		
100	4	43	56	
250		5	54	55
500			10	51
1000				5

[a] Incubated for 18 hours at 30°. [b] DL-Phenylalanine and DL-tyrosine, 40 γ/ml. each.

2-Amino-5-methylhexanoic acid (IV) is also a higher homolog of leucine in which an isobutyl grouping replaces the isopropyl group of the natural amino acid; however, it was not found to be an effective antimetabolite in the test systems studied. The introduction of a methylene group within the carbon skeleton of leucine, instead of at the end of the chain as in III, produces an analog which has the terminal isopropyl structure of leucine; however, its inactivity suggests that a rather specific carbon–carbon distance is required between the amino-carboxyl groups and the branching side chain in order to permit enzyme interaction of the analog at the site of leucine utilization. The presence of terminal methyl group(s) is not essential to induce leucine antagonism since certain β-alicyclic substituted alanine derivatives have been found to be competitive leucine antagonists.[1,4]

Experimental[8]

Microbiological Assays.—For the assays using *Leuconostoc dextranicum* 8086, a previously described amino acid medium[9] was employed, except that the phenylalanine, tyrosine and leucine were omitted from the basal media and supplemented as indicated in the tables. The medium was further modified by adding 0.02 γ/ml. of pantethine, by increasing the salts A concentration fourfold, and by adding 0.2 γ/ml. of calcium pantothenate. For *Escherichia coli* 9723 an inorganic salts–glucose medium[10] was used, and this procedure has been reported in detail.[11]

In all assays the amino acid analogs were dissolved in sterile water and added to sterile assay tubes without being heated. The amount of growth was determined turbidimetrically in terms of galvanometer readings adjusted such that distilled water reads 0, and an opaque object 100.

Ethyl 2-Acetamido-2-cyanoisoheptanoate.—To a solution of 2.8 g. of sodium dissolved in 300 ml. of anhydrous ethyl alcohol was added 20 g. of ethyl acetamidocyanoacetate followed by 26 g. of 3-methyl-1-iodobutane. After stirring at room temperature for a few hours, the reaction mixture was heated to reflux for 24 hours, and the precipitate which formed on cooling was filtered. The filtrate was reduced to about one-fourth its original volume *in vacuo*, and then poured into four volumes of ice-water. The resulting precipitate was recovered, and recrystallized from acetone–water to yield 15.7 g. of product, m.p. 112–114°.

Anal. Calcd. for $C_{12}H_{20}N_2O_3$: C, 59.98; H, 8.39; N, 11.66. Found: C, 60.34; H, 8.37; N, 11.68.

2-Amino-5-methylhexanoic Acid.—A mixture of 14 g. of ethyl 2-acetamido-2-cyanoisoheptanoate and 50 ml. of 10% sodium hydroxide was heated in a steel beaker for 48 hours. The resulting reaction mixture was then taken to *p*H 5 with concentrated hydrochloric acid and reduced to dryness *in vacuo*, after which the residue was extracted repeatedly with ethanol to recover the organic material. Evaporation of the solvent yielded a solid which was recrystallized from acetone–water to yield a total of 5.55 g. of product, m.p. 266–267° dec.[12]

Anal. Calcd. for $C_7H_{15}NO_2$: N, 9.65. Found: N, 9.54.

2-Amino-4-methylhexanoic Acid.—To a solution of 1.54 g. of sodium dissolved in 200 ml. of ethanol was added 14 g. of ethyl acetamidomalonate followed by 9.9 g. of 2-methyl-1-bromobutane, and the mixture was heated to reflux for 48 hours to yield a dark-colored reaction mixture. Attempts to recover the intermediate condensation product as a crystalline solid were unsuccessful and the alcohol soluble residue was then hydrolyzed directly by heating on a steam-cone for 18 hours in the presence of 25 ml. of concentrated hydrochloric acid. The resulting solution was reduced to dryness *in vacuo*, and ethanol was repeatedly added and evaporated to remove the excess hydrochloric acid. The resulting solid material was taken up in water, adjusted to *p*H 5 with 10% sodium hydroxide, and placed in the refrigerator. There was recovered 3.5 g. of product which was recrystallized from hot water, m.p. 253–255° dec.

Anal. Calcd. for $C_7H_{15}NO_2$: C, 57.90; H, 10.41; N, 9.65. Found: C, 57.66; H, 10.44; N, 9.57.

(6) J. Edelson, J. D. Fissekis, C. G. Skinner and W. Shive, THIS JOURNAL, **80**, 2698 (1958).

(7) W. M. Harding and W. Shive, *J. Biol. Chem.*, **206**, 401 (1954).

(8) All melting points are uncorrected. The chemical analyses were carried out either in the authors' laboratories, or by Drs. Weiler and Strauss, Oxford, England. The methallylglycine was kindly furnished by Dr. T. J. McCord, Abilene Christian College. All chromatograms were made using the ascending technique, and the spots were developed with ninhydrin reagent.

(9) L. T. H. Dien, J. M. Ravel and W. Shive, *Arch. Biochem. Biophys.*, **49**, 283 (1954).

(10) E. H. Anderson, *Proc. Natl. Acad. Sci., U. S.*, **32**, 120 (1946).

(11) F. W. Dunn, J. M. Ravel and W. Shive, *J. Biol. Chem.*, **219**, 809 (1956).

(12) T. Curtius and W. Wirbatz, *J. prakt. Chem.*, **125**, 267 (1930), report a m.p. of 280° for this derivative prepared by another procedure.

Ethyl 2-Acetamido-2-cyano-4-methylhexanoate.—Using the condensation procedure for the intermediate described above, 1.54 g. of sodium, 200 ml. of ethanol, 11.3 g. of ethyl acetamidocyanoacetate and 12.5 g. of 2-methyl-1-bromobutane were allowed to react to yield 14 g. of crude product, which, after recrystallization from water, had a m.p. of 100–101°.

Anal. Calcd. for $C_{12}H_{20}N_2O_3$: C, 59.98; H, 8.39; N, 11.66. Found: C, 60.20; H, 8.76; N, 11.57.

Hydrolysis of this product by the procedure described above gave a sample of 2-amino-4-methylhexanoic acid which was identical with the material characterized above.

2-Methyl-2-butene-1-ol.—To a solution of 26.2 g. of tiglaldehyde dissolved in 100 ml. of methanol was added, with external cooling, 4 g. of sodium borohydride in 200 ml. of methanol. After the addition was complete, the reaction mixture was allowed to come to room temperature, and was finally heated for 30 minutes on a steam-cone. The solution was then reduced to about one-half the original volume and acidified with concentrated hydrochloric acid to about *p*H 3, after which 250 ml. of water was added and the resulting solution was extracted three times with 75-ml. portions of ether. The combined ether phase was washed with potassium carbonate solution, and dried over anhydrous potassium carbonate. After removal of the solvent, the residue was fractionally distilled to yield 15 g. of product, b.p. 136–139°, $n^{24}D$ 1.440.[13]

2-Bromomethyl-2-butene.—A mixture of 40 g. of 2-methyl-2-butene-1-ol and 5 ml. of pyridine was cooled in an isopropyl alcohol–Dry Ice-bath and 24 ml. of phosphorus tribromide was added slowly, after which the reaction mixture was allowed to warm to room temperature and finally stirred overnight. Upon the addition of water, an organic phase which separated was recovered, and the resulting aqueous phase was extracted with ether. The combined organic phases were washed with dilute sodium hydroxide followed by water, and finally dried over calcium sulfate. After removal of the solvent, the residue was fractionally distilled to yield 48 g. of lachrymatory product, b.p. 43–47° (44 mm.).

Anal. Calcd. for C_5H_9Br: C, 40.29; H, 6.09. Found: C, 40.17; H, 6.20.

Ethyl 2-Acetamido-2-cyano-4-methyl-4-hexenoate.—To a solution of 7 g. of sodium dissolved in 250 ml. of ethanol, 50 g. of ethyl acetamidocyanoacetate was added, and then 48 g. of 2-bromomethyl-2-butene, and the reaction mixture was stirred at room temperature for three days. After filtration, the filtrate was reduced to a small volume *in vacuo*, and upon the addition of water to the residue an oil formed which solidified upon standing. This solid was subsequently crystallized from ethanol–water to yield 40.6 g. of product, m.p. 95–96°.

(13) A. Guillemonat, *Compt. rend.*, **200**, 1416 (1935), reported a b.p. of 136–138°, $n^{24}D$ 1.441, for this compound prepared through a different procedure.

Anal. Calcd. for $C_{12}H_{18}N_2O_3$: C, 60.48; H, 7.61; N, 11.76. Found: C, 60.49; H, 7.49; N, 11.86.

2-Acetamido-4-methyl-4-hexenoic Acid.—A mixture of 31 g. of ethyl 2-acetamido-2-cyano-4-methyl-4-hexenoate and 100 ml. of 10% sodium hydroxide was heated to reflux in a stainless steel beaker for two days. The resulting reaction mixture was treated with Darco G-60, filtered, and the filtrate was acidified with concentrated hydrochloric acid to yield a precipitate. The solid finally was crystallized from water to yield 6 g. of product, m.p. 112–113°.

Anal. Calcd. for $C_9H_{15}NO_3$: C, 58.36; H, 8.16; N, 7.56. Found: C, 58.44; H, 8.40; N, 7.81.

2-Amino-4-methyl-4-hexenoic Acid.—A mixture of 7 g. of ethyl 2-acetamido-2-cyano-4-methyl-4-hexenoate and 100 ml. of 20% sodium hydroxide was heated to reflux in a stainless steel beaker for 92 hours. The reaction mixture was neutralized with hydrochloric acid, treated with Darco G-60, and finally evaporated to dryness *in vacuo*. The solid residue was extracted continuously with ethanol for 72 hours using a Soxhlet extractor and, upon cooling, the alcohol extract yielded some solid material which was discarded. Addition of ether to the alcohol solution precipitated 2.6 g. of solid which was taken into water, the inorganic salts were removed with acetone, and the filtrate was evaporated to dryness *in vacuo*. The resulting residue was dissolved in 10 ml. of water and placed on a column containing an intimate mixture of 100 g. of Darco G-60 and 100 g. of Celite. The column was subsequently washed with 800 ml. of water, after which the ninhydrin active material was eluted with 50% ethyl alcohol. The alcohol eluate was evaporated to dryness to yield a yellow-colored solid which was crystallized from ethanol–acetone–water to yield 150 mg. of product, m.p. 219° dec.

Anal. Calcd. for $C_7H_{13}NO_2$: C, 58.72; H, 9.15; N, 9.78. Found: C, 58.39; H, 9.54; N, 9.53.

Hydrogenation of the above compound gave a product which was identical with 2-amino-4-methylhexanoic acid in several paper chromatographic solvent systems.

Lactone of 2-Amino-4-methyl-4-hexenoic Acid.—A mixture of 1.6 g. of ethyl 2-acetamido-2-cyano-4-methyl-4-hexanoate and 10 ml. of concentrated hydrochloric acid was heated to reflux for about 18 hours, after which the reaction mixture was reduced to dryness *in vacuo*. The residue was freed of residual hydrochloric acid by the repeated addition and evaporation of ethyl alcohol. The resulting solid was taken up in ethanol, and the insoluble material was discarded. Upon the addition of ether to the alcohol solution a precipitate formed which was subsequently crystallized from ethanol–ether to yield 160 mg. of material, m.p. 147–48°.

Anal. Calcd. for $C_7H_{13}NO_2 \cdot HCl$: C, 46.77; H, 7.86; N, 7.80. Found: C, 46.52; H, 7.80; N, 8.01.

Using 65% pyridine as the solvent, the R_f of the lactone above was 0.90, whereas that of the corresponding unsaturated amino acid was 0.80.

AUSTIN, TEXAS

[CONTRIBUTION FROM THE DEPARTMENT OF CHEMISTRY, THE UNIVERSITY OF FLORIDA]

Pyrazolines. III. The Stereochemistry of the Decomposition of 2-Pyrazolines[1]

BY W. M. JONES

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The stereochemical consequences of the thermal decomposition of the two isomeric 3,4-dicarbomethoxy-5-phenyl-2-pyrazolines (I and II) were investigated. The major cyclopropane product was found to have a different geometrical configuration from the primary cyclopropane product resulting from the thermal decomposition of 3,5-dicarbomethoxy-4-phenyl-2-pyrazoline (VII), thus vitiating the commonly accepted theory that the geometrical configuration of the cyclopropane product resulting from the decomposition of a 2-pyrazoline is determined by its relative thermodynamic stability. The geometrical configurations of these 2-pyrazolines and a consistent path for their decomposition are discussed.

One of the classic methods employed for the synthesis of cyclopropanes is the decomposition of 1- or 2-pyrazolines.[2] Although the thermal decomposition of 1-pyrazolines has been clearly shown to occur stereospecifically,[3,4] the decomposition of 2-pyrazolines has been reported to give a mixture of the possible stereoisomeric cyclo-

(1) Preceding paper, W. M. Jones, THIS JOURNAL, **81**, 3776 (1959).

(2) For an excellent discussion of pyrazolines, see T. L. Jacobs in R. C. Elderfield, "Heterocyclic Compounds," John Wiley and Sons, Inc., New York, N. Y., Vol. 5, 1957, Chapter 2.

(3) K. von Auwers and F. Konig, *Ann.*, **496**, 27 (1932).

(4) *Ibid.*, **496**, 252 (1932).