The occurrence of \( \beta \)-aminoisobutyric acid in human urine was first reported by Crumpler et al. (1) in 1951. They commented that some persons consistently excrete considerable amounts of AIB, and suggested that this may be a genetically determined characteristic. Further studies have confirmed that the characteristic of excreting AIB is inherited as a recessive trait (2). One of the possible precursors for AIB is methylmalonic semialdehyde, an intermediate in valine metabolism (11). Recently, the occurrence of ethylmalonic acid as an intermediate in isoleucine degradation has been reported (12). This raised the possibility that \( \alpha \)-ethyl-\( \beta \)-alanine might be a natural amino acid, arising from transamination of \( \alpha \)-ethylmalonic semialdehyde. \( \alpha \)-Ethyl-\( \beta \)-alanine has been prepared and its behavior in the Beckman-Spinco amino acid analyzer established. No definite evidence for the occurrence of this amino acid has been observed with urine from 57 individuals; these included pathological, intermittent, and familial AIB excretors and nonexcretors.

**EXPERIMENTAL PROCEDURE**

Isolation of \((-\beta\)-AIB—A total of 14.7 liters of urine that contained 9.04 g of creatinine was collected from a Japanese woman. The pooled urine, which contained 1.53 g of AIB, was acidified to pH 5 with glacial acetic acid, filtered, and then divided into nine portions for desalting. Each portion was passed through a column (4 \( \times \) 40 cm) of Dowex 50-X8 (H\(^+\) form, 100 to 200 mesh), the resin was washed with 900 ml of water, and the amino acids were eluted with 1 liter of 2 N \( \text{NH}_3\text{OH} \). The ammoniocolic eluates were concentrated under reduced pressure to a volume of about 100 ml each and were then combined.

The desalted solution of amphotolines was acidified to pH 5, and acidic amino acids were removed by passing the solution through a column (7 \( \times \) 50 cm) of Amberlite CG-45 (acetate form) (100 to 200 mesh) and washing the resin with water. The first 975 ml of the effluent were discarded, and the next 2480 ml were collected and concentrated under reduced pressure to a volume of 800 ml. To remove basic amino acids, the solution was then passed through a column (7 \( \times \) 30 cm) of Dowex 50 (ammonium form) (100 to 200 mesh), and the resin was washed with water. The effluent from 505 to 2965 ml was collected, concentrated to 780 ml under reduced pressure, and divided into three equal portions. Each portion was passed through a column (7 \( \times \) 45 cm) of the pyridine form of Dowex 50 (100 to 200 mesh), the resin was washed with 750 ml of water to remove most of the neutral amino acids, and AIB was eluted from the resin with 1 N pyridine; AIB emerged between 1300 and 2430 ml. The pyridine eluates were combined and concentrated to dryness under reduced pressure, the residue was dissolved in 40 ml of hot water, and the solution was cooled in a refrigerator overnight.
Glycocyamine (1.50 g) crystallized and was collected, and the filtrate was concentrated to an oily residue that weighed 4.43 g. The constituents of this residue were investigated by paper chromatography; it contained approximately 1.5 g of AIB, 0.1 g of phenylalanine and tyrosine, 2.0 g of creatine, and 0.4 g of creatinine.

For further purification, this mixture was dissolved in 50 ml of water, and its components were acetylated by the addition of 2.5 g of acetic anhydride and 2 N NaOH over a period of 30 minutes to the cooled solution, which was maintained at pH 9 to 10. The resulting solution was acidified to pH 5.5 by the addition of glacial acetic acid and the acetylated amino acids were adsorbed on a column (4 x 32 cm) of Amberlite IR-400 (OH form). Creatine and creatinine were removed by washing the resin with 200 ml of water, and acetyl-AIB was eluted with 2 N acetic acid; it was collected in the eluate between 200 and 450 ml. The eluate was concentrated to dryness under reduced pressure, and the residue was suspended in 400 ml of water. The residue was washed with two 25-ml portions of water, and its components were acetylated by the addition of 2.5 g of acetic anhydride and 2 N acetic acid and the mixture was washed with two 25-ml portions of water, and the AIB was eluted with 3 N NaOH. Five hundred ml. of the eluate was concentrated to dryness in a vacuum, and the crystalline residue was recrystallized from 180 ml of 95% ethanol. A total of 10.0 g of n-AIB was obtained; m.p. 174-178°. An additional 0.47 g (total yield, 61%) was obtained from the mother liquor.

For further purification, this mixture was dissolved in 50 ml of water, and the AIB was eluted with 3 N acetic acid; it was collected in the eluate between 200 and 450 ml. The eluate was concentrated to dryness under reduced pressure, and the crystalline residue was recrystallized from 180 ml of hot dichloroethane; yield, 49.2 g, m.p. 96-97°. A second crop of 5.3 g, m.p. 96-97°, was obtained; total yield, 74.3%.

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\[
C_6H_5NO_2
\]
Calculated: N 9.58
Found: N 9.56

The melting point was not altered upon admixture with synthetic acetyl-(-)-AIB.

Of the isolated acetyl-AIB, 0.83 g was hydrolyzed by refluxing it for 3 hours in 20 ml of 2 N HCl; the solution was concentrated to dryness under reduced pressure, and the residue was dissolved in 10 ml of water and applied to a column (2 x 5 cm) of Amberlite IR-120 (H⁺ form). The resin was washed with 50 ml of water, and the amino acid was eluted with 40 ml of 2 N NH₄OH. The eluate was concentrated to dryness and the crystalline residue was crystallized from 20 ml of hot absolute ethanol; 0.31 g was recovered; m.p. 191-194°, not depressed by admixture with synthetic (-)-AIB; \([\alpha]_D^{19} = -15.3° (c, 1, H_2O)\).

\[
C_6H_4NO_2
\]
Calculated: N 13.6
Found: N 13.4

Ethyl-α-cyanopropionate—In 300 ml of absolute ethanol, 90 g (0.5 mole) of ethyl-α-bromopropionate (Matheson, Coleman and Bell) and 25 g (0.57 mole) of sodium cyanide were refluxed for 20 hours. The reaction mixture was cooled, and sodium cyanide and sodium bromide were removed by filtration. The filtrate was concentrated to a small volume, 200 ml of ether were added, the mixture was washed with two 25-ml portions of water, and the ether was removed. The residue from evaporation of the ether was distilled to yield 27.4 g (43% of theory); b.p. 85-97° at 18 mm.

\[\text{DL-AIB} \rightarrow \text{To 21.5 g (0.102 mole) of ethyl-α-cyanopropionate in 300 ml of hot isopropyl alcohol, and the solution was chilled overnight: 20.7 g of crystals were collected; m.p. 181-184°. Recrystallization of this crop from 100 ml of isopropyl alcohol yielded 17.1 g; m.p. 184-187°; [\alpha]_D^{19} = -100.6° (c, 1, H_2O). Another recrystallization from 100 ml of isopropyl alcohol yielded 15.10 g; m.p. 186-188°; [\alpha]_D^{19} = -101.6° (c, 1, H_2O). Subsequent recrystallizations did not lead to any further change in physical properties.}

The cinchonidine salt was dissolved in 50 ml of water, 10 ml of concentrated NH₄OH were added, the resulting mixture was cooled, and the precipitated cinchonidine was filtered and washed with three 20 ml portions of cold water. The filtrate and washings were combined, washed with two 100-ml portions of chloroform, acidified to pH 1 by the addition of concentrated HCl, and saturated with NaCl. Acetyl(-)-AIB was extracted into six 850-ml portions of ethyl acetate and the combined extracts were concentrated to dryness. The residue was recrystallized from 180 ml of hot dichloroethane; yield, 49.2 g, m.p. 96-97°. A second crop of 5.3 g, m.p. 96-97°, was obtained; total yield, 74.3%.

\[
C_6H_5NO_2
\]
Calculated: N 9.58
Found: N 9.56

N-Acetyl(-)-AIB—Cinchonidine (30 g, 0.102 mole) and acetyl-DL-AIB (14.76 g, 0.102 mole) were dissolved in 300 ml of hot isopropyl alcohol, and the solution was chilled overnight: 20.7 g of crystals were collected; m.p. 181-184°. Recrystallization of this crop from 100 ml of isopropyl alcohol yielded 17.1 g; m.p. 184-187°; [\alpha]_D^{19} = -100.6° (c, 1, H_2O). Another recrystallization from 100 ml of isopropyl alcohol yielded 15.10 g; m.p. 186-188°; [\alpha]_D^{19} = -101.6° (c, 1, H_2O). Subsequent recrystallizations did not lead to any further change in physical properties.

The cinchonidine salt was dissolved in 50 ml of water, 10 ml of concentrated NH₄OH were added, the resulting mixture was cooled, and the precipitated cinchonidine was filtered and washed with three 20 ml portions of cold water. The filtrate and washings were combined, washed with two 100-ml portions of chloroform, acidified to pH 1 by the addition of concentrated HCl, and saturated with NaCl. Acetyl(-)-AIB was extracted into six 200-ml portions of ethyl acetate and the combined extracts were concentrated to dryness under reduced pressure. The crystalline residue was recrystallized from 80 ml of dichloroethane to yield 4.14 g of product; m.p. 80-82°; [\alpha]_D^{19} = -29.8° (c, 1, absolute ethanol). Recrystallization did not change these properties.

\[
\text{C}_6\text{H}_5\text{NO}_2
\]
Calculated: N 9.58
Found: N 9.40

(−)-AIB—N-Acetyl(−)-AIB (13.0 g) was refluxed in 200 ml of 2 N HCl for 3 hours and the acid was removed under reduced conditions.
pressure. The crystalline residue was taken up into 20 ml of water and passed through a column (2 × 22 cm) of Amberlite CG-120 (H+ form) (100 to 200 mesh). The resin was washed with 100 ml of water and the AIB was eluted with 3 N NaOH. The first 75 ml of eluate were discarded and the following 50 ml were collected and concentrated to dryness. The crystalline AIB was recrystallized from 70 ml of 95% ethanol to yield 0.55 g of long rectangular plates, m.p. 194°-196°; [α]D +15.4° (c, 1, H2O).

C6H13NO2
Calculated: N 13.6
Found: N 13.3

An additional 1.54 g, m.p. 192°-195°, was obtained from the mother liquor; total yield, 88%.

(+)AIB—The mother liquor from the first crop of the cinchonidine salt of acetyl-(-)-AIB was concentrated to 60 ml and 2.25 g of material, which crystallized when the solution was cooled, were collected and discarded. The filtrate was concentrated to dryness and the oily residue was dissolved in 100 ml of water. Free acetyl-(+)AIB was obtained from the salt as described above; yield, 5.73 g; m.p. 76°-86°; [α]D +24.5° (c, 1, absolute ethanol). Attempts to obtain an optically pure specimen of the salts of acetyl-(+)AIB with brucine, quinine, quinidine, and cinchonine were unsuccessful. Impure acetyl-(-)-AIB (15.6 g) was hydrolyzed and the hydrochloride was converted to the free amino acid as described above for the isolated (-)-AIB. After recrystallization from 100 ml of 96% alcohol, 9.75 g (90%) were recovered; m.p. 183°-192°; [α]D +12.1° (c, 1, H2O). From the mother liquor, two additional small crops of somewhat better optical purity were obtained. Recrystallization did not effect a significant improvement in the product, so all of the crops and dried mother liquors were combined, dissolved in 9.5 ml of concentrated HCl, and concentrated to dryness. The residue was dissolved in 100 ml of absolute ethanol, 150 ml of ether were added, and the solution was allowed to stand at room temperature for 6 hours; 9.0 g of shining plates were obtained; m.p. 131°-140°; [α]D +10.1° (c, 1, H2O). This material was recrystallized by dissolving it in 100 ml of absolute ethanol and adding 100 ml ether; yield 7.24 g (50% based on the impure acetyl-(+)AIB; m.p. 133°-137°; [α]D +10.8° (c, 1, H2O).

C6H13NO2Cl
Calculated: N 10.0
Found: N 9.84

For comparison, (-)-AIB hydrochloride was prepared in the same manner from 100 mg of pure (-)-AIB; m.p. 134°-138°; [α]D -10.7° (c, 1, H2O).

Of the pure (+)-AIB hydrochloride, 7.1 g were dissolved in 30 ml of water and passed through a column (2 × 17 cm) of Amberlite CG-120 (H+ form). The column was washed with 100 ml of water and the amino acid was eluted with 120 ml of 3 N NaOH. The last 50 ml of the eluate were collected and evaporated under reduced pressure. The residue was recrystallized from 150 ml of 99% ethanol to yield 4.2 g of long rectangular plates; m.p. 192°-194°; [α]D +15.4° (c, 1, H2O).

C6H13NO2
Calculated: N 13.6
Found: N 13.4

Another crop of 0.62 g, m.p. 190°-192°, was obtained from the mother liquor.

Ethyl α-cyanoacetate Ethyl α-bromocetoate (95.5 g, 0.506 mole) and sodium cyanide (29.0 g, 0.592 mole) were refluxed in 300 ml of absolute ethanol for 18 hours. The reaction mixture was processed as described for ethyl α-cyanopropionate; yield, 48.7 g (69% of theory); b.p. 90°-104° at 18 mm.

α-Ethyl-β-alanine—Ethyl α-cyanobutyrate (21.2 g; 0.15 mole) was reduced and the reaction mixture was worked up as described for the preparation of AIB. The amino acid was purified by adsorption on a column (4 × 25 cm) of Amberlite CG-120 (H+ form). The resin was washed with 500 ml of water, and the amino acid was eluted with 3 N NaOH; it emerged in the fractions between 300 and 500 ml of eluate. This fraction was concentrated to dryness and the crystalline residue was recrystallized from 200 ml of 80% ethanol; yield, 10.7 g of rhombic plates, m.p. 221°-222° (decomposition). An additional 2.7 g, m.p. 218°-222° (decomposition) was obtained from the mother liquor; total yield, 76%.

C6H13NO2
Calculated: N 12.0
Found: N 11.8

In the Beckman-Spinco amino acid analyzer with the system of Spackman, et al. (13), α-ethyl-β-alanine emerges with the basic amino acids. With a 54 cm column of Amberlite IR-120 eluted with 0.38 N citrate buffer, pH 4.26, at 30°, it emerges 27 ml after the phenylalanine-tyrosine peak. The color yield with ninhydrin is 38% of that of lysine.

DISCUSSION

Previously, Crumpler et al. (1) isolated AIB from the urine of an excretor and reported it to be levorotatory and to melt at 183°-184°; and Fink et al. (3) isolated a small amount, melting at 181°-184° from the urine of a cancer patient. Crumpler et al. (1), racemized their isolated material for comparison with synthetic AIB, which melts at 177°. In the present work the isolation of a larger amount of material made possible a more effective purification. The properties of the isolated material, melting at 191°-194°, specific rotation −15.3° in water, compare favorably with natural AIB isolated from iris bulbs, [α]D −17° (14); the resolved (+)- and (−)-AIB, [α]D +15.4° and −15.4° do ascribed above of m.p. 192°-194° and 194°-196°, respectively; and the (−)-AIB prepared by Balenović [α]D −14°, m.p. 173°-175° (10).

Some difficulty was encountered in the isolation of acetyl-δ-l-alanine, because both forms made crystalline salts with most alkaloids, and they could not be separated effectively by fractional crystallization. Further difficulty lay in the fact that N-acetyl-δ-l-aspartic acid is a racemic compound and has a higher melting point and lower solubility than either pure isomer. Neither slightly optically impure acetyl derivatives nor free amino acids could be improved by recrystallization. These difficulties were met by assuring the purity of the cinchonidine salt of acetyl-(-)-AIB, and by recrystallization of impure (+)-AIB as the hydrochloride.

Balenović (10) has succeeded in relating the configuration of

3 This melting point as given is possibly a typographical error, and should perhaps be 193°-195°.
(-)-AIB to that of (-)-2-methylbutanol-1, which, in turn, has been related to L(-)-glyceraldehyde. Some confusion might be expected to arise from the assignment of (-)-AIB to a configurational family. It might be looked upon as an a-aminomethylpropionic acid, in which case it would belong to the L-family as a homologue of L-alanine. It seems more desirable however, to consider it to be a relative of beta-alanine; in this case it would belong to the d-family and probably should be named d-(-)-alpha-methyl-beta-alanine. With this latter nomenclature, natural d(-)-AIB may be more readily related to other amino acids. For example, it would be formed by decarboxylation of erythro-beta-methyl-L-aspartic acid, an epimer of the three-beta-methyl-L-aspartic acid formed by Clostridium tetanomorphum (15). In general, the L or natural forms of the amino acids have a bitter taste and the D forms are sweet. It is of interest that natural d(-)-AIB has a faintly bitter taste and L(+)-AIB is faintly sweet.

Since D(-)-AIB is formed by the biological degradation of dihydrothymine (7), the natural isomer is of obvious importance as an intermediate for the preparation of natural dihydrothymine and beta-ureidoisobutyric acid for studies of thymine metabolism.

**SUMMARY**

(-)-beta-Aminoisobutyric acid has been isolated from the urine of a person genetically determined as an excretor of the amino acid. A preparation of both of the optically active forms of beta-aminoisobutyric acid is described. The preparation of DL-alpha-ethyl-beta-alanine is described.

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The Preparation and Isolation of d-(—)-β-Aminoisobutyric Acid
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