[CONTRIBUTION FROM THE FURMAN CHEMICAL LABORATORY, VANDERBILT UNIVERSITY]

The Resolution of Amino Acids. I. Phenylalanine and Valine[1]

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Existing methods for preparing optically active amino acids are often unsatisfactory, especially when both forms are required in high purity. This paper describes convenient new chemical resolutions of phenylalanine and valine. The special utility for this purpose of the N-acetyl derivatives and of certain simple active amines, particularly α-fenchylamine, is discussed and illustrated.

The active forms of the natural amino acids are required in an increasingly wide variety of investigations. It is established that the D- and L-forms are not always biologically equivalent, but studies concerned with the role of either active form often still employ the DL-form because the active form in question is not otherwise readily available. Isolation of rigorously pure L-forms from protein hydrolysates is, with some exceptions, still difficult and uncertain.[3] The pure D-forms are available only by racemization or synthesis, followed by resolution. In view of this situation and of the recent commercial availability of many synthetic racemic amino acids, it has seemed desirable to devote further study to the problem of resolution of the racemic forms. This paper represents the first of a series of studies recently completed in this field.

Most chemical resolutions of amino acids have employed the classical procedure of Fischer,[4] based upon resolution of the N-benzoyl or N-formyl derivatives by means of alkaloids. N-Formyl derivatives have been preferred, mainly because the final hydrolysis proceeds under conditions mild enough to preclude racemization. Other acyl derivatives, including the N-acetyl derivatives, and resolving agents other than alkaloids, have had limited use.

In the present work it has seemed desirable to stress (1) attainment of maximum antipodal purity by careful purification and characterization of intermediates; (2) selection of methods capable of giving *both* active forms of the amino acid in high purity; (3) use of new procedures and agents, particularly the use of the simpler active bases to replace or supplement the bulky and expensive alkaloids; and (4) development, when feasible, of practical procedures capable of large scale use.

In the preliminary phases of the work N-formylphenylalanine was found to be resolved fairly well by means of the (−)-α-phenylethylamine and (−)-α-fenchylamine salts. It was observed, however, that the formyl derivatives of this and several other amino acids undergo partial hydrolysis during the crystallizations involved in the resolution and attempted purification. This experience and similar indications frequently noted in the literature suggest that many formyl derivatives are not suitable for rigorous purification. On the other hand, it has been found that the acetyl derivatives of many amino acids do meet the requirements of satisfactory intermediates and it has been possible to resolve several of these with simple, non-alkaloid amines. The active α-fenchylamines, now readily available in both forms,[5] have been particularly useful.

Early attempts to prepare acetamino acids were unsatisfactory. More recently the combined work of several groups of investigators has led to reliable methods for preparing both the active and racemic modifications by acetylation with acetic anhydride in acetic acid[6,7,8] or in aqueous alkali.[9,10] The N-acetyl derivatives of most of the natural amino acids and their racemic forms have been prepared by these methods, although some have been imperfectly characterized. It may be noted also that recent syntheses of amino acids through acetaminomalonic esters[11] and acetamidocyanoacetic esters[12] yield the acetamino acids as isolable intermediates.

The earliest resolution through an acetamino derivative, that of α-aminophenylacetic acid,[13] was unsatisfactory because of racemization during hydrolysis. Successful chemical resolutions through the acetyl derivatives, however, have been reported for cystine,[14] tryptophan,[15,16,17] tyrosine[18] and valine.[19] Alkaloids were used in most instances, except that N-acetyltryptophan was resolved with (+)-α-phenylethylamine.[15] This is one of the few instances[20,21] of the use of a non-alkaloid agent for resolving acylamino acids. A remarkably general biochemical method based upon selective enzymatic hydrolysis of acetamino acids and related derivatives has been developed recently by Greenstein and co-workers.[22]

(1) From the Ph.D. thesis of Lacy R. Overby, September, 1949.

(2) du Pont Fellow, 1948-1949.

(3) M. S. Dunn and L. B. Rockland, in "Advances in Protein Chemistry," Vol. III, Academic Press, New York, N. Y., 1947, p. 295.

(4) (a) E. Fischer, *Ber.*, **32**, 2451 (1899); (b) E. Fischer and O. Warburg, *ibid.*, **38**, 3997 (1906).

(5) A. W. Ingersoll and H. D. DeWitt, THIS JOURNAL, **73**, 3360 (1951); preceding paper.

(6) F. Knoop and J. G. Blanco, *Z. physiol. Chem.*, **146**, 267 (1925).

(7) M. Bergmann and L. Zervas, *Biochem. Z.*, **203**, 280 (1928).

(8) J. J. Kolb and G. Toennies, *J. Biol. Chem.*, **144**, 193 (1942).

(9) V. du Vigneaud and C. E. Meyer, *ibid.*, **98**, 295 (1932); **99**, 143 (1932).

(10) R. L. M. Synge, *Biochem. J.*, **33**, 1913, 1918 (1939).

(11) H. R. Snyder, *et al.*, THIS JOURNAL, **66**, 350, 500 (1944); **67**, 310 (1945).

(12) N. F. Albertson and B. F. Tullar, *ibid.*, **67**, 502 (1945).

(13) O. Neubauer and O. Warburg, *Z. physiol. Chem.*, **70**, 3 (1910).

(14) L. Hollander and V. du Vigneaud, *J. Biol. Chem.*, **94**, 243 (1931); V. du Vigneaud, R. Dorfmann and H. S. Loring, *ibid.*, **98**, 577 (1932).

(15) V. du Vigneaud and R. R. Sealock, *ibid.*, **96**, 511 (1932); V. du Vigneaud, R. R. Sealock and C. Van Etten, *ibid.*, **98**, 565 (1932).

(16) A. C. Shabica and M. Tishler, THIS JOURNAL, **71**, 3251 (1949).

(17) C. P. Berg, *J. Biol. Chem.*, **100**, 79 (1933).

(18) R. R. Sealock, *ibid.*, **166**, 1 (1946); *Biochemical Preparations*, Vol. I, 71 (1949).

(19) A. H. Gordon, A. J. P. Martin and R. L. M. Synge, *Biochem. J.*, **37**, 79 (1943).

(20) C. R. Harington, *ibid.*, **22**, 1429 (1928).

(21) W. Windus and C. S. Marvel, THIS JOURNAL, **53**, 3490 (1931).

(22) J. P. Greenstein, *et al.*, (a) *J. Biol. Chem.*, **178**, 503 (1949); (b) **179**, 1169 (1949); (c) **180**, 473 (1949).

In the present work N-acetyl-DL-phenylalanine and N-acetyl-DL-valine were prepared in 90–96% yields by a modification of the method of du Vigneaud and Meyer.[9] The more rapid acetylations in acetic acid[6,8] gave somewhat inferior yields of less pure products. Attempted resolutions with α-phenylethylamine, ephedrine, brucine, cinchonine and quinine were unsuccessful, but resolutions giving both forms pure were easily effected with the active α-fenchylamines. When the (−)-amine was used, for example, the salt of the acetyl-D-amino acid was much the less soluble in both instances and was readily obtained pure in 80–93% yields. The accompanying salts of the acetyl-L-amino acids are very soluble and remained in the mother liquors. The pure salts and those in the liquors were decomposed with alkali as usual, with nearly quantitative recovery of the amine, the pure acetyl-D-amino acids and the partially resolved acetyl-L-amino acids.

Pure acetyl-L-phenylalanine was readily obtained from the partially resolved material by one or two crystallizations from acetone, in which the accompanying DL-form is more soluble. Complete resolution was thus effected with only one form of the resolving agent. In the case of acetyl-L-valine this was not possible, since here the DL-form is less soluble than the active form. Acetyl-L-valine, however, was readily purified through the (+)-α-fenchylamine salt. It also was feasible to complete the resolution by means of *dl*-α-fenchylamine, since the solubility order in the three-salt system[23] involved is (+)-B·L-A < *dl*-B·DL-A < (−)-B·L-A. It was found, furthermore, that the racemic amine is readily resolved by active acetylvalines and acetylphenylalanines.

The active and racemic forms of the acetyl derivatives are easily crystallized and quite stable to ordinary manipulations, including repeated crystallizations from water and organic solvents. They were readily obtained in high purity and were rather fully characterized. The active forms were readily hydrolyzed by brief boiling with dilute hydrochloric or hydrobromic acid and the corresponding amino acids were obtained pure in good yields.

Phenylalanine has been resolved previously in several ways. Resolution through the formyl derivative with brucine by the method of Fischer and Schoeller[24] as modified by du Vigneaud and Meyer[9] appears to be the best previous chemical method. Sterically specific synthesis of the anilide of N-acetyl-L-phenylalanylglycine induced by cysteine-activated papain[25] and, especially, the asymmetric hydrolysis of the isopropyl ester[26] by a pancreatic enzyme appear to be effective biochemical methods. Valine has been resolved through the brucine salts of the formyl[27] and acetyl[19] derivatives. Both of these amino acids are resolved by the enzymatic procedures of Greenstein[22a] although details have not been given. The methods described in the present paper would appear to be at least as convenient as previous methods and perhaps more readily adaptable to the complete purification of both active forms in good yield.

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Experimental

Preparation of N-Formyl-DL-phenylalanine.—The method was a simplification of that of Fischer and Warburg.[4b] DL-Phenylalanine (82.5 g.) was dissolved in 100 cc. of 90% formic acid and boiled gently under reflux for two hours. An additional 100 cc. of formic acid was added and the solution allowed to cool. Formylphenylalanine then crystallized in readily filtrable form and was essentially pure (m.p. 165–166°) after filtering, washing with a little formic acid and drying in a desiccator over solid sodium hydroxide. A second pure crop was obtained similarly after concentrating the filtrate by distillation to 75 cc. The yield was 83 g. (87%). Crops obtained by further concentration were slightly colored and contained some free phenylalanine or its formate as shown by failure to dissolve completely in hot acetone. This material was best combined with similar material from other runs and crystallized from fresh formic acid. Working in this manner, the yield is nearly quantitative. Crystallization from acetone left the melting point unchanged at 165–166° (uncor.)[28] in agreement with reported values.[9,24]

Resolution of N-Formyl-DL-phenylalanine (a) with (−)-α-Fenchylamine.—Formylphenylalanine (19.3 g., 0.1 mole) was dissolved in 35 cc. of water containing 0.1 mole of sodium hydroxide and the solution mixed with a nearly boiling solution of (−)-α-fenchylamine hydrochloride (19.0 g., 0.1 mole) in 60 cc. of water. Crystallization occurred promptly, giving 14.0 g. of the nearly pure salt of formyl-D-phenylalanine, m.p. 177–178°, $[\alpha]^{26}D$ −33.04° (*c* 4, water). Further crystallization occurred in the filtrate overnight, giving 9.6 g. of fairly pure salt of formyl-L-phenylalanine, $[\alpha]^{26}D$ +26.5°. Systematic recrystallization of these crops and further crops from the original solution rather readily gave 15.4 g. of salt with m.p. 177–178°, $[\alpha]^{26}D$ −33.05° and 16.4 g. with m.p. 164–165°, $[\alpha]^{26}D$ +27.53°; calculated for each salt, 17.4 g.

The salts were decomposed by a generally applicable procedure. The powdered salt was suspended in about twice its weight of water and an equal volume of sulfur-free benzene in a separatory funnel and a few drops of phenolphthalein solution was added. Concentrated sodium hydroxide solution was then gradually added with vigorous shaking and occasional cooling until the solid dissolved and a faint permanent pink color appeared in the aqueous layer. The benzene solution of the amine was removed and the aqueous layer was extracted five or six times with 25-cc. portions of benzene. The united benzene extracts were washed once with a little water, dried with solid sodium hydroxide and distilled through a moderately effective column for recovery of the benzene and amine. With care to exclude carbon dioxide, the recovery was nearly quantitative. The aqueous layer and washings were promptly acidified with the calculated amount of concentrated hydrochloric acid and the liberated acyl derivative was isolated.

In the present instance the aqueous layer from the levorotatory salt gave a nearly quantitative precipitate of crystalline N-formyl-D-phenylalanine. This was dried and recrystallized from acetone; yield 83.5%. However, the product was found to contain traces of (insoluble) free phenylalanine. This was readily removed, but further traces appeared in subsequent crystallizations or when samples were made up in ethanol for rotation determinations. The rotation, $[\alpha]^{26}D$ −72.7° (*c* 1, 95% ethanol) and m.p. 163–164°, indicate slight impurity since recorded values[9] are $[\alpha]^{26}D$ −75.2° and m.p. 167°. Samples warmed for some time in ethanol or methanol gave increased amounts of

(23) A. W. Ingersoll and E. G. White, THIS JOURNAL, **54**, 274 (1932).
(24) E. Fischer and W. Schoeller, *Ann.*, **357**, 1 (1907).
(25) O. K. Behrens, D. G. Doherty and M. Bergmann, *J. Biol. Chem.*, **136**, 61 (1940).
(26) K. A. J. Wretlind, *ibid.*, **186**, 221 (1950).
(27) E. Fischer, *Ber.*, **39**, 2320 (1906).
(28) Melting points in this paper were taken with calibrated short-range thermometers without further correction.

the free amino acid. Similar behavior was later observed with formyl-DL-norleucine and formyl-DL-leucine.

The dextrorotatory salt gave N-formyl-L-phenylalanine with $[\alpha]^{26}D$ +60.9° and m.p. 161–162°. This contained free amino acid, but the low values indicate that this salt was not completely purified in the resolution.

(b) With (−)-α-Phenylethylamine.—Formylphenylalanine (96.5 g., 0.5 mole) was suspended in 200 cc. of water and the amine (60.6 g., 0.5 mole) added. The salt dissolved readily on warming and on cooling 46.0 g. separated promptly as needles, $[\alpha]^{26}D$ +29.7° (*c* 1, water). One recrystallization gave 24.6 g. with $[\alpha]^{26}D$ +32.8° and m.p. 175–176°, values not further changed on recrystallization. Successive crops from the original solution were systematically recrystallized and about 45% of the calculated amount of dextrorotatory salt was obtained pure. The later crops were levorotatory. At this point free phenylalanine appeared in some of the solutions and the fractionation was discontinued. The dextrorotatory salt was decomposed as previously described. The resulting N-formyl-L-phenylalanine, $[\alpha]^{26}D$ +73.7 (*c* 1, 95% ethanol), was obtained in 95% yield, but repeated crystallization did not completely remove traces of free phenylalanine.

Hydrolysis and isolation of the amino acid in the usual manner,[24] followed by one crystallization from water gave pure L-phenylalanine, $[\alpha]^{27}D$ −35.2° (*c* 1.8, water); $[\alpha]^{27}D$ −7.32 (*c* 3.5, *N* HCl) in agreement with reported values.[24]

Preparation of N-Acetyl-DL-phenylalanine.—DL-Phenylalanine was acetylated in 1-mole lots by the procedure previously described for acetyl-L-leucine.[29] Most of the acetylphenylalanine separated during acidification. After an additional hour at 5–10° the solid was filtered sharply by suction and washed with two 100-cc. portions of ice-water by slurrying and filtering. After drying, the product was already essentially pure; m.p. 152°; yield 195 g. (94%). The filtrate is best discarded. However, it was extracted for 24 hours with chloroform in a continuous counter-current extractor. The extract was evaporated to dryness under reduced pressure to remove chloroform, water and acetic acid. Hot acetone then extracted an additional 8 g. of product from the brown residue.

Recrystallization of N-acetyl-DL-phenylalanine from water, acetone, ethyl acetate and chloroform gave an anhydrous product (neut. equiv., 207); m.p. 152.5–153°. Literature values[30,9] range from 143 to 151°. The substance forms glistening plates from water and characteristic long hexagonal tablets from dilute acetone solutions. The solubilities (expressed throughout this paper as g./100 cc. solution at 25 ± 0.5°) are: water, 0.73; acetone, 4.31; ethyl acetate, 0.79; chloroform, 0.34. It is conveniently recrystallized from acetone.

Preparation of N-Acetyl-DL-valine.—The procedure described in the section above was followed. In 1-mole runs the initial deposit after acidification was about 80% of the theoretical yield, hence extraction of the filtrate with chloroform was essential and increased the yield of recrystallized product to an average of 92% in three runs. The melting point of the initial crop (147–148°) was raised to 148–149° by one crystallization from water or acetone and was not further changed by repeated recrystallizations from these and other solvents. Synge[31] reports m.p. 147–148°. The substance is anhydrous (neut. equiv., 159.5) and forms thin rhomboidal or diamond-shaped plates from water or acetone. The solubilities are: water, 3.71; acetone, 1.97; ethyl acetate, 0.40; chloroform, 0.30.

Resolution of N-Acetyl-DL-phenylalanine.—N-Acetyl-DL-phenylalanine (48.3 g., 0.233 mole) was suspended in 350 cc. of water, exactly neutralized to phenolphthalein with sodium hydroxid and the solution heated to boiling. A nearly boiling solution of (−)-α-fenchylamine hydrochloride (44.3 g., 0.223 mole) in about 150 cc. of water was added. Crystallization of coarse needles began at once. The solution was digested in a boiling water-bath for 30 minutes and filtered by suction while hot. The crystals were washed on the filter with two 50-cc. portions of boiling water and dried. The salt thus obtained (29.4 g.) is the nearly pure salt of acetyl-D-phenylalanine [(−)-B·D-A salt], $[\alpha]^{22}D$ −43.6° (*c* 6, methanol). The filtrate on cooling gave 12.9 g. of salt with rotation −1.8°. After evaporation to 200 cc. the filtrate slowly deposited 18.1 g. of irregular granules with rotation +21.2°. A final small crop from sirupy liquors had $[\alpha]^{22}D$ +38° but no attempt was made to purify completely the very soluble (−)-B·L-A salt. Digestion of the second crop with 100 cc. of boiling water left undissolved 4.2 g. with rotation −37.3° and systematic recrystallization of the third crop gave 4.7 g. of similar salt.

The combined levorotatory crops (38.3 g.) were crystallized from 250 cc. of methanol in several crops and thus gave 35.2 g. of the pure (−)-B·D-A salt (87% yield), m.p. 214–215°; $[\alpha]^{25}D$ −46.5° (*c* 4, methanol), values not changed by further crystallization. Methanol can be used as solvent in the resolution but is less convenient than water.

N-Acetyl-D-phenylalanine and (−)-α-fenchylamine were recovered from the salt (35.2 g.) by the general procedure already given, except that the salt was dissolved in minimum methanol (150 cc.) before alkalinization. The solution of the sodium salt was acidified with a few drops of acetic acid and evaporated to 150 cc. to remove methanol before addition of hydrochloric acid. The chilled solution deposited practically pure acetyl-D-phenylalanine. The substance was recrystallized from 175 cc. of acetone in several crops totalling 19.6 g. or 81% based on the racemic form taken. It forms coarse tablets or crusts from acetone, m.p. 171–172°; $[\alpha]^{25}D$ −47.5° (*c* 4, abs. ethanol); −40.3° (*c* 4, methanol); −38.1° (*c* 0.6, water). Slightly higher rotation values in absolute ethanol have been reported[9] but our values were repeatedly checked on samples crystallized from various solvents. The solubilities are: water, 0.85; acetone, 4.14; ethyl acetate, 0.93; chloroform, 0.16.

N-Acetyl-L-phenylalanine was recovered from the mother liquors of the resolution. The crude product (24 g., calcd., 27 g.) had $[\alpha]^{22}D$ +37.8° (*c* 4, ethanol) and hence contained about 80% of the L-form (dextrorotatory) and 20% of DL-form. Although the L-form is only slightly less soluble in acetone than the DL-form it crystallizes more rapidly, as also noted by Martin and Synge,[32] and is readily purified by two crystallizations. In this way there was obtained 18.5 g. of pure L-form (77% based on the racemic form taken), m.p. 171–172°; $[\alpha]^{25}D$ +47.5° (*c* 4, ethanol). Within experimental error the solubilities were identical with those given for the D-form. The sirupy, slightly colored acetone mother liquors slowly deposited a small amount of nearly inactive product.

D-Phenylalanine.—Pure N-acetyl-D-phenylalanine (5.0 g.) was boiled five hours under reflux with one and one-half equivalents of normal hydrochloric acid. The solution was evaporated to dryness under reduced pressure and the residue taken up in 40 cc. of 95% ethanol. The amino acid was precipitated by dropwise addition of concentrated aqueous ammonia to *p*H 6, filtered and washed freely with ethanol. The dry product (3.8 g., 98%) was already pure, $[\alpha]^{25}D$ +35.3° (*c* 1.6, water); +7.70° (*c* 4, *N* HCl); +3.48° (*c* 2, 6 *N* HCl), values not changed by recrystallization. In later experiments hydrolysis was effected in two hours with 1.2 equivalents of 3 *N* hydrobromic acid.

L-Phenylalanine.—The hydrolysis of N-acetyl-L-phenylalanine and isolation of the amino acid were carried out as just described. The initial product had $[\alpha]^{25}D$ −34.78°, changing after crystallization from water to $[\alpha]^{25}D$ −35.16° (*c* 1.6, water); −7.72° (*c* 4, *N* HCl); −3.48° (*c* 2, 6 *N* HCl). The reported value[24] in water solution is −35.1°.

Resolution of N-Acetyl-DL-valine.—Crystalline salts were formed with α-phenylethylamine and with ephedrine, but no resolution was effected by crystallization from various solvents. Resolution was easily effected with (−)-α-fenchylamine. In a typical experiment the amine (153 g., 1 mole) and acetylvaline (160 g., 1 mole) were dissolved by heating in 1800 cc. of water. Slow cooling gave massive hexagonal prisms of the N-acetyl-D-valine salt and additional crops of this salt (total 156 g.) were obtained by concentrating the filtrate in stages to 200 cc. The viscous solution then gradually solidified but the more soluble salt was not obtained pure. The less soluble salt was already substantially pure; recrystallization from aqueous methanol gave 146 g. (93%) of the pure salt, m.p. 216°, $[\alpha]^{25}D$ −4.22° (*c* 8, methanol). The solubilities are: water, 4.5; methanol, 8.5. The salt can be recrystallized from a large volume

(29) H. D. DeWitt and A. W. Ingersoll, THIS JOURNAL, **73**, 3359 (1951).

(30) A. H. Gordon, A. J. P. Martin and R. L. M. Synge, *Biochem. J.*, **35**, 1358 (1941).

(31) R. L. M. Synge, *ibid.*, **33**, 1913 (1939).

(32) A. J. P. Martin and R. L. M. Synge, *ibid.*, **35**, 117 (1941).

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

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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).