of water but some volatilization of amine attends the dissolution of the salt and subsequent evaporations of the liquors. Loss from this cause may be avoided by distilling the original solution and recrystallization liquors under reduced pressure. Alternatively the salt may be dissolved in about 3 parts of hot methanol and the solution diluted with an equal volume of hot water. The salt is recovered by successive cooling and distilling of solvent without serious loss of amine. The less soluble salt can be crystallized from methanol but the original salt mixture is not readily resolved in this solvent.

N-Acetyl-D-valine was recovered from its salt (146 g.) by the general procedure already described. About 80% separated on addition of an exact equivalent of hydrochloric acid. The remainder was obtained by evaporating the filtrate to dryness and extracting the residue with acetone. The yield after recrystallization was 70.5 g. (88% based on the racemic form originally taken). The compound was purified by crystallization from water (laminated rhomboidal plates) or acetone (massive rhombs) and then had m.p. 164–165°; neut. equiv., 160; $[\alpha]^{25}D$ −0.5° (*c* 12, methanol); −3.4° (*c* 12, ethanol); −9.36° (*c* 4, glac. acetic acid); +20.05° (*c* 4, water). Synge[19] reports m.p. 164°; $[\alpha]^{25}D$ +4.0° (*c* 2, ethanol) for N-acetyl-L-valine. The solubilities are: water, 6.94; acetone, 5.54; ethyl acetate, 0.73; chloroform, 0.24. It may be noted that although the active form melts distinctly higher than the DL-form it is nevertheless more soluble in most solvents.

N-Acetyl-L-valine.—The crude substance (82 g.) obtained from mother liquors of the resolution had $[\alpha]^{25}D$ +8.27° (*c* 8, glac. acetic acid) and hence contained about 93% of the L-form. It could not be purified by crystallization from common solvents except that the less soluble DL-form was partially removed in the head fractions. It was purified by two methods. (a) A portion (50 g., 0.313 mole) was combined with 48 g. (0.313 mole) of (+)-α-fenchylamine in 300 cc. of methanol and 400 cc. of water and several crops of salt totalling 79 g. (89.5%) were obtained as previously described. The salt had $[\alpha]^{25}D$ +4.18° (*c* 8, methanol) and otherwise closely resembled the enantiomorphous form described above. Decomposition gave 38.6 g. of pure N-acetyl-L-valine, m.p. 164–165°; $[\alpha]^{25}D$ −20.08° (*c* 4, water) and other properties substantially identical with those of the D-form. The salts in the mother liquors gave 9.4 g. of crude acetylvaline containing excess D-form.

(b) A composite sample of crude N-acetyl-L-valine (54.4 g., 0.34 mole) calculated to contain 80% of the L-form and 20% of the DL-form was combined in aqueous methanol with 93 g. (0.61 mole) of DL-α-fenchylamine and 0.27 mole of hydrochloric acid. The proportions were calculated to produce 84.5 g. of N-acetyl-L-valine-(+)-fenchylamine salt, 21.3 g. of N-acetyl-DL-valine-DL-fenchylamine salt and 51.1 g. of (−)-fenchylamine hydrochloride. Fractionation in the usual manner gave 72 g. (85.2%) of the pure N-acetyl-L-valine-(+)-amine salt, $[\alpha]^{25}D$ +4.17° (*c* 8, methanol). Decomposition of this gave 34 g. of pure N-acetyl-L-valine and 33 g. of pure (+)-fenchylamine. Intermediate fractions in the resolution, rich in DL-acid-DL-amine salt, could not be purified. This salt was prepared from the pure components in a separate experiment and had m.p. 182–185°. The solubilities are: water 13.4; methanol, 25.3. The combined intermediate fractions and mother liquors of the resolution gave on decomposition 18.0 g. of mixed acetylvalines and 57 g. of mixed fenchylamines suitable for further processing.

D-Valine.—Pure N-acetyl-D-valine (8.0 g.) was hydrolyzed and the amino acid recovered as described for D-phenylalanine, except that two equivalents of hydrochloric acid were used. The initial product (5.9 g.) had $[\alpha]^{25}D$ −5.93° (*c* 4, water). Recrystallization from water gave 3.0 g., $[\alpha]^{25}D$ −6.1° (*c* 4, water); −23.6° (*c* 4.2, *N* HCl); −27.4° (*c* 4.2, 6 *N* HCl). A sample from another resolution had $[\alpha]^{25}D$ −27.7° (*c* 4, 6 *N* HCl). The value −27.1° (*c* 1, 6 *N* HCl) has been reported.[22a]

L-Valine.—N-Acetyl-L-valine (6 g.) similarly gave L-valine (4.3 g.) which, without recrystallization had $[\alpha]^{25}D$ +6.3° (*c* 4, water); +23.4° (*c* 4, *N* HCl); +27.4° (*c* 4, 6 *N* HCl). These values were not substantially changed by recrystallization.

Other Resolutions.—In other experiments, not reported in detail, it was shown that N-acetyl-DL-valine was readily resolved when neutralized with one-half equivalent of sodium hydroxide and one-half equivalent of either (−)- or (+)-α-fenchylamine, the less soluble salt being obtained in about 80% yield. Similarly, pure DL-α-fenchylamine or partially active samples recovered from the resolutions described above were resolved by means of pure active acetylvalines or acetylphenylalanines or by judiciously chosen partially active samples of these.

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[CONTRIBUTION FROM THE FURMAN CHEMICAL LABORATORY, VANDERBILT UNIVERSITY]

The Resolution of Amino Acids. II. Isoleucine, Alloisoleucine, Leucine and Norleucine[1]

BY W. A. HOOPER HUFFMAN[2] AND A. W. INGERSOLL

The general objects of this series of studies have been described in the first paper.[3] The present paper describes the extension of the work to the principal members of the leucine family, all of which have been resolved into both active forms through the N-acetyl derivatives. The antecedent separation of DL-isoleucine and DL-alloisoleucine from synthetic mixtures was effected by fractional crystallization of the acetyl derivatives. A partial conversion of N-acetyl-DL-alloisoleucine to N-acetyl-DL-isoleucine is described. The procedures afford all isomeric forms of the amino acids and their acetyl derivatives in high purity.

The amino acids of the leucine family have been rather difficult to obtain in high purity and their derivatives have been incompletely characterized. This has been especially true of isoleucine and alloisoleucine and of the less common stereoisomers of leucine and norleucine. Since the N-acetyl derivatives of phenylalanine and valine were shown[3] to be well suited for chemical resolution and rigorous purification, the use of acetyl derivatives for these purposes has been extended to the leucine family. Synthetic DL-leucine and DL-norleucine readily afforded the corresponding acetyl derivatives by simple acetylation. All available samples of "DL-isoleucine," however, contained the diasteroisomeric DL-alloisoleucine and it was necessary to effect an antecedent separation of these or their derivatives.

Separation of N-Acetyl-DL-isoleucine and N-Acetyl-DL-alloisoleucine.—Syntheses leading to isoleucine probably always produce more or less of the diastereoisomeric alloisoleucine. Early workers[4,5,6] usually ignored this possibility but appar-

(1) Taken from the Ph.D. thesis of W. A. H. Huffman, August, 1950.

(2) du Pont Fellow, 1949–1950.

(3) L. R. Overby and A. W. Ingersoll, THIS JOURNAL, **73**, 3363 (1951). Paper I.

(4) L. Bouveault and R. Locquin, *Bull. soc. chim. France*, [3] **35**, 965 (1906).

(5) R. Locquin, *ibid.*, [4] **1**, 595 (1907).

(6) F. Ehrlich, *Ber.*, **41**, 1453 (1908).

ently obtained fairly pure DL-isoleucine by repeated crystallization of the mixture from water or aqueous alcohol, in which DL-isoleucine is the less soluble. Our own and other recent work[7] indicates that this tedious process may yield pure isoleucine but not pure alloisoleucine. Mixed crystal formation may be involved, since we have found the solubilities (g./100 cc. of water at 25°) to be 2.02 and 5.35, respectively. Abderhalden and Zeisset[8] apparently obtained both racemic forms pure by combining fractional crystallization and distillation of the esters. Fractionation of the copper salts has been suggested[9] and a separation based on the markedly lesser solubility of sodium isoleucinate in lower alcohols has been patented recently.[10]

In the present work it has been found that the mixed acetyl derivatives, obtained in 83–88% yields by acetylating synthetic "DL-isoleucine" from various sources, are rather readily separated by fractional crystallization from water and then acetone. The sparingly soluble N-acetyl-DL-alloisoleucine is easily obtained as small hexagonal plates, m.p. 166°, while the very soluble N-acetyl-DL-isoleucine separates slowly from acetone in massive rhombic tablets, m.p. 119°. Semi-quantitative separation of the mixed derivatives from two commercial preparations showed apparent isoleucine:alloisoleucine ratios of 74:26 and 28:72. The separation procedure was applied also to samples of isoleucine prepared by the method of Marvel[11] and systematically recrystallized seven times from water. A composite of head fractions contained about 90% isoleucine; the headmost fraction was nearly pure. The proportions of the isomers apparently may differ markedly for different methods of preparation.

Epimerization of N-Acetyl-DL-alloisoleucine.—Many optically active α-acetamino acids are known to be racemized by excess acetic anhydride in warm alkali solution.[12] When we applied these conditions to N-acetyl-DL-alloisoleucine and fractioned the product, it was found that about 37% conversion to N-acetyl-DL-isoleucine had been effected, presumably by partial inversion of configuration about the α-carbon atom.

Resolutions.—Locquin[5] resolved synthetic isoleucine through the formyl derivatives with brucine. This appears to be still the method of choice of recent workers.[13,14] Abderhalden and Zeisset[8] repeated this resolution and extended it to alloisoleucine. In the present work N-acetyl-DL-isoleucine was resolved with (−)-α-fenchylamine in water. The less soluble salt is that of N-acetyl-D-isoleucine, from which this derivative was recovered in 65% yield. The more soluble fractions of the salt gave optically impure N-acetyl-L-isoleucine. This is distinctly less soluble than the accompanying DL-form and was easily purified in 62% yield by crystallization from water or acetone. An attempted resolution with (−)-α-phenylethylamine was unsuccessful.

In the case of N-acetyl-DL-alloisoleucine attempted resolutions with (−)-α-fenchylamine, (−)-α-phenylethylamine, brucine, cinchonine, (−)-ephedrine and (−)-desoxyephedrine were unsuccessful, but resolution was fairly readily effected with quinine in acetone. Slightly impure N-acetyl-L-alloisoleucine from the sparingly soluble salt was readily purified. In this instance the DL-form (m.p. 166°) is less soluble than the L-form (m.p. 155.5°) but the latter crystallizes in characteristic needles which are readily separated. Crude N-acetyl-D-alloisoleucine derived from the more soluble quinine salt was similarly purified.

The four active acetamino acids were characterized (see Experimental) and then separately hydrolyzed to the corresponding amino acids in the usual manner. Numerical rotation values in each instance were equal to or higher than reported values.[8] The resolution methods are regarded as reliable with respect to purity and at least equal in convenience to earlier methods.

After this work was complete a report by Greenstein and associates[15] described a somewhat similar separation of N-acetyl-DL-isoleucine and N-acetyl-DL-alloisoleucine. The former was resolved by asymmetric enzymatic hydrolysis. Epimerization of the active forms by acetylation, followed by enzymatic resolution of the epimeric mixtures afforded all four of the active isomeric forms. The data given in the abstract do not permit extensive comparison of properties at this time.

N-Acetyl-DL-leucine and N-acetyl-DL-norleucine were resolved with (−)-α-fenchylamine and, less conveniently, with (−)-α-phenylethylamine. In both instances the less soluble salt is that of the acetyl-D-amino acid, from which this derivative and the corresponding amino acid were recovered. The more soluble salts gave the optically impure acetyl-L-amino acids. N-Acetyl-L-leucine is less soluble and N-acetyl-L-norleucine is more soluble than the accompanying DL-form, but both were readily purified by crystallization. It may be noted that in these resolutions (and that of N-acetyl-DL-isoleucine) either active form desired may be obtained in the initial step by selecting the appropriate form of the resolving agent, both forms of which are readily available.[16] The resolutions are regarded as reliable but only moderately convenient. They enabled us to prepare and characterize the entire set of twelve stereoisomeric modifications of the amino acids of the leucine family and their acetyl derivatives. Revised or supplementary values for many of the constants are recorded in the Experimental part.

Experimental

All melting points were taken with the same calibrated A.S.T.M. thermometer without further correction. Solu-

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bility values are the average of duplicate determinations expressed as grams per 100 cc. of solution at 25 ± 0.5°.

Preparation of Racemic Acetamino Acids.—Acetylation with acetic anhydride in aqueous alkali was employed as previously described,[3] including exhaustive extraction of the acidified aqueous liquors with chloroform. This was particularly important with the mixtures of acetylisoleucine and acetylalloisoleucine in order to minimize distortion of the original proportions by losses due to differences in solubility. Yields of recrystallized products ranged from 80 to 92%. Neutralization equivalents within ±0.4 unit of the calculated value were determined in each instance.

N-Acetyl-DL-leucine forms long needles in water, massive flat prisms in acetone; solubilities in water, 1.88; acetone, 3.78; ethyl acetate, 0.78; m.p. 159–160°, literature, 157[17] to 161°.[18]

N-Acetyl-DL-norleucine forms irregular masses in water, large, nearly cubical, rhombs in acetone; solubilities in water, 3.60; acetone, 23.4; ethyl acetate, 2.22; m.p. 107–108°, literature, 108°.[19]

N-Acetyl-DL-isoleucine forms irregular masses in water, very large rhomboidal tablets by slow deposition from acetone; solubilities in water, 6.50; acetone, 26.4; ethyl acetate, 5.47; m.p. 118–119°.

N-Acetyl-DL-alloisoleucine forms aggregates of hexagonal plates from water or acetone, distinct hexagonal plates from ethyl acetate; solubilities in water, 1.09; acetone, 2.11; ethyl acetate, 0.46; m.p. 165–166°.

Separation of N-Acetyl-DL-isoleucine and N-Acetyl-DL-alloisoleucine.—The mixtures studied were obtained from three samples of "isoleucine." (a) A commercial sample labeled "DL-isoleucine with DL-alloisoleucine" (2 moles) was acetylated and the product was systematically recrystallized four times from (initially) 12 parts of water. The product was 303.4 g. (87.5%) of which head fractions totalling 132 g. were pure N-acetyl-DL-alloisoleucine, m.p. 165–166°. Three further crystallizations of the remaining material gave only 38 g. more with m.p. 165–166°. The remainder was crystallized systematically from acetone. The head crops were crusts of nearly pure allo derivative; the sirupy liquors slowly deposited nearly pure N-acetyl-DL-isoleucine, m.p. 117–123°, in transparent masses or large rhombs, sometimes 2–3 cm. across. Appropriate recrystallization eventually gave 203.5 g. (70%) of pure N-acetyl-DL-alloisoleucine, 79 g. (27%) of pure N-acetyl-DL-isoleucine, m.p. 117–118° (from acetone), m.p. 118–119° (from water or ethyl acetate) and 14.8 g. (3%) of semi-crystalline residual material. The exhaustive fractionation required to achieve semi-quantitative separation was much simplified for preparative use. In later runs three crystallizations of crude product from water removed about 65% of the total with m.p. above 163°. This was digested briefly with an equal weight of hot acetone, which after cooling was filtered off and used similarly on intermediate and foot fractions. The amounts representing substantially pure isomers were then about 68% and 20%. The remaining intermediate fractions and foot liquors were combined for further fractionation with similar fractions from other runs.

(b) A commercial sample labeled "DL-isoleucine" (0.25 mole) was similarly acetylated and the product fractionated. The results indicated 74% acetylisoleucine and 26% acetylalloisoleucine in a total yield of 74.3%.

(c) DL-Isoleucine was obtained as by Marvel[11] but crystallized from water alone. The mixture (134 g.) after seven systematic crystallizations gave (1) 64 g. with solubilities ranging from 2 to 4, (2) 18.5 g. with greater solubilities, and (3) foot liquors containing 48 g., including some ammonium bromide. The three portions were acetylated separately and the products crystallized systematically from acetone. Fractions from (1) were substantially pure N-acetyl-DL-isoleucine, with melting points ranging from 125° to 117°. Combination with material from (2) and further fractionation eventually gave only 3.6 g. with m.p. 165–166°, 78.7 g. with m.p. 123° to 117° and 7 g. of brown semi-crystalline residue. The more soluble portion of the product from (3) was accidentally lost but it was possible to determine that over half of it was N-acetyl-DL-alloisoleucine, m.p. 165–166°. The results indicate that a fairly sharp separation of the two amino acids was effected by seven crystallizations from water.

Partial Epimerization of N-Acetyl-DL-alloisoleucine.—Substantially pure N-acetyl-DL-alloisoleucine, m.p. 163° (86.6 g., 0.5 mole) was dissolved in 250 cc. of 2 *N* sodium hydroxide and 510 g. (5 moles) of acetic anhydride was added in portions with shaking. The already warm mixture was held at 60 ± 2° for six hours. After keeping overnight at room temperature the solution was evaporated to 225 cc. at 60–70° under reduced pressure and diluted to 300 cc. with water. The product was obtained as usual by precipitation with acid followed by extraction of the filtrate with chloroform. Fractionation in acetone gave back 96.5% of the original weight consisting of 47.9 g. with m.p. 162–163°, 3.7 g. of intermediate fractions and 32.3 g. (37.2%) with m.p. below 120° and otherwise identical with N-acetyl-DL-isoleucine. A similar experiment carried out for three hours at 35–40° with six mole-equivalents of acetic anhydride resulted in only 17.2% conversion to N-acetyl-DL-isoleucine.

Resolution of N-Acetyl-DL-isoleucine.—Carefully purified material, m.p. 118–119° (0.68 mole) was dissolved in 1300 cc. of water with (−)-α-fenchylamine. Overnight needles separated and were filtered and washed with a little water. Stepwise concentration of filtrate and washings to about 500 cc. gave similar crops; the liquor was set aside. The moist salt was boiled briefly with 1.5 parts of water (insufficient for complete solution) and filtered after keeping overnight. The dry salt (96.8 g., 87.6%) had $[\alpha]^{25}D$ −7.5° (*c* 4, methanol) and was the nearly pure salt of N-acetyl-D-isoleucine. The digestion procedure represents a simplification designed to expedite removal of most of the more soluble salt. Preliminary experiments had shown that the less soluble salt could be fully purified in about 70% yield ($[\alpha]^{25}D$ −7.7°) by two or more further crystallizations from water or 2-propanol, but considerable evaporation and other manipulation was required. On the other hand the slight impurity of the salt obtained by the abbreviated procedure was of little consequence, since the correspondingly impure N-acetyl-D-isoleucine obtained by decomposition is less soluble than the DL-form and is readily purified.

N-Acetyl-D-isoleucine.—The slightly impure salt (95.8 g.) was decomposed with aqueous alkali and the amine extracted with benzene in the usual manner.[3] The crude acetamino acid was liberated with one equivalent of hydrochloric acid and crystallized in several crops. Brief systematic recrystallization from water gave 41.8 g. (71% based on the DL-form originally taken) of pure N-acetyl-D-isoleucine. This crystallizes in long flattened needles from water or rectangular plates from acetone, m.p. 150–151°; $[\alpha]^{25}D$ +8.2° (*c* 2, water); −11.3° (*c* 4, methanol); −16.3° (*c* 2, abs. ethanol); −25.2° (*c* 2, acetone); solubilities in water, 4.15; acetone, 11.76; ethyl acetate, 2.80. For a sample prepared by resolution of isoleucine[5] and acetylation of the D-form Synge[20] reports m.p. 150–151°; $[\alpha]^{20}D$ −15.6° (*c* 2.3, ethanol). The mother liquors of the recrystallization gave 12.2 g. of nearly pure N-acetyl-DL-isoleucine, m.p. 120°.

N-Acetyl-L-isoleucine.—The mother liquors of the resolution gave by similar procedures 36.0 g. (54.2%) of pure N-acetyl-L-isoleucine and 27.3 g. of slightly impure DL-form. The active form had m.p. 150–151°; $[\alpha]^{25}D$ −8.2° (*c* 2, water); +11.4° (*c* 4, methanol) and solubilities substantially identical with those of the antipode.

The resolution of N-acetyl-DL-isoleucine was attempted with (−)-α-phenylethylamine. The salt formed moderately soluble fine needles in water, methanol and ethanol but was not resolved. It was nearly insoluble in acetone and ethyl acetate.

Resolution of N-Acetyl-DL-alloisoleucine.—Powdered pure material (52 g., 0.3 mole) was suspended in 500 cc. of gently boiling acetone and 113.5 g. (0.3 mole) of quinine trihydrate was added in small portions. The quinine dissolved gradually and the boiling solution became filled with fine needles consisting mainly of the N-acetyl-L-alloisoleucine salt. After boiling for an hour the suspension was allowed to cool and the salt (84 g.) was collected by filtration. A further crop (25 g.) was obtained after concentration to about 100 cc. The pale brown, sirupy liquor was set aside

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and the solid crops were digested separately in 2 parts of acetone. The head crop resisted solution and gave back 71.4 g., m.p. 181–183°. A sample crystallized readily from water, but the liquor and crystals (m.p. 185–187°) were discolored. A sample (2 g.) dissolved completely on prolonged boiling in 225 cc. of acetone and separated as well formed needles, m.p. 184–186°, solubility in acetone, 0.46; $[\alpha]^{25}D$ +126° (*c* 2, methanol). The intermediate crop (25 g.) and digestion liquors contained rather soluble material which could not be effectively fractionated.

N-Acetyl-L-alloisoleucine.—A part of the sparingly soluble salt (53 g.) was decomposed with alkali and the quinine extracted with chloroform. The recovered acetamino acid (18.8 g.) was crystallized from 3 parts of water in several crops ranging in rotation between +11° and +17° (*c* 4, methanol) and consisting mainly of rosettes of long pointed needles. Systematic recrystallization, assisted by combination of similar fractions and selective seeding, soon gave a head fraction (1.7 g.) of almost pure N-acetyl-DL-alloisoleucine, intermediate fractions (5.5 g.) of mixed forms and 9.8 g. (53%) of pure N-acetyl-L-alloisoleucine, m.p. 155–155.5°; $[\alpha]^{25}D$ −5.3° (*c* 2, water); +17.3° (*c* 4, methanol), values not changed by further crystallization from water or acetone. It forms small rectangular plates from acetone or ethyl acetate; solubilities in water, 4.37; acetone, 6.14; ethyl acetate, 1.20.

N-Acetyl-D-alloisoleucine.—The intermediate fractions and mother liquors of the resolution were freed from acetone and separately decomposed. The acetamino acid fractions recovered from both parts were found to be similar and were combined for fractionation. The crude material (29.6 g.) on fractionation gave 10.3 g. of the DL-form, 5.4 g. of mixed forms and 7.8 g. of pure N-acetyl-D-alloisoleucine, m.p. 154–155.5°; $[\alpha]^{25}D$ −17.2° (*c* 4, methanol); solubilities in water, 4.44; acetone, 5.90; ethyl acetate, 1.22.

Attempted resolutions with other bases were unsuccessful. Brucine, cinchonine and (−)-desoxyephedrine formed noncrystalline salts. (−)-α-Fenchylamine, (−)-α-phenylethylamine and (−)-ephedrine formed well-crystallized salts in water but no resolution occurred in this or other common solvents.

Resolution of N-Acetyl-DL-leucine.—(a) The racemic acid (0.2 mole) and (−)-α-fenchylamine (0.2 mole) were combined in 250 cc. of hot water and the salt was crystallized in several fractions. No satisfactory resolution occurred, even though the reciprocal resolution of the racemic amine with the L-acetamino acid[16] is easily effected in water. However, fractionation in methanol (2–3 cc./g.) after four series gave 23.5 g. (72%) of pure N-acetyl-D-leucine salt, $[\alpha]^{25}D$ +7.7–7.9° (*c* 4, methanol) identical in other properties with its antipode.[16] The more soluble salt was not purified. In later experiments it was found more expeditious to form the salt in methanol and, after brief fractionation, to take for decomposition somewhat impure salt (rotation +7.0° or higher).

N-Acetyl-D-leucine.—Decomposition of pure or somewhat impure salt in the usual manner and crystallization of the acetamino acid from 15 parts of water gave the pure D-form; the more soluble DL-form, if present, was recovered from the later fractions. The substance forms long needles from water or thin rhombic plates from acetone, m.p. 185–186°; $[\alpha]^{25}D$ + 24.2° (*c* 4, methanol); solubilities in water, 0.81; acetone, 1.73; ethyl acetate, 0.34, in agreement with reported values.[20]

N-Acetyl-L-leucine was obtained similarly in 50–70% yield from foot fractions of the resolution. It has m.p. 185–186°, $[\alpha]D$ −24.1° and other properties in close agreement with reported values for its antipode and for samples prepared from natural leucine.[21,22]

(b) The racemic acid (69.3 g.) was combined in 450 cc. of hot water with an equivalent amount of (−)-α-phenylethylamine and the salts were crystallized in several fractions ranging in rotations erratically from +8.3 to −9.1° (*c* 4, methanol). There was little difference in appearance and solubility of the various fractions upon recrystallization from water or the lower alcohols. Combination of fractions of similar rotations and recrystallization from 3–4 parts of water soon gave 31 g. (52%) of nearly pure dextrorotatory salt, $[\alpha]^{25}D$ +9.9° (*c* 4, methanol); +15.3° (*c* 4, water); solubility in water, 4.7. Decomposition and purification as previously described gave 17 g. (49%) of pure N-acetyl-D-leucine. The antipode was obtained similarly in smaller yield from levorotatory fractions.

Resolution of N-Acetyl-DL-norleucine.—(a) The acid and (−)-α-fenchylamine (0.15 mole) were dissolved in 200 cc. of water and the salts crystallized in several fractions. Three systematic recrystallizations gave 15.7 g. (67%) of coarse needles $[\alpha]^{25}D$ −12.7° (*c* 4, methanol) and more soluble fractions of fine needles or liquors with lower levo or dextro rotations.

N-Acetyl-D-norleucine was recovered from the less soluble salt as usual (61% yield) and crystallized from 3–4 parts of water as long needles, m.p. 114–115.5°; $[\alpha]^{25}D$ +5.0° (*c* 4, water); −0.8° (*c* 2, ethanol); +19.6° (*c* 4, methanol); solubilities in water, 7.84; ethyl acetate, 13.4. The solubility in acetone was too great to permit crystallization. Synge[20] reported m.p. 112–114°, $[\alpha]^{23}D$ −0.2° (*c* 2.4, ethanol).

N-Acetyl-L-norleucine was recovered similarly in impure form from the more soluble fractions. Crystallization removed the less soluble DL-form (8.2 g.) as coarse masses and gave the L-form (5.5 g.) as long needles, m.p. 114–115.5°; $[\alpha]^{25}D$ −19.4° (*c* 4, methanol).

(b) A resolution was effected with (−)-α-phenylethylamine by essentially the same procedure as described in (a) above. The less soluble salt (55% yield) has $[\alpha]^{25}D$ −14.8° (*c* 4, methanol) and gave N-acetyl-D-norleucine $[\alpha]^{25}D$ +19.4° (*c* 4, methanol). The antipode obtained from the more soluble salts has m.p. 114–115°, $[\alpha]^{25}D$ −19.3°.

The Amino Acids.—Each of the racemic and active acetamino acids (5 g.) was boiled two hours under reflux with 11.6 cc. (1.2 equivs.) of 3 *N* hydrobromic acid and the solution then evaporated to dryness under reduced pressure in a boiling water-bath. The residue was taken up in 25 cc. of methanol and the amino acid obtained by dropwise addition of 27% aqueous ammonia to about *p*H 6, filtration and washing with warm methanol. Additional small crops were obtained by reworking the filtrates: yields 90–98%. The amino acids were then purified by one or more crystallizations from water or aqueous methanol.

The racemic amino acids were partially characterized by solubility determinations in water at 25°; DL-isoleucine, 2.02; DL-alloisoleucine, 5.35; DL-leucine, 1.02; DL-norleucine, 1.13. The active amino acids were characterized by the specific rotation values recorded in Table I for several solvents.

TABLE I

SPECIFIC ROTATIONS OF AMINO ACIDS, $[\alpha]^{25}D$

	Water	1.007 *N* HCl (*c* 4)	5.990 *N* HCl (*c* 4)	Ref.[d]
D-Isoleucine	−12.2[a]	−36.6	−40.7	5, 8, 14
L-Isoleucine	+12.2[a]	+36.7	+40.7	5, 8
D-Alloisoleucine	−15.6[b]	−34.8	−38.4	8
L-Alloisoleucine	+15.7[b]	+34.9	+38.5	8
D-Leucine	+10.6[b]	−14.9	−15.2	20
L-Leucine	−10.7[b]	+14.6	+15.2	21, 22
D-Norleucine	− 6.0[c]	−22.1	−23.7	20
L-Norleucine	+ 6.1[c]	+21.8	+23.5	20

[a] *c* = 3.2. [b] *c* = 2. [c] *c* = 1. [d] References to comparable literature values.

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