# Diastereomers of 2-Amino-3-methylaminobutyric Acid, Aza Analogs of Isoleucine[1]

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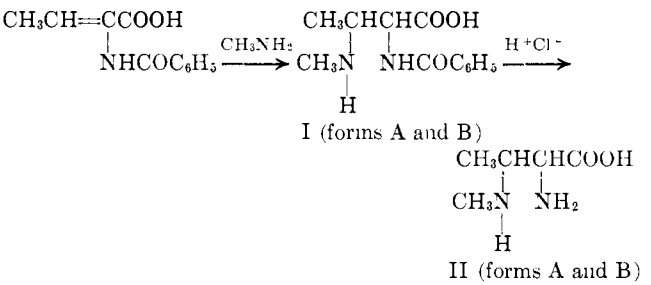
Under the experimental conditions herein described, 2-benzamidocrotonic acid was condensed with methylamine to yield a 3:5 *M* mixture of two diastereomeric racemates of 2-benzamido-3-methylaminobutyric acid. Subjection of this mixture to ethanol extraction resulted in the separation of two different forms, A and B, although the homogeneity of the respective forms was not established. The two forms thus obtained were separately hydrolyzed with HCl to give the respective diastereomers of 2-amino-3-methylaminobutyric acid (4-azaisoleucine), while maintaining the same designations, forms A and B. Form B of 4-azaisoleucine, although not a highly effective antagonist, is inhibitory toward *Escherichia coli* 9723 at a concentration level of about 0.4 mg/ml, and the inhibition is competitively reversed by isoleucine. In contrast, form A even at a concentration level of 2 mg/ml does not exert any appprecinble inhibitory effect on the growth of the same test organism.

Previous investigations have demonstrated that the introduction of a nitrogen atom in place of carbon at the 4 position of an aliphatic amino acid was successful in producing aza analogs with biologically antagonistic properties. For example, 2-amino-3-dimethylaminopropionic acid (4-azaleucine) inhibits the growth of *Escherichia coli* 9723 and *Leuconostoc dextranicum* 8086, and its toxicity is reversed specifically and competitively by leucine.[2] 2-Amino-3-(2-aminoethylamino)propionic acid (4-azalysine) has been found to be a specific and competitive antagonist of lysine in *E. coli* and several lactobacilli.[3]

In the present investigation 2-amino-3-methylaminobutyric acid, the 4-aza analog of isoleucine, was of particular interest since this type of structural variation has produced effective amino acid antagonists, and a comparably substituted 4-thia analog of isoleucine, 2-amino-3-methylthiobutyric acid, was reported recently to be a potent inhibitory analog of isoleucine.[4] Accordingly, a procedure was found whereby the two diastereomeric forms of 2-amino-3-methylaminobutyric acid were prepared, and their physical and microbiological properties were determined as subsequently described. Although not a highly effective antagonist, one of the diastereomers of 4-azaisoleucine was found to antagonize the utilization of isoleucine for the growth of *E. coli* 9723 in a competitive manner.

It was preferable to employ a synthetic procedure whereby the corresponding N-benzoyl derivative of the desired amino acid was prepared directly as the key intermediate, since it has previously been shown[5-10] that benzoylation of a mixture of two diastereomeric forms of an amino acid followed by fractional recrystallization of the N-benzoyl derivatives based on their differential solubilities in alcohol provides one of the most useful methods of separating diastereomers. Thus, the condensation of 2-benzamidocrotonic acid with an excess of 40% aqueous methylamine yielded approximately a 3:5 *M* mixture of the two diastereomeric racemates of 2-benzamido-3-methylaminobutyric acid (I).




I (forms A and B)




II (forms A and B)

At this stage, an effective separation was accomplished by subjecting the mixture of I to an ethanol extraction as described in the Experimental Section. The two diastereomeric forms were purified by recrystallization to constant melting point and infrared spectrum; however, the homogeneity of the respective forms was not established. Diastereomer designations of amino acids and their derivatives based on differences in melting point, solubility, and infrared spectral behavior have been previously reported.[11]

The two diastereomeric forms (A and B) of I were separately hydrolyzed in the presence of dilute HCl to yield the respective diastereomers of 2-amino-3-methylaminobutyric acid (II), while maintaining the same designations, form A and B. Both forms of the diastereomers were isolated and purified as their dihydrochloride acid salts.

The microbiological properties of the two diastereomeric forms of 2-amino-3-methylaminobutyric acid were determined using *E. coli* 9723 as the test organism. The form B aza analog was observed to inhibit the growth of *E. coli* at a concentration level of 0.4 mg/ml, which is a much higher concentration level than that previously reported for the corresponding 4-aza analogs of leucine[2] and lysine[3] under comparable assay conditions. The growth inhibitory effect of 2-amino-3-methylaminobutyric acid (form B) toward *E. coli* was reversed competitively by isoleucine giving an inhibition index (ratio of the concentrations of analog

(1) This investigation was supported in part by Public Health Service Research Grant No. AM-4040-06, from the National Institute of Arthritis and Metabolic Diseases, and by an undergraduate research participation grant (GY-217) from the National Science Foundation.

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to isoleucine just necessary for inhibition of growth of about 300, as shown in Table I. In contrast, form A of 4-azaisoleucine, even at a concentration level of 2.0 mg/ml, was not inhibitory toward the growth of *E. coli*.

Table I

Reversal of 4-Aza-dl-isoleucine (Form B) by Isoleucine for *Escherichia coli* 9723[a]

| dl-Isoleucine, μg/ml | 4-Aza-dl-isoleucine MIC,[b] μg/ml |
|---|---|
| 0 | 0.4 |
| 0.6 | 2.0 |
| 2.0 | 6.0 |
| 6.0 | 20.0 |

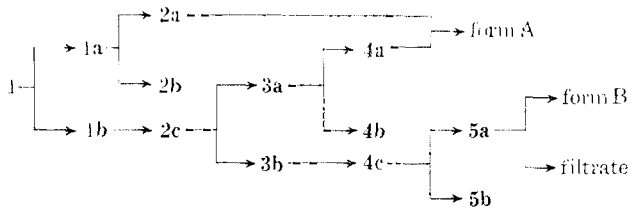
[a] Incubated at 37° for 15 hr. [b] Minimum concentration for complete inhibition of growth.

This study demonstrates that substitution of a nitrogen atom in place of the carbon atom at the 4 position of isoleucine has been successful in producing a moderately effective antagonist of isoleucine. The difference in microbiological results of the two diastereomers with *E. coli* suggests a stereochemical specificity for the antagonism of isoleucine.

## Experimental Section[12]

**Organic Syntheses. 2-Benzamido-3-methylaminobutyric Acids.**—A solution of 8 g of 2-benzamidocrotonic acid in 80 ml of 40% methylamine was allowed to stand at 40° for 12 days. The reaction mixture was taken to dryness *in vacuo*, then ethanol was added and evaporated repeatedly to remove excess amine. The residual material containing the two diastereomeric forms (A and B) were separated by the use of the fractional recrystallization shown in Scheme I.

Scheme I




a = solids, b = extracts or filtrates, c = residues

**Form A.**—The diastereomeric mixture (**1**; 19.2 g) isolated above was extracted with 100 ml of boiling absolute ethanol. The insoluble material (**1a**) (5.0 g) was removed by filtration and washed with ether. Recrystallization was accomplished by heating a suspension of the solid in absolute ethanol to boiling and then adding a minimum amount of water to the boiling suspension until solution was complete. After chilling in a refrigerator overnight, there was obtained 2.2 g of crystalline solid (**2a**), mp 200–201°. Further recrystallizations of this material from aqueous ethanol failed to change the melting point.

*Anal.* Calcd for $C_{12}H_{16}N_2O_3 \cdot 0.5H_2O$: C, 58.77; H, 6.98; N, 11.42. Found: C, 59.09; H, 7.37; N, 11.54.

The ethanolic extract (**2b**) and ether washings were combined and taken to dryness by removal of the solvents under reduced pressure. The residue (**2c**) was extracted with 50 ml of boiling absolute ethanol, and 2.1 g of insoluble material (**3a**), mp 200–201°, was collected on a filter and washed with ether. Recrystallization of **3a** from aqueous ethanol yielded 1.5 g of **4a**, mp 201–202°. A mixture (1:1) of **4a** and **2a** obtained above failed to give any melting point depression. The two solids were combined to give a total of 3.7 g (40%) of form A.

**Form B.** The more soluble B form was recovered by evaporating the alcoholic extract (**3b**, from residue **2c**) to dryness *in vacuo* to yield 3.0 g of residue (**4c**). The residual material was suspended in 100 ml of boiling ethyl acetate, and then absolute ethanol was added dropwise to effect solution. After chilling in a refrigerator overnight, the white solid was removed by filtration, washed with ethyl acetate, and dried (vacuum, $CaCl_2$). There was recovered 2.0 g of solid (**5a**), mp 174–176°, which was recrystallized from ethanol–ethyl acetate to yield 1.75 g (19%) of form B, mp 176–178°. Further recrystallization of the product failed to alter the melting point; however, mp 180–183° was observed for a mixture of forms A and B (1:1).

*Anal.* Calcd for $C_{12}H_{16}N_2O_3$: C, 61.00; H, 6.82; N, 11.85. Found: C, 61.03; H, 6.99; N, 11.96.

The yield of both diastereomers was about 5.45 g for a total recovery of 59%. The infrared absorption spectra of these diastereomeric amino acid derivatives are quite distinct. The 3.05-μ strong band present in the spectrum of form A appears as a shoulder on the strong band at 3.35 μ of form B. From 4 to 7 μ, the spectra of the two compounds are practically identical, whereas the remainder of the spectra, from 7 to 16 μ, is quite dissimilar.

**4-Azaisoleucine (2-Amino-3-methylaminobutyric Acid) Dihydrochloride. Form A.**—A 1.0-g sample of 2-benzamido-3-methylaminobutyric acid (form A) was added to 52 ml of 3 *N* HCl, and the mixture was heated to reflux for 12 hr. The reaction mixture was cooled and extracted (twice with 25 ml of ether to remove the benzoic acid. The aqueous layer was taken to dryness *in vacuo*, and ethanol was repeatedly added and distilled to remove excess HCl. The residue was then dissolved in 20 ml of absolute ethanol, and to the resulting solution was added an equal volume of ethyl acetate until a slight turbidity persisted. After standing in the refrigerator for 4 hr, the solid material was separated by centrifugation, washed with ethyl acetate, and dried (under vacuum, $P_2O_5$). There was obtained 0.5 g (57%) of crude hygroscopic product, mp 179–181° dec. $R_f$ in 4-butanol–acetic acid–water (3:1:1), 65% pyridine, and 95% methanol was 0.13, 0.54, and 0.47, respectively. For elemental analyses, a sample was carefully dried ($P_2O_5$, under vacuum) to constant weight and carefully transferred in a dry atmosphere.

*Anal.* Calcd for $C_5H_{12}N_2O_2 \cdot 2HCl$: C, 29.28; H, 6.88; N, 13.65. Found: C, 29.66; H, 7.11; N, 13.63.

**Form B.** A suspension of 0.68 g of 2-benzamido-3-methylaminobutyric acid (form B) in 36 ml of 3 *N* HCl was heated to reflux for 8 hr. After cooling and extracting the reaction mixture twice with 25-ml portions of ether, the aqueous phase was taken to dryness *in vacuo*. Excess HCl was removed from the residue by repeated addition and evaporation of ethanol. The residual solid was leached with 50 ml of absolute ethanol and filtered to yield 0.35 g (59%) of product, mp 187–189° dec. A 1:1 mixture of forms A and B melted at 180–185°. $R_f$ values of form B in 4-butanol–acetic acid–water (3:1:1), 65% pyridine, and 95% methanol were 0.14, 0.58, and 0.46, respectively.

*Anal.* Calcd for $C_5H_{12}N_2O_2 \cdot 2HCl$: C, 29.28; H, 6.88; N, 13.65. Found: C, 29.10; H, 6.82; N, 13.55.

The two 4-aza analogs of isoleucine thus obtained gave the typical purple ninhydrin color and decomposed at their melting points. The paper chromatographic behavior of the two diastereomers appears to be essentially identical in several solvent systems, but they are distinguishable by an appreciable difference in melting point behavior, hygroscopic character, solubility behavior in absolute ethanol, and infrared spectra data. In the 2–7.5-μ region, form B posses more bands than does its A diastereomer, and this difference holds for the spectra beyond 8 μ as well. Further, the microbiological properties of the two analogs are different as subsequently indicated.

**Microbiological Assays.** For *Escherichia coli* 9723, a previously described inorganic salts medium[13] was employed, and the organism was incubated at 37° for about 16 hr. The amino

(12) All melting points are uncorrected. The paper chromatograms were determined by the ascending technique using the solvents indicated, and the spots were developed with ninhydrin reagent. The infrared spectra were determined on a Beckman Instruments, Inc., Model IR-8 spectrophotometer in KBr pellets and at a concentration of 0.5%. The elemental analyses were determined by Messrs. D. B. Howell, G. D. Smith, and D. L. Thaw of this laboratory and the International Chemical and Nuclear Corporation City of Industry, Calif. The authors are indebted to Mrs. D. Howell and Mr. G. Chase who assisted in the microbiological assay and spectrophotometric work, respectively.

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acid analogs were dissolved in sterile water and added aseptically to the previously autoclaved assay tubes. In all assays the amount of growth was determined photometrically at 625 mμ with a Bausch and Lomb Spectronic 20 spectrophotometer, in terms of absorbance readings of the turbid culture medium against a blank of uninoculated medium set a zero absorbance. For *E. coli* the data in Table I are recorded as absorbance readings which are related to the milligrams of dry cells calculated from a standard curve of milligrams of dry cells per milliliter *vs.* absorbance readings.

# Lincomycin. VI. 4′-Alkyl Analogs of Lincomycin. Relationship between Structure and Antibacterial Activity[1]

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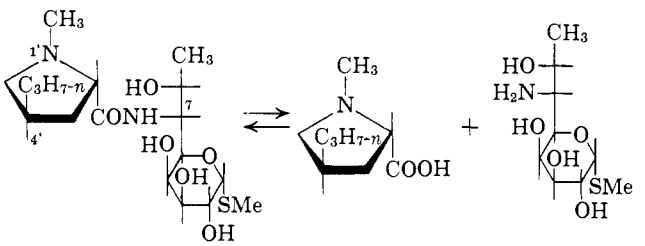
The partial synthesis of a series of 4′-alkyl analogs of lincomycin and 1′-demethyl-1′-ethyllincomycin is reported. The *in vitro* antibacterial activity of some of these compounds was three to four times that of lincomycin. Replacement of the 7-hydroxyl group of these compounds by chlorine further enhanced the antibacterial activity. Relationships are drawn between structure and *in vitro* and *in vivo* antibacterial activity.

Lincomycin, a water-soluble antibiotic,[2] is orally effective in man for the treatment of diseases caused by gram-positive organisms.[3] The elucidation of the structure of lincomycin (**1**) showed that it was not chemically related to any of the major antibiotics.[4]

Lincomycin may be cleaved into an amino acid fragment, *trans*-1-methyl-4-*n*-propyl-L-proline (**2**) and an amino sugar, methyl thiolincosaminide (**3**). These fragments may be recombined to yield lincomycin by employing one of the standard methods for amide formation.[5] The unique chemical stability of lincomycin

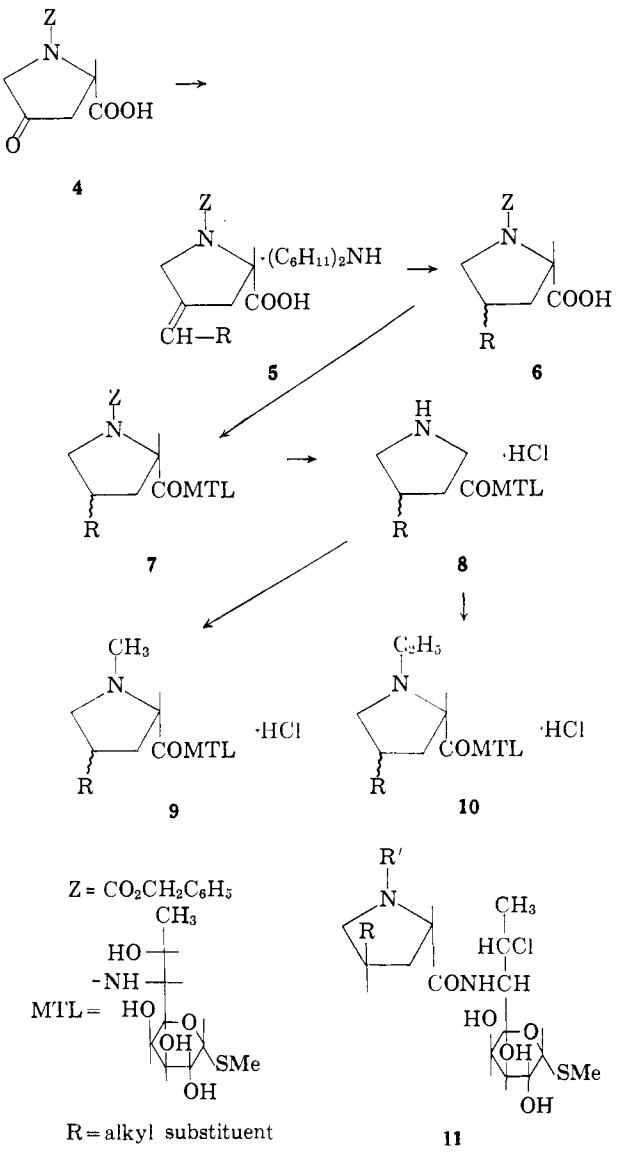


**1** **2** **3**

and the cleavage–recombination sequence established the antibiotic as a highly desirable substrate in which to study the effect of chemical modification on antibacterial activity. The synthesis and antibacterial properties of lincomycin analogs having various alkyl groups at N-1′ and C-4′, and in some cases having the 7-hydroxyl replaced by chlorine, are now described.

The method for preparation of 4′-alkyl analogs of lincomycin was a modification of the previously described partial synthesis of lincomycin.[6] The synthetic scheme is outlined in Chart I.

CHART I




**4** **5** **6** **7** **8** **9** **10** **11**

1-Carbobenz-

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