dried over magnesium sulfate in the refrigerator, the solvent evaporated, and the residue dried thoroughly over phosphorus pentoxide at 1–2 mm.[30] For further purification the crude sulfinic acid was dissolved in ether and extracted with 5% sodium bicarbonate solution. The basic solution was treated with charcoal, acidified, and the precipitated acid either filtered or removed by ether extraction.

The ferric sulfinates of the acids also were employed for isolation and purification of the products. An excess of 3% ferric chloride solution was added to an aqueous solution of the sodium salt of the acid and the pH adjusted to 1 using hydrochloric acid.[16] The ferric sulfinate was collected by vacuum filtration, washed repeatedly with acid solution followed by water, and dried. The original acid could be recovered by treating the ferric sulfinate with dilute ammonium hydroxide and filtering the ferric hydroxide precipitate. Yields of unstable acids could be determined by the quantitative formation of the ferric sulfinate.[16]

Chloromercurinaphthylphenylmethanes were prepared conveniently from the sulfinic acids.[3b] 8-Benzyl-1-naphthalenesulfinic acid formed a chloromercuri compound only when heated for 2 hr. at 70–80°.

1-Chloromercuri-2-naphthylphenylmethane.—*N*-Bromosuccinimide treatment[31] of 1-bromo-2-methylnaphthalene formed 1-bromo-2-bromomethylnaphthalene (m.p. 103.5–106.5°, lit.[31] m.p. 103.5–105.5°) in 60% of the theoretical quantity. 1-Bromo-2-naphthaldehyde (m.p. 117.5–119.5°, lit.[32] m.p. 119–120°) was prepared[32] from this dibromide in 22% yield. Following the procedure of Evans,[33] 1-bromo-2-naphthaldehyde was treated with phenylmagnesium bromide and the resulting carbinol directly reduced with phosphorus and iodine to 2-benzyl-1-bromonaphthalene (b.p. 200–204° at 1.0 mm., m.p. 40.5–42.0°, lit.[33] m.p. 39–40°) in 39% yield. Mercuric chloride and the Grignard from 2-benzyl-1-bromonaphthalene provided 39% yield of 1-chloromercuri-2-naphthylphenylmethane, m.p. 147.0–148.5°. Mixture melting point of this compound and the chloromercuri derivative of 2-benzyl-1-naphthalene sulfinic acid was not depressed and the infrared spectra of the two compounds were identical.

Acknowledgment.—The authors are indebted to Phillips Petroleum Company and the National Cancer Institute, National Institutes of Health (grant CY-4536), for financial support of this project.

(30) Some of the sulfinic acid products were especially labile to heat and air; they slowly decomposed during the isolation and drying process forming base-insoluble residue.

(31) M. S. Newman and A. S. Kosak, *J. Org. Chem.*, **14**, 375 (1949).

(32) D. M. Hall and E. E. Turner, *J. Chem. Soc.*, 1242 (1955).

(33) E. A. Evans, *ibid.*, 2790 (1957).

The Synthesis and Resolution of Methylleucines

JOHN C. SHEEHAN AND M. GERTRUDE HOWELL[1]

Department of Chemistry, Massachusetts Institute of Technology, Cambridge 39, Massachusetts

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β-Methylleucine and N,β-dimethylleucine were synthesized from 2,3-dimethylbutyraldehyde by a Strecker procedure. Separation of the diastereomeric N,β-dimethylleucines was accomplished by fractional crystallization. The N-acetyl-β-methylleucines were separated and one racemate was resolved by acylase. In addition to 2,3-dimethylbutyraldehyde, 2,3-dimethylbutyraldol and a dimeric ether were produced by the rearrangement of 2,3-dimethyl-1,2-butanediol.

During the elucidation of the structure of the peptide antibiotic Etamycin, N,β-dimethylleucine (I) was discovered.[2] This new amino acid is of biogenetic interest since the corresponding aldehyde, derived from hypochlorite degradation of the amino acid, is structurally and stereochemically identical to the aldehyde obtained from the ozonization of the ergosterol side chain.[2]

In this communication, the synthesis of the β-methylleucines (II),[3] the separation of the diastereomeric N-acetyl derivatives and the resolution of one isomer are reported. Also, the formation and separation of the diastereomeric N,β-dimethylleucines are described. The β-methylleucines represent promising intermediates for the synthesis of the four stereoisomeric N,β-dimethylleucines.

β-Methylleucine was synthesized in 66% over-all yield from 2,3-dimethylbutyraldehyde (III) by a modified Strecker procedure. The amino acid mixture was precipitated fractionally from water–acetone to remove the excess ammonium chloride. Although no attempt was made to separate the diasteroisomers, it is probable that some fractionation was achieved as indicated by changes in crystalline form, in the infrared spectra, and in the solubility of the various fractions.

Since the diastereomeric amino acids were not separated quantitatively, it was not established whether the isomers were formed in relatively equal quantities. However, treatment of the acetyl derivatives with excess acetic anhydride would be expected to equalize any unfavorable ratio by racemization at the α-center of the intermediate azlactone.[4] This facile isomerization and the amenability of the acetylamino acids to resolution by acylase indicates the advantages of approaching the N,β-dimethylleucines from the β-methylleucines.

N-Acetyl-β-methylleucine (IV) was obtained in 92% yield by the reaction of excess acetic anhydride with II. One diastereoisomer (IV–isomer I) was purified by fractional recrystallization[5] from water and from acetone. Purification of the second isomer (IV–isomer II) was accomplished by repeated precipitation from acetone–benzene or by very slow deposition from acetone.[6]

(1) Aided by a contract from the Office of Naval Research, Biochemistry Branch.

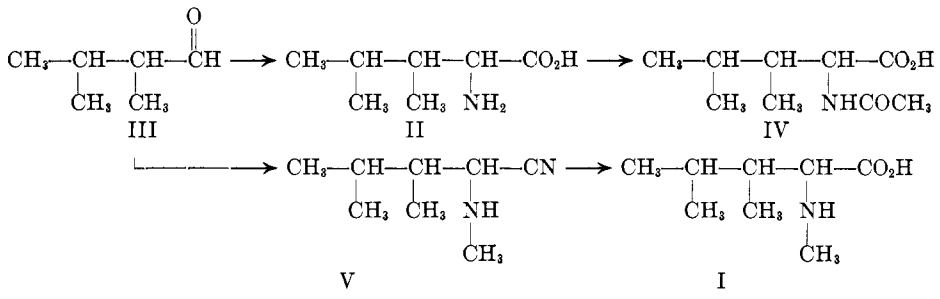
(2) J. C. Sheehan, H. G. Zachau, and W. B. Lawson, *J. Am. Chem. Soc.*, **80**, 3349 (1958).

(3) The synthesis of the ureido derivative of this amino acid by an alternate route has been reported by P. E. Gagnon, P. A. Boivin, and H. M. Craig, *Can. J. Chem.*, **29**, 70 (1951).

(4) J. P. Greenstein, S. M. Birnbaum, and L. Levintow, *Biochem. Prep.*, **3**, 84 (1953).

(5) Unless the amino acids are in a high state of purity the acylated derivatives are difficult to crystallize.

(6) The solvents employed were identical to those used for the separation of the acetyl isoleucines reported by W. A. H. Huffman and A. W. Ingersoll, *J. Am. Chem. Soc.*, **73**, 3366 (1951). Greenstein, Birnbaum, and Levintow[4] have described the preferential precipitation of N-acetylalloisoleucine and the subsequent purification of the acylamino acid by recrystallization from acetic acid–water. This latter procedure was not applicable to the N-acetyl-β-methylleucines since the isopropyl group confers increased solubility. Once crystallization was induced, the entire acetylation product solidified and no preferential deposition was obtained.



Infrared spectra (potassium bromide) of the two isomeric N-acetyl-β-methylleucines are surprisingly different, affording a precise means of following the separation. The major absorption bands for isomer I occur at 1705, 1620, 1560, and 1260 cm.$^{-1}$. The corresponding values for isomer II are 1725, 1640, 1550, and 1235 cm.$^{-1}$. In addition, there are major differences in the regions of 1425–1475 cm.$^{-1}$, 1300–1350 cm.$^{-1}$, 1150–1175 cm.$^{-1}$, and 950–1050 cm.$^{-1}$. A single band at 1715 cm.$^{-1}$ is found in the spectrum of a nearly equal mixture of isomers. As the separation progressed, this peak was resolved and the presence of the contaminating isomer was indicated by a shoulder at the frequency at which the pure compound absorbs. Of more value were the changes in the fingerprint region which accompanied the purification. The melting points of the pure isomers differed by 13° but gave only a general indication of the extent of purity of the two derivatives.

The treatment with acylase of IV–isomer I afforded 68% of pure amino acid, $[\alpha]^{25}D$ +38°. The rotation of the naturally occurring N,β-dimethylleucine was +39° and it is known that methylation of the amino group does not change significantly the rotation of the amino acid.[2] By analogy to the isoleucines,[5] it would be expected that the isomeric β-methylleucine would possess a rotation similar in both magnitude and sign to that exhibited by the diastereoisomer. Thus, no configurational assignment of the naturally occurring I can be made on the basis of rotational data. The N-acetyl-β-methylleucine recovered from the enzymatic resolution had a very low rotation, $[\alpha]^{25}D$ −1°. However, it seems probable that this material was at least partially resolved since the melting point of the recovered compound was raised by 18° from that of the pure starting material.

It has been observed that a reverse solubility relationship exists between the diastereomeric isoleucines and the N-acetyl derivatives.[6] It seems probable that a similar relationship exists for the β-methylleucines. The infrared spectrum of the amino acid obtained by acylase cleavage of IV–isomer I is very similar to that of the more soluble fractions of II.

The synthesis of N,β-dimethylleucine also was achieved by a Strecker procedure. When two molecular proportions of methylamine were employed to suppress iminobisnitrile formation,[7] α-methylamino-β-methylleucylnitrile (V) was isolated in 78% yield. α-Methylamino-β-methylleucylnitrile was hydrolyzed by the procedure developed by Cook and Cox for the conversion of alkylamino nitriles to the amino acids.[7] The infrared spectrum of the product isolated after five hours of heating with 40% sulfuric acid indicated a mixture of I and the amino amide (VI). After the hydrolysis was continued for an additional four hours, 62% of N,β-dimethylleucine and 14% of N,β-dimethylleucylamide were isolated. The resistance of the amide to hydrolysis finds precedent in the report that forty hours of treatment with hot sulfuric acid is required for the complete hydrolysis of N-methylvaleronitrile.[7]

A crude separation of the diastereomeric N,β-dimethylleucines was accomplished by fractional recrystallization from water. The less soluble isomer, arbitrarily designated isomer I, was purified by repeated crystallization from water–acetone, water–methanol, and water followed by chromatography and sublimation. The more soluble amino acid, isomer II, obtained in smaller quantities from the synthesis, was recrystallized from methanol–acetone, ethanol–acetone, and methanol. Chromatography on Dowex 50 and sublimation did not enhance the purity of the amino acids.

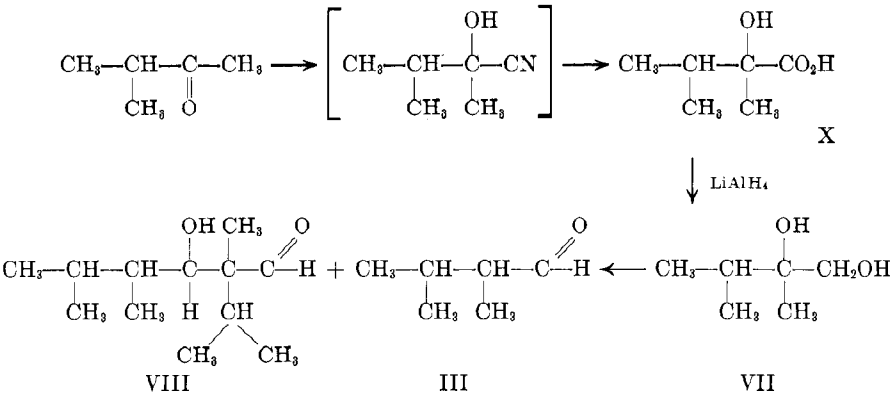
The separation of the isomeric amino acids was followed by the changes which appeared in the infrared spectra. Although the spectra of the two pure substances are not so dissimilar as those of the N-acetyl-β-methylleucines, the differences are sufficient to provide a convenient means of determining the purity of the materials. The shape and intensity of the bands in the region of 1375–1450 cm.$^{-1}$ are significantly different. Isomer I has a single absorption peak at 1325 cm.$^{-1}$, while a doublet at 1345 cm.$^{-1}$ and 1315-cm.$^{-1}$ appears in the spectrum of isomer II. The resolution of this doublet occurs during the separation and, in the spectrum of the pure material, the band at the lower frequency is slightly more intense than the 1345-cm.$^{-1}$ peak. Intensity reversals are noted in the region of 1100–1175 cm.$^{-1}$ and a band is present at 700 cm.$^{-1}$ in the spectrum of isomer I, which is absent in that of the other amino acid.

2,3-Dimethylbutyraldehyde (III)[8] was synthesized by the acid-catalyzed rearrangement of 2,3-dimethyl-1,2-butanediol (VII).[8d] In addition to III, 2,3-dimethylbutyraldol (VIII) was invariably a product of the reaction. Addition of 2,4-dinitrophenylhydrazine reagent[9] to the aldol unexpectedly afforded the corresponding derivative of III. An initial product of the acid-catalyzed rearrangement of VII was found to be a dimeric ether for which either structure IXA or IXB is

(7) A. H. Cook and S. F. Cox, *J. Chem. Soc.*, 2342 (1949).

(8) (a) H. Brunner and E. H. Farmer, *ibid.*, 1039 (1937); (b) F. C. Whitmore and P. L. Meunier, *J. Am. Chem. Soc.*, **63**, 2199 (1941); (c) R. A. Barnes and W. M. Budde, *ibid.*, **68**, 2339 (1946); (d) M. B. Green and W. J. Hickinbottom, *J. Chem. Soc.*, 3262 (1957).

(9) R. L. Shriner and R. C. Fuson, "The Systematic Identification of Organic Compounds," John Wiley and Sons, Inc., New York, N. Y., 1948, p. 171.



suggested.[10] Lithium aluminum hydride reduction of 2,3-dimethyl-2-hydroxybutyric acid (X)[11] afforded VII. The cyanohydrin of methyl isopropyl ketone was hydrolyzed to produce X.

IXA IXB

Experimental[12]

2,3-Dimethyl-2-hydroxybutyric Acid (X).—The cyanohydrin of methyl isopropyl ketone was prepared by the general method of Green and Hickinbottom.[8d] To the chilled cyanohydrin [obtained from 258 g. (3 moles) of methyl isopropyl ketone, 165 g. (3.2 moles) of sodium cyanide, and 380 g. (2 moles) of sodium metabisulfite] was added 350 ml. of cold, concentrated hydrochloric acid. Anhydrous hydrogen chloride was passed into the solution for 6 hr. and the mixture was stored for 8 hr. at 25°. The solution was refluxed for 8 hr. after 200 ml. of water had been added. The solid, which crystallized upon cooling, was separated by filtration and digested with hexane. Continuous ether extraction of the aqueous filtrate for two 24-hr. intervals, followed by drying and concentration of the combined hexane and ether solutions, afforded 244.5 g. (62%) of 2,3-dimethyl-2-hydroxybutyric acid, m.p. 73.5–75°. The melting point was raised to 75.5–76.5° after recrystallization from hexane (lit.[11] m.p. 75–77°).

Interruption of the hydrolysis after 45 min. gave, in addition to X (79 g., 20%), 169 g. (43%) of 2,3-dimethyl-2-hydroxybutyramide. The amide was recrystallized from acetone–water and dried under reduced pressure, m.p. 85–86° (lit.[13] m.p. 84–85°).

2,3-Dimethyl-2-hydroxybutyramide (47.6 g., 0.364 mole) was suspended in 250 ml. of 20% sodium hydroxide. Solution was complete as reflux temperature was reached but heating was continued for 8 hr. The cooled mixture was acidified cautiously and extracted continuously with ether for 24 hr. After drying and removal of the solvent, 44 g. (93%) of X crystallized, m.p. 73–75°.

2,3-Dimethyl-1,2-butanediol (VII).—A suspension of 36.4 g. (0.94 mole) of lithium aluminum hydride in 700 ml. of anhydrous ether was heated at reflux temperature under a nitrogen atmosphere for 30 min. and then cooled to 25°. To the efficiently stirred slurry, 65 g. (0.5 mole) of 2,3-dimethyl-2-hydroxybutyric acid in 200 ml. of anhydrous ether was added dropwise followed by 150 ml. of anhydrous ether. After 48 hr. of heating, the mixture was cooled to 25° and 200 ml. of water cautiously introduced. The gelatinous precipitate was separated by filtration on a bed of Filter-Cel and washed copiously with ether and water. The combined filtrates were continuously extracted with ether, and the extract dried and concentrated. Distillation of the residue through a 6-in. Vigreux column afforded 53.3 g. (92%) of VII, b.p. 85° (15 mm.), $n^{25}D$ 1.4474 [lit.[8d] b.p. 91–93° (25 mm.)].

2,3-Dimethylbutyraldehyde (III).—The general method developed by Green and Hickinbottom[8d] for the rearrangement of 1,2-glycols and allylic alcohols was employed. The product, obtained from the interaction of 20.3 g. (0.17 mole) of VII and 965 ml. of 0.4 *N* sulfuric acid, was distilled through a semimicro column to yield 12 g. (70%) of 2,3-dimethylbutyraldehyde, b.p. 110–112°, $n^{25}D$ 1.4005 (lit.[8c] b.p. 112–114°, $n^{25}D$ 1.3998). A higher-boiling residue (2.5 g., 16%) remained and was distilled fractionally. The composition and molecular weight of an intermediate cut, b.p. 59–60.5° (1.5 mm.), $n^{25}D$ 1.4325, were consistent with those of 2,3-dimethylbutyraldol.

Anal. Calcd. for $C_{12}H_{24}O_2$: C, 71.95; H, 12.08; mol. wt., 200. Found: C, 71.95; H, 12.05; mol. wt., 201 (Rast).

Addition of 2,4-dinitrophenylhydrazine reagent[9] to the aldol gave orange platelets, m.p. 115–117°, raised to 123–124° by recrystallization from ethanol–water and ethanol. The molecular weight and composition were in agreement with those of 2,3-dimethylbutyraldehyde 2,4-dinitrophenylhydrazone.

Anal. Calcd. for $C_{12}H_{16}N_4O_4$: C, 51.42; H, 5.75; N, 19.99; mol. wt., 280. Found: C, 51.19; H, 5.87; N, 20.05; mol. wt., 267 (Rast).

A mixture melting point with an authentic sample of 2,3-dimethylbutyraldehyde 2,4-dinitrophenylhydrazone[9] (m.p. 123–123.5°) showed no depression.

After heating at 100° for 45 min., the cloudy suspension formed from the interaction of 15.9 g. (0.134 mole) of 2,3-dimethyl-1,2-butanediol and 750 ml. of 0.4 *N* sulfuric acid was cooled to 25°, saturated with sodium chloride, and extracted with three 50-ml. portions of pentane. After drying and removal of the solvent, the residue was distilled through a semimicro column to afford 8.2 g. (62%) of product, b.p. 72–74° (25 mm.). The distillate was purified by chromatography and redistilled, b.p. 75° (25 mm.), $n^{25}D$ 1.4372. The infrared spectrum of the product (pure liquid) shows no absorption in the carbonyl and hydroxyl stretching regions but has strong absorption attributed to C–O stretching at 1090 $cm.^{-1}$. The molecular weight and composition of the pure material were consistent with that of a dimeric ether.

Anal. Calcd. for $C_{12}H_{24}O_2$: C, 71.95; H, 12.08; mol. wt., 200. Found: C, 72.21; H, 12.03; mol. wt., 203 (Rast).

The dimeric ether, upon heating with 0.4 *N* sulfuric acid, afforded a mixture (31%) of 2,3-dimethylbutyraldehyde and the aldol.

β-Methylleucine (II).—A solution of 9.7 g. (0.182 mole) of ammonium chloride in 24 ml. of water (40°) was added to 8.1 g. (0.165 mole) of sodium cyanide in 17 ml. of water. Ammonium hydroxide (11 ml., 0.182 mole) was added and the solution was stirred and cooled while 2,3-dimethylbutyraldehyde (16.5 g., 0.165 mole) in 24 ml. of methanol was added dropwise. The stoppered flask was immersed for 4.5 hr. in a water bath at 55–60° with occasional shaking. The mixture was cooled to 0.5° (ice–salt bath) and acidified with 200 ml. of chilled, 12 *N* hydrochloric acid. Anhydrous hydrogen chloride was passed into the solution at 0–5° for 5 hr. until saturation. After storage at room temperature for 8 hr., the mixture was diluted with 50 ml. of water and refluxed for 6 hr. The solvent was removed under reduced pressure and the residue redissolved in water and the solution

(10) The epoxidation of 2,4,4-trimethyl-2-pentene and of 2,4,4-trimethyl-1-pentene also produced dimeric ethers and 1,4-dioxane structures were tentatively assigned by A. Byers and W. J. Hickinbottom, *J. Chem. Soc.*, 1328 (1948); W. J. Hickinbottom, *ibid.*, 1331 (1948). Green and Hickinbottom[8d] have reported acetal formation during the rearrangement of 2-methyl-2-propen-1-ol.

(11) W. H. Perkin, *ibid.*, 1486 (1896).

(12) All melting points are corrected and the boiling points are uncorrected. The microanalyses are by Dr. S. M. Nagy and associates at Massachusetts Institute of Technology.

(13) S. L. Shapiro, I. M. Rose, E. Roskin, and L. Freedman, *J. Am. Chem. Soc.*, **81**, 386 (1959).

again was concentrated to dryness. Digestion of the precipitate in 200 ml. of boiling absolute ethanol, followed by filtration, removed the bulk of the inorganic salts which were redigested with an additional 100 ml. of boiling absolute ethanol. The ethanolic filtrates were combined and concentrated to dryness. The residue was dissolved in 100 ml. of 20% ammonium hydroxide and the ammoniacal solution was concentrated to dryness. The residual solid was triturated with a small amount of water and the undissolved portion was collected by filtration, dissolved in boiling water, and treated with Norit. Addition of acetone and cooling gave several crops of crystalline amino acid, 15.8 g. (66%).

N-Acetyl-β-methylleucine (IV).—A suspension of β-methylleucine (1.0 g., 6.9 mmoles) in 10.4 ml. of glacial acetic acid and 1.62 ml. (17.3 mmoles) of acetic anhydride was heated at reflux temperature for 2 min. (drying tube). The clear solution was cooled to room temperature and concentrated under reduced pressure. Water was added and the solution again concentrated to a heavy oil which crystallized. It was advantageous to remove traces of acetic acid by further flushing with water.

Recrystallization from the minimum amount of boiling water afforded as a first crop 130 mg. of needle-shaped crystals, m.p. 163–165°. Two additional crops of needles, 340 mg. and 100 mg., were collected, m.p. 163–165°. Concentration of the mother liquors to dryness left 600 mg. of crystalline material, m.p. 153–158°. The total yield of acetylated amino acid was 92%. The higher-melting fractions were recrystallized twice from acetone and then from water to give one pure diastereomer, arbitrarily designated I, m.p. 171.8–172.2°.

Anal. Calcd. for $C_9H_{17}O_3N$: C, 57.73; H, 9.15; N, 7.48. Found: C, 57.84; H, 9.18; N, 7.53.

The 600-mg. lower-melting residue was crystallized repeatedly from acetone–benzene to give rosettes, m.p. 157–157.5°.

Anal. Calcd. for $C_9H_{17}O_3N$: C, 57.73; H, 9.15; N, 7.48. Found: C, 57.89; H, 9.18; N, 7.39.

Resolution of N-Acetyl-β-methylleucine, Isomer I.—The procedure described by Greenstein, Birnbaum, and Levintow[5] for the resolution of D,L-N-acetylalloisoleucine was employed with modification. Finely divided N-acetyl-β-methylleucine, isomer I, m.p. 171–172° (0.75 g., 4 mmoles), was suspended in 30 ml. of water and 3 *N* lithium hydroxide added dropwise until pH 7.5 was attained. To the solution, 0.055 g. of hog-kidney acylase (General Biochemicals) was introduced with gentle stirring. Incubation of the golden-colored solution at 37° for 20 hr. was followed by acidification to pH 5 with glacial acetic acid. After stirring for 1 hr. with 0.22 g. of Norit, the mixture was filtered through a thin layer of Norit on a no. 1 Whatman filter paper. The filtrate was lyophilized, the residue dissolved in water and adsorbed on Dowex 50W-X8. After copious washing with water, the amino acid (0.198 g., 68%) was eluted with 2 *N* ammonium hydroxide.

The amino acid was suspended in water, warmed, and a small amount of insoluble material removed by filtration. The filtrate was concentrated to dryness, the residue crystallized from ethanol-ether and dried at 77° (0.1 mm.), $[\alpha]^{25}D$ +38° (1.3% in 5 *N* hydrochloric acid). An analytical sample was prepared by recrystallization from ethanol, m.p. 237–240° dec.

Anal. Calcd. for $C_7H_{15}NO_2$: C, 57.90; H, 10.41; N, 9.65. Found: C, 57.69; H, 10.35; N, 9.62.

The N-acetyl-β-methylleucine, 0.128 g. (34%), recovered from the aqueous washings,[14] was crystallized as rosettes from water, m.p. 189.2–190.5°, $[\alpha]^{25}D$ −1° (2.3% in ethanol).

α-Methylamino-β-methylleucylnitrile (V).—The procedure of Cook and Cox[7] for the synthesis of α-methylamino-*n*-valeronitrile was employed with modification. To 12.8 g. (0.128 mole) of chilled (5°) 2,3-dimethylbutyraldehyde under a nitrogen atmosphere was added 11.2 g. (0.064 mole) of sodium metabisulfite in 22 ml. of water. After 30 min. of stirring, 32 ml. (0.256 mole) of methylamine (40% in water) was run rapidly into the heterogeneous medium. Potassium cyanide (8.3 g., 0.128 mole) was introduced after an additional 30 min., and the two-phase system was stirred for 1 hr. The product was extracted with three 50-ml. portions of ether and dried. The solvent was removed through a semimicro column and the residue distilled to give 13.8 g. (78%) of the methylamino nitrile, b.p. 85–87° (4 mm.), $n^{25}D$ 1.4409. An analytical sample was prepared by redistillation, b.p. 44° (0.5 mm.).

Anal. Calcd. for $C_8H_{16}N_2$: C, 68.52; H, 11.50; N, 19.98. Found: C, 68.57; H, 11.69; N, 20.15.

Hydrolysis of α-Methylamino-β-methylleucylnitrile.—α-Methylamino-β-methylleucylnitrile (9.73 g., 0.0695 mole) was added dropwise, with stirring, to 7.6 ml. of chilled (0°) concentrated sulfuric acid. The viscous solution was heated at 100° for 1 hr., cooled to 50°, and 11 g. of ice cautiously introduced. After 5 hr. of refluxing, the solution was chilled and carefully neutralized with 11.04 g. (1 equivalent) of sodium hydroxide in 25 ml. of water. The resultant suspension was lyophilized and triturated with ether. The residue was digested with three 150-ml. portions of boiling anhydrous methanol. Concentration of the combined methanolic solutions to dryness and trituration with ether yielded a solid (8.67 g.) which, on the basis of the infrared spectrum, appeared to be a mixture of amino acid and amino acid amide. After an additional 4 hr. of refluxing in 50 ml. of 10% sulfuric acid followed by neutralization, the suspension was extracted with 50 ml. of pentane and the amide (1.52 g., 14%), m.p. 124–126°, was collected from the aqueous mixture by filtration. Recrystallization from ligroin and from carbon tetrachloride raised the melting point to 128.2–129.2°. An analytical sample was obtained by recrystallization from carbon tetrachloride, m.p. 128.5–129.8°.

Anal. Calcd. for $C_8H_{18}N_2O$: C, 60.72; H, 11.47; N, 17.70. Found: C, 61.00; H, 11.47; N, 17.66.

The aqueous filtrate was lyophilized and the residue digested with three 100-ml. portions of boiling anhydrous methanol. Concentration of the methanolic filtrates afforded 6.08 g. (62%) of crude N-β-dimethylleucine (mixture of diastereoisomers). The total yield of amino amide and amino acid was 76%.

Separation of N-β-Dimethylleucines (I).—A crude separation of diastereoisomers was achieved by fractional crystallization from water–acetone. After initial crystallization, the mother liquors were warmed and acetone added to the cloud point. The process was repeated until the introduction of a large volume of acetone gave no precipitation. The resulting solution was concentrated to dryness to await purification of the more soluble isomer.

The early fractions were composed of one diastereoisomer of good purity (arbitrarily designated I). Recrystallization from water–acetone, water–methanol, and water, followed by adsorption on Dowex 50, elution with 2 *N* ammonium hydroxide and sublimation (170°, 0.1 mm.) gave material which was analytically pure, m.p. 247–249° dec.

Anal. Calcd. for $C_8H_{17}NO_2$: C, 60.34; H, 10.76; N, 8.80. Found: C, 60.46; H, 10.76; N, 8.79.

The separation was followed by inspection of the infrared spectra which were consistently sharp in the latter stages of the purification.

The soluble material (designated isomer II) was purified by fractional recrystallization from methanol–acetone, ethanol–acetone, and methanol, adsorption on Dowex 50 followed by elution with 2 *N* ammonium hydroxide and analytical sublimation (170°, 0.1 mm.), m.p. 239–241° dec.

Anal. Calcd. for $C_8H_{17}NO_2$: C, 60.34; H, 10.76; N, 8.80. Found: C, 60.05; H, 10.65; N, 8.75.

The purification of isomer II was followed also by the sharpening of the infrared spectra.

The intermediate crops were combined and fractionally recrystallized to give more of the respective isomers.

(14) In this experiment, the complete recovery of the acetyl derivative was not attempted.