THE PREPARATION OF L-LEUCINE AND ITS BEHAVIOR IN SOME NON-AQUEOUS SOLVENTS

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Bergmann and Stein (1) have described a procedure for the isolation and purification of naturally occurring L-leucine based upon the precipitation and recrystallization of the slightly soluble salt of this amino acid with naphthalene-β-sulfonic acid. The product obtained was stated to be 97 per cent pure and to be free from methionine, a common contaminant of less pure preparations of L-leucine (2). We have found that L-leucine so prepared contains significant amounts of those amino acids which are oxidized by bromine in acid solution, and that a naphthalene sulfonate of superior purity can be obtained if the crude leucine is first treated with bromine water. The use of the preliminary bromine oxidation and of more dilute solutions than were used by Bergmann and Stein for the recrystallization of the naphthalene-β-sulfonate gave L-leucine preparations of exceptional purity when judged by qualitative tests and solubility determinations. Characteristic physical constants of L-leucine so prepared are given elsewhere in this paper.

In the determination of the optical rotation of α-amino acids in aqueous acid solutions, it has been assumed at times that the concentration of the aqueous acid is not critical provided sufficient acid has been added to assure formation of the amino acid cation. Dunn *et al.* (3) have pointed out that the specific rotation of solutions of L- and D-alanine in hydrochloric acid is dependent upon the hydrochloric acid concentration, and the data presented in Fig. 1 clearly show a similar dependency in the case of either hydrochloric or sulfuric acid solutions of L-leucine.

When solutions of L-leucine in 100 per cent sulfuric acid were allowed to stand at 25° for periods exceeding 1 month, no change in specific rotation was observed. Furthermore, the L-leucine recovered from these sulfuric acid solutions was indistinguishable from L-leucine not so treated. As either the lack of formation of the doubly charged cation, $[RCHNH_3C(OH)_2]^{++}$, or the stability of this ion, in respect to the loss of a proton from the α-carbon atom in sulfuric acid solutions, may have been responsible for the optical stability noted, the cryoscopic properties of sulfuric acid solutions of L-leucine were investigated. It was found that the freezing point

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depression of these solutions was 2.2 times that of a non-electrolyte, which would indicate that although the reaction

$$[RCHNH_3CO_2]^{\pm} + H_2SO_4 \rightarrow [RCHNH_3CO_2H]^{+} + [HSO_4]^{-}$$

is essentially complete there is relatively little ionization of the type

$$[RCHNH_3CO_2H]^{+} + H_2SO_4 \rightarrow [RCHNH_3C(OH)_2]^{++} + [HSO_4]^{-}$$

The effectiveness of the positively charged ammonium group in preventing appreciable protonation of the carboxyl group of the L-leucine cation in 100

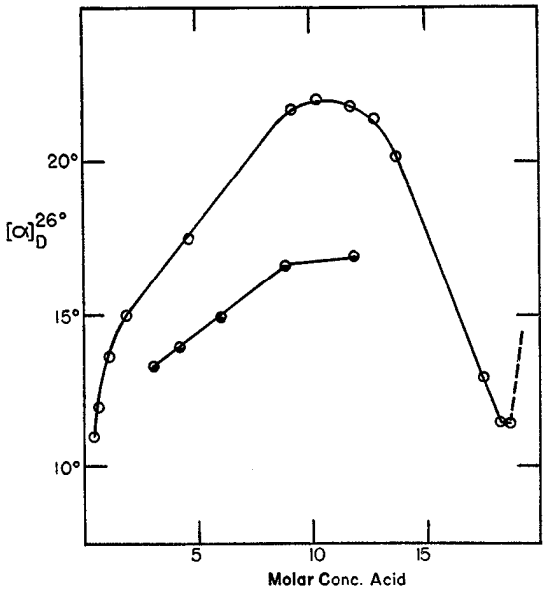

FIG. 1. Specific rotation of L-leucine in aqueous hydrochloric and sulfuric acid solutions. ○, sulfuric acid; ◒, hydrochloric acid.

per cent sulfuric acid can be appreciated when it is remembered that both acetic acid and monochloroacetic acid are completely ionized in this solvent (4).

It was observed that, when L-leucine was titrated with perchloric acid in glacial acetic acid solution (5), the specific rotation of the solution increased rapidly with added increments of perchloric acid until 1 equivalent of the acid had been added (Fig. 2). Kolthoff and Willman (6) have argued that a dipolar ion, such as glycine, would be expected to behave as a strong base in glacial acetic acid solutions because of the greater acid strength of acetic acid as compared to water and of an assumed near equivalence of the acid strengths of amino acid cation and acetic acid in solutions of the latter

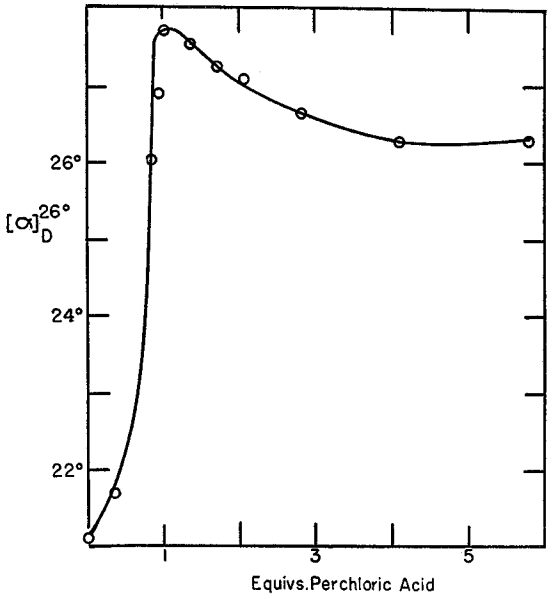

FIG. 2. Titration of L-leucine with perchloric acid in glacial acetic acid

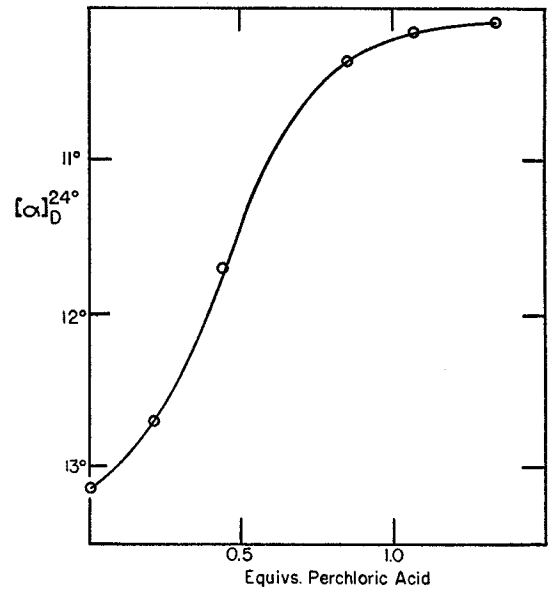

FIG. 3. Titration of *d*-α-phenylethylamine with perchloric acid in glacial acetic acid.

substance. These authors conclude, from conductivity measurements, that glycine dissolved in glacial acetic acid is present as $[CH_2NH_3CO_2H]^+$-$[Ac]^-$ and that the degree of dissociation of this ion pair is of the same order as that of potassium or ammonium acetate in the same solvent. The observed change in the specific rotation of a glacial acetic acid solution of L-leucine upon the addition of perchloric acid in the same solvent and particularly the abrupt change in the trend of the specific rotation noted at the equivalence point can be interpreted to suggest negligible or limited dissociation of the ion pair $[C_4H_9CHNH_3CO_2H]^+[Ac]^-$ in glacial acetic acid.

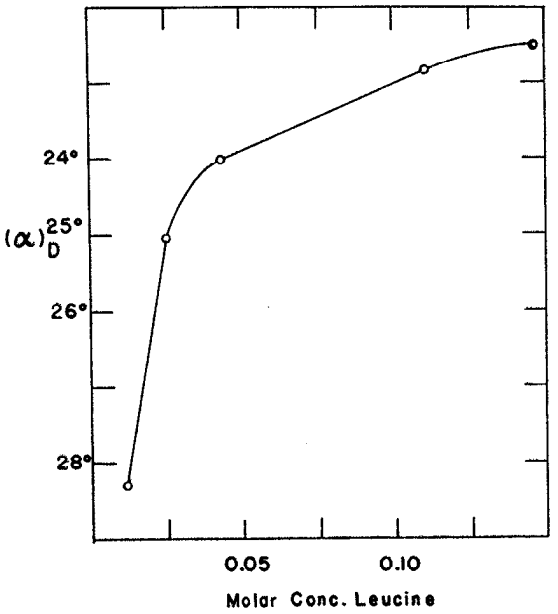

FIG. 4. Specific rotation of L-leucine in glacial acetic acid at 25°

If one assumes that the amino acid is completely ionized in glacial acetic acid solutions, the observed changes in the specific rotation of these solutions upon the addition of perchloric acid could be ascribed to the replacement of acetate ion by perchlorate ion in the undissociated ion pair. That such an effect is possible is demonstrated by the titration of a partially resolved sample of *d*-α-phenylethylamine in glacial acetic acid by perchloric acid in the same solvent (Fig. 3). In the latter case there can be no doubt that the α-phenylethylamine is completely ionized in glacial acetic acid solutions. Although rigorous evidence as to the nature of the ion species present in a glacial acetic acid solution of L-leucine is lacking, present knowledge (4, 7–9) would appear to offer little support to the

view that the amino acid can exist in glacial acetic acid solutions as the dipolar ion.

In the course of these studies, it was observed that the specific rotation of L-leucine in glacial acetic acid is strikingly dependent not only upon temperature but also upon the amino acid concentration (Fig. 4). Furthermore, it was observed that lowering the molal freezing point of solutions of L-leucine in glacial acetic acid varies with the amino acid concentration, as is shown in Fig. 5. These observations suggest that even at relatively low concentration there is extensive association of the ion pairs present in a glacial acetic acid solution of L-leucine.

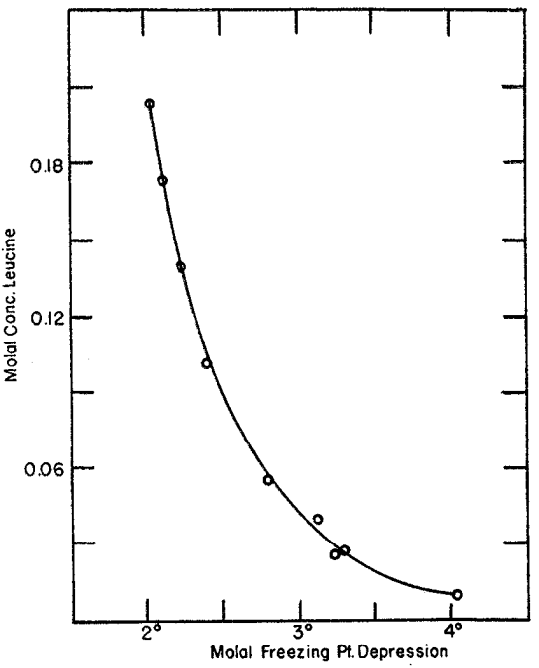

FIG. 5. Freezing point depression of solutions of L-leucine in glacial acetic acid

EXPERIMENTAL

Amperometric Titration of Leucine Preparations (*10*)—The titration of 100 mg. samples gave the following values, expressed as moles of Br_2 consumed per gm. of amino acid: a technical leucine (Lemke), 1.31×10^{-3}; a purified leucine (Hoffmann-La Roche), 3.66×10^{-4}; and leucine prepared by the method of Bergmann and Stein (1), 1.11×10^{-5}. Recrystallization of the naphthalene-β-sulfonate, beyond that advocated by Bergmann and Stein, did not prove effective in removing bromine-oxidizable impurities.

Preparation of L-*Leucine*—Saturated bromine water was added, slowly

and with stirring, to a 10 per cent (weight per volume) solution of technical leucine (Lemke) in 1 M[1] hydrochloric acid until the c lor persisted for 20 minutes after the last addition. The excess bromine was destroyed with sodium bisulfite prior to the addition of naphthalene-β-sulfonic acid as directed by Bergmann and Stein (1). The naphthalene-β-sulfonate, m.p. 189–191.5°, $[\alpha]_D^{25} = +13.3° \pm 0.1°$, 4.3 per cent (weight per volume) in methyl cellosolve, was recrystallized four times from a 7.5 per cent (weight per volume) aqueous solution and the L-leucine, obtained by the decomposition (1) of the four times recrystallized naphthalene-β-sulfonate, m.p. 191–192°, $[\alpha]_D^{25} = +13.2° \pm 0.1°$, 3.8 per cent (weight per volume) in methyl cellosolve, was recrystallized twice from 33 per cent (by volume) aqueous ethanol. The yield of the twice recrystallized L-leucine, based upon the weight of the starting material, varied between 10 and 14 per cent. Qualitative tests for the presence of sulfur (11) and of tyrosine (12) in the twice recrystallized L-leucine were negative. Examination of the absorption spectra of a 0.4 per cent (weight per volume) aqueous solution of the amino acid revealed no specific absorption in the 260 to 270 mμ region. Analysis for total nitrogen gave 10.60 ± 0.09 per cent or 99.3 ± 0.8 per cent of theory.

Solubility of L-*Leucine in Water*—An excess of twice recrystallized L-leucine was equilibrated with redistilled water at 25.05° ± 0.05°. Aliquots were withdrawn over a period extending from 5 to 30 days and the solvent was evaporated at 105°. Seventeen determinations gave a mean value of 2.152 ± 0.006 gm. per 100 gm. of water for the solubility at 25°. The undissolved leucine remaining from the above series of solubility measurements was utilized for a second series of measurements, and in this instance twelve determinations, conducted as described above, gave a mean value of 2.15 ± 0.01 gm. per 100 gm. of water at 25°. The value of 2.15 ± 0.01 gm. per 100 gm. of water for the solubility of L-leucine in water at 25° obtained above is to be compared with the value of 2.19 gm. per 100 gm. of water at 25° given by Stoddard and Dunn (9) and the value of 2.20 gm. per 100 gm. of water at 25° reported by Hlynka (13).

Optical Rotation of L-*Leucine in Aqueous Systems*—The optical rotation of the twice recrystallized L-leucine was determined in 6.02 M hydrochloric acid, with the leucine concentration varying from 2.0 to 5.0 per cent (weight per volume) and the temperature from 18–35°. The specific rotation of L-leucine in 6.02 M hydrochloric acid was found to be independent of the leucine concentration between the limits studied. The variation of the specific rotation with temperature was found to be linear within the above limits, the temperature coefficient having a value of 0.07° per 1°, in reason-

[1] Molar and molal are used in a conventional sense only and are not to be construed as indicative of the actual molecular species that may be present in solution.

able agreement with the value of 0.063° per 1° reported by Stoddard and Dunn (9) for L-leucine in 6.08 M hydrochloric acid.

The dependence of the specific rotation of L-leucine upon the concentration of the aqueous hydrochloric or sulfuric acid used as a solvent was studied, and the data obtained are presented in Fig. 1. In order to compare values for the specific rotation of L-leucine in aqueous hydrochloric acid solutions reported by others with those obtained in this study, the former values were interpolated to a temperature of 25° and a hydrochloric acid concentration of 6.0 M. The values so obtained are given in Table I.

Behavior of L-Leucine in Sulfuric Acid—The optical stability of L-leucine in 100 per cent sulfuric acid was determined by preparing a 0.1524 M so-

TABLE I

Specific Rotation of L-Leucine in 6.0 M Hydrochloric Acid at 25°

Author	Value in literature	Interpolated value*
	degrees	*degrees*
Bergmann and Stein (1)	15.33 (21% HCl, 24°)	15.24
Stoddard and Dunn (9)	15.21 (6.08 M HCl, 25°)	15.16
Dunn and Courtney (14)	15.1 (6.0 " " 25.9°)	15.03
Thomas and Niemann (this paper)	14.85 (6.02 " " 25°)	14.84

* Interpolated to 6.00 M HCl and 25°.

lution of this amino acid in 100 per cent sulfuric acid and observing the specific rotation of the solution immediately after preparation and after it had stood at room temperature in an air-tight container for 8 weeks. No significant change was observed. The solution was then diluted with water, and the amino acid was recovered by precipitation with naphthalene-β-sulfonic acid and compared with L-leucine naphthalene-β-sulfonate of known purity. The determination of the mixed melting point, nitrogen content, and specific rotation failed to disclose any differences.

The cryoscopic properties of L-leucine in 100 per cent sulfuric acid were studied by the method described by Hammett and Deyrup (15). The freezing point depression of 0.03 to 0.12 molal solutions of L-leucine in 100 per cent sulfuric acid was found to be 13.5° ± 0.2° per mole of amino acid or approximately 2.2 ± 0.05 times the depression caused by a non-electrolyte.

Behavior of L-Leucine in Glacial Acetic Acid—The specific rotation of glacial acetic acid solutions containing varying amounts of perchloric acid and 1.0 gm. of twice recrystallized L-leucine per 50 gm. of solution was determined at 26°. These data are given in Fig. 2. For comparative

purposes the specific rotation of solutions of *d*-α-phenylethylamine, $[\alpha]_D^{24} = +37.6°$, containing 0.50 gm. of the amine in 25 ml. of acetic acid-perchloric acid of varying perchloric acid concentration, was determined at 24° (Fig. 3). The freezing point depression of solutions of twice recrystallized L-leucine in glacial acetic acid was determined with the aid of a Beckmann thermometer. The results of these cryoscopic measurements are presented in Fig. 5.

SUMMARY

A procedure for the preparation of L-leucine is described and certain physical properties, useful for establishing the identity or purity, have been redetermined. The behavior of L-leucine in sulfuric acid and glacial acetic acid solutions has been investigated.

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