[CONTRIBUTION FROM THE FURMAN CHEMICAL LABORATORY, VANDERBILT UNIVERSITY]

The Preparation of Pure N-Acetyl-L-leucine and L-Leucine[1]

BY H. D. DEWITT AND A. W. INGERSOLL

N-Acetyl-L-leucine was prepared primarily for use as a resolving agent. This paper calls attention to the ease with which this compound can be obtained pure in good yield from technical leucine and suggests it as a convenient source of pure L-leucine. Physical constants of eight acetamino acids are recorded and considered with reference to the characterization and possible separation of amino acids.

Preparations rich in L-leucine can be obtained from many sources. A refined by-product fraction from the manufacture of sodium glutamate is cheaply available and was used in this work. Stoddard and Dunn[2] previously used similar material and purified the leucine by crystallization as the hydrochloride. The method is apparently quite effective if large end fractions are rejected. Purification through various sparingly soluble aromatic sulfonates[3] is also effective, but appears to involve handling large volumes and the sacrifice or tedious recovery of costly reagents. Thomas and Niemann[4] detected L-methionine in L-leucine purified through the β-naphthalenesulfonate. This was removed by oxidation with bromine, recrystallization as β-naphthalenesulfonate and double recrystallization from aqueous ethanol. The yield of pure leucine was small.

In the present work technical leucine (*ca.* 70%) was acetylated in 131-g. lots under non-racemizing conditions by a simplification of the method of du Vigneaud and Meyer.[5] Substantially pure N-acetyl-L-leucine was obtained in 80–95-g. yields after one crystallization of the crude product from water or aqueous methanol. Two or three further crystallizations from 33% methanol gave, with small loss, a product with properties in close agreement with reported values[6] and with those of a sample prepared in this Laboratory by resolution of the synthetic DL-form. The properties were not altered by successive crystallizations from water, methanol and acetone or by attempted further purification through salts.

The intermediate fractions and mother liquors contained other acetamino acids. Those likely to be present have been characterized in other work in this Laboratory and the properties are listed in the accompanying table. The solubilities suggest that, barring mixed crystal formation, only acetyl-L-phenylalanine might be difficult to separate from acetyl-L-leucine. It was shown in separate experiments on artificial mixtures containing 5% of acetyl-L-phenylalanine that this separation is fairly readily effected by two to four crystallizations from acetone or aqueous methanol and that acetyl-DL-leucine, which could arise from partial racemization, is easily separated by crystallization from water. The possible preparative separation of certain groups of acetamino acids by systematic crystallization is being further studied.

COMPARISON OF ACETAMINO ACIDS

N-Acetyl deriv. of	M.p., °C.	$[\alpha]^{25}D$ *c* 4, CH_3OH	Solubility[a] Water	Acetone
L-Leucine	185–186	−24.1	0.81	1.53
L-Phenylalanine	171–172	−40.3	0.85	4.14
DL-Leucine	160–161		1.93	7.10
L-Glutamic acid	196–197	− 5.6	3.64	0.21
L-Isoleucine	150–151	+11.4	4.32	11.85
L-Valine	164–165	− 0.4	6.95	5.48
L-Tyrosine	153–154	+53.1	22.7	V.s.[b]
L-Methionine	104–105	−20.4	30.7	29.5

[a] Expressed as g./100 cc. solution at 25 ± 0.2° with 1–2% precision. [b] Very soluble.

Pure N-acetyl-L-leucine was readily hydrolyzed by brief heating with hydrobromic acid and L-leucine isolated by customary methods. Crystallization from aqueous methanol gave 85–90% yields of L-leucine having properties in close agreement with reported values. In view of the probable purity of the acetylleucine, this product is believed to be essentially pure.

Experimental

Preparation of N-Acetyl-L-leucine.—The original procedure[5] was modified so as to keep the volume of the reaction mixture at a minimum and thus avoid the need to evaporate to dryness and extract the product. Technical L-leucine[7] (131 g., 1 mole, assuming pure leucine) was suspended in 350 cc. of water and acetylated with 3 moles of acetic anhydride and a solution of 7 moles of sodium hydroxide in 350 cc. of water. The reagents were added during two hours, keeping the mixture always slightly alkaline, with stirring and cooling to 5–15° by an ice–salt-bath and cooling coil. After 20 minutes the cold mixture was gradually acidified with 7 moles of 37% hydrochloric acid, chilled overnight and filtered by suction. The product (90–110 g. after washing with successive 200-cc. portions of cold water and acetone) was crystallized (carbon) from 9 parts of 33% methanol. By slow cooling from this solvent about 65% of the solute separates in characteristic large translucent prisms, m.p. 183 to 185°; $[\alpha]^{25}D$ −22.3° to −23.9° (*c* 4, methanol). Additional crystalline material totalling over 90% of the crude weight was obtained by evaporating the filtrate in stages. Small intermediate fractions with slightly lower rotations or less characteristic habit were set aside for recrystallization, usually with similar material from other runs. The final liquors gave a mixture of poorly formed crystals and pale yellow waxy solid which was reserved for later study. A partial examination of intermediate and foot fractions showed the presence of acetyl-L-methionine and small amounts of acetyl-L-isoleucine and L-phenylalanine. The yield depends on the extent to which intermediate fractions are reworked. It is possible without much work to obtain 80–95 g. (70–80%) from head fractions and from one recrystallization of collected intermediate frac-

(1) Taken from the Ph.D. thesis of H. D. DeWitt, September, 1950.

(2) M. P. Stoddard and M. S. Dunn, *J. Biol. Chem.*, **142**, 329 (1942). Earlier work is reviewed.

(3) M. Bergmann and W. H. Stein, *ibid.*, **129**, 609 (1939); W. H. Stein, S. Moore, G. Stamm, C. Chou and M. Bergmann, *ibid.*, **143**, 121 (1942); S. Moore and W. H. Stein, *ibid.*, **150**, 113 (1943).

(4) D. W. Thomas and C. Niemann, *ibid.*, **175**, 241 (1948).

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

(6) A. J. P. Martin and R. L. M. Synge, *Biochem. J.*, **35**, 91 (1941).

(7) Furnished by Corn Products Refining Co. A partial analysis supplied by the manufacturer gave the following minimum percentages: leucine, 69.5; isoleucine, 3.9; methionine, 7.8; valine, 1.7; glutamic acid, 2.0; phenylalanine, 0.9; and others not determined.

tions. Several runs carried out with only 2 moles of acetic anhydride and 5 moles of alkali gave considerably lower yields.

Purification and Criteria of Purity.—The material described above is pure enough after one recrystallization for use as a resolving agent.[8] For use in the preparation of L-leucine it was recrystallized three times more from water (15 cc./g.), or preferably by dissolving in 2.5 cc./g. of methanol and adding 5 cc./g. of hot water. If the solution became faintly cloudy on cooling the treatment with carbon was repeated. The product then had m.p. 185–185.5°; $[\alpha]^{25}D$ −24.0 ± 0.2° (*c* 4, methanol); $[\alpha]^{25}D$ −23.0° (*c* 4, abs. ethanol). Martin and Synge[6] report m.p. 183–184°; $[\alpha]^{24}D$ −22.6° (*c* 3.3, ethanol). A sample crystallized from acetone and then from water had m.p. 185–185.5°; $[\alpha]^{25}D$ −24.1° (*c* 4, methanol). It forms distinctive square tablets from dilute acetone solutions. A sample recovered from use in a resolution in which a sparingly soluble salt had been recrystallized and a sample obtained by resolution of the synthetic racemic form[9] both had m.p. 185–186°; $[\alpha]^{25}D$ −24.2°.

(8) A. W. Ingersoll and H. D. DeWitt, THIS JOURNAL, **73**, 3360 (1951).

(9) Unpublished results of Mr. W. A. H. Huffman.

Anal. Calcd. for $C_7H_{14}ON{\cdot}CO_2H$: N, 8.09; neut. equiv., 173. Found: N, 8.1; neut. equiv., 172.5.

Preparation of L-Leucine.—Thrice crystallized acetylleucine, $[\alpha]^{25}D$ −24.0° (17.3 g., 0.1 mole) was boiled under reflux with 35 cc. (0.12 mole) of 3 *N* hydrobromic acid. Solution was complete in 45 minutes but heating was continued for two hours. The colorless solution was diluted with 100 cc. of hot methanol and brought to *p*H 6 with aqueous ammonia. After cooling, the precipitate was collected on a filter and washed freely with warm methanol. The product (10.8 g.) had $[\alpha]^{25}D$ +15.3° (0.9905 g. made up to 25 cc. in 5.99 *N* hydrochloric acid). The filtrate was taken to dryness *in vacuo* and the residue digested with 100 cc. of hot methanol. Undissolved material (1.7 g.) resembled the first crop.

Several preparations made in this manner were combined, dissolved in 20 parts of water and crystallized after adding 30 parts of methanol. The product had $[\alpha]^{25}D$ +15.1° (*c* 4, 5.99 *N* HCl). A sample prepared by resolution had +15.2° under the same conditions. Stoddard and Dunn[2] give +15.2° and Thomas and Niemann[4] give +14.84° at 25° in approximately 6 *N* hydrochloric acid. Tests for ammonia, halogens, methionine and tyrosine were negative.

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The Preparation and Resolution of DL-α-Fenchylamine

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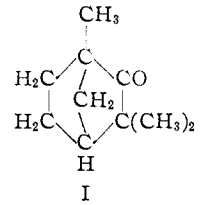
Convenient procedures are described for preparing *dl*-α-fenchylamine and for its resolution into both active forms by means of N-acetyl-L-leucine, a new resolving agent. A resolution with active mandelic acid and other procedures for obtaining the active amines also are described. The active amines are useful resolving agents.

Resolutions of *dl*-mandelic acid and *dl*-malic acid by active fenchylamines have been noted previously.[1] Further studies have shown that these amines (now designated as the α-fenchylamines) form salts of exceptional crystallizing power with a wide variety of racemic acids, many of which are resolved into their antipodes. In some ten resolutions completed thus far[2] the active α-fenchylamines have shown considerable advantages over the commonly used alkaloids, especially with respect to resolving power, chemical stability, ease of recovery, moderate molecular weight and availability of both forms.

Wallach,[3] by reduction of the oximes or by the Leuckart reaction, prepared a levorotatory fenchylamine from (+)-fenchone of fennel oil and the corresponding dextrorotatory amine from (−)-fenchone of thuja oil. These oils contain only 15–20% of the respective active fenchones and these and other sources are expensive and not sufficiently available to provide an adequate supply of the desired amines. On the other hand, a commercial *dl*-fenchone is abundantly available from the fenchyl alcohols of pine stump oil and related sources. We have readily converted this into crude *dl*-fenchylamine by a modified Leuckart synthesis.[4] The separation and resolution of this crude amine afford both active α-fenchylamines in large amounts.

Fenchone (I) may be expected to give rise to two diastereoisomeric (α- and β-) fenchylamines (II) having inverse configurations of the $CHNH_2$ group. It is apparent from our results and other



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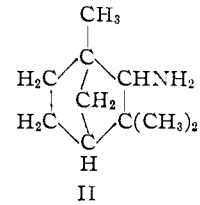

II

recent work[5] that the active amines characterized by Wallach were the predominant α-isomers. The crude amine from commercial *dl*-fenchone was found to contain 10 to 25% of the β-isomer, depending on reaction conditions. The isolation of pure amines from the mixture was further complicated by the presence in the commercial fenchone of 10–12% of (+)-fenchone, some camphor and other unidentified ketones. Accordingly, the crude amine contains the corresponding active and racemic α- and β-fenchylamines together with small amounts of bornylamines, neobornylamines and other minor amines.

The key to the separation of pure active and racemic forms of the α-amine from this mixture and from partially active fractions obtained in subsequent resolution procedures was found in the

(1) H. L. Dickison and A. W. Ingersoll, THIS JOURNAL, **61**, 2477 (1939).

(2) For examples see L. R. Overby and A. W. Ingersoll, *ibid.*, **73**, 3363 (1951).

(3) O. Wallach, *et al.*, *Ann.*, **259**, 324 (1890); **263**, 140 (1891); **269**, 358 (1892); **276**, 317 (1893).

(4) A. W. Ingersoll, J. H. Brown, C. K. Kim, W. D. Beauchamp and G. Jennings, THIS JOURNAL, **58**, 1808 (1936).

(5) W. Hückel, H. Kindler and H. Wolowski, *Ber.*, **77B**, 220 (1944); *C. A.*, **39**, 3273 (1945).