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Resolution of DL-Tryptophan

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ABSTRACT

A process for the resolution of a mixture of DL- tryptophan. The ratio of one desired enantiomorph to the other enantiomorph of said amino acid is increased in the crystalline compound obtained as compared to the ratio in the starting material .The resolved crystals can be separated as they have different morphological forms. Here the resolution of the tryptophan amino acids using their own D-form and L-form. The DL-tryptophan, D-tryptophan, L-tryptophan is converted to its methane sulphonate salts using methane sulphonic acid. The optical rotation of the DL-form as well as the optical rotation of the D-form and L-form added salt is checked by using polarimeter. The experiment shows a improvement in the specific optical rotation values. In another method instead of using methane sulphonate salts the D-form and L-form was taken as such.

Keywords: Amino acids, Resolution, DL-Tryptophan.

INTRODUCTION

Amino acids are the building blocks of proteins and found in structural tissues of the human body[1,2]. A number of fascinating roles associated with amino acids include pH regulation [3] neurotransmitter functioning [4] pain control, cholesterol mechanism, inflammation control, detoxification, regulators of gene expression [5] etc. Their deficiency may cause certain diseases such as, gastrointestinal insufficiencies, inadequacy of proteins, inflammatory responses, detoxification impairments, cardiovascular disease, neurological dysfunction, and inborn errors of metabolism etc. Amino acid is a molecule containing unique structure coexisted acidic COOH and basic NH_2. Except glycine existing in nature, 19 kinds of amino acids are divided as L-form and D-form by optical activity. L-form of amino acid is digested and synthesized in the living body, but D-form having an opposite optical activity cannot be. Also these abilities and effects often depend on the optical activity. Therefore proper asymmetric synthesis method would provide economically and scientifically valuable molecules with high optical purity[6]. Amino acids can also be isolated as salts[7].

It is well known that amino acids are fundamental in the field of nutrition and psychological chemistry. Of more than twenty isolated amino acids a small number have been shown to be indispensable for the maintenance of life. Man requires eight such indispensable amino acids. It has been further established that the L-optical isomers are the only ones found in proteins and produced by nature. Since the D-amino acids

are either not at all or only partially found in nature so it is desirable to separate the D-amino acids from mixtures of synthetically obtained DL-acids. From the literature and the available data it has been found that resolution of racemic acids has been mostly done by enzymatic methods[8,9]. In enzymatic process high cost is involved and they are highly sensitive to changes in physical and chemical conditions[10]. They are easily denatured by the small increase in temperature and are highly susceptible to poisons and changes in pH. Therefore the conditions they work must be tightly controlled. Enzymes substrate mixture must be uncontaminated by the other substances that might affect the reaction. The high cost of enzyme isolation and purification discourages their use.

Motivated by the above concerns we tried to do the resolution by normal chemical methods using the same D-form and L- form of the DL-amino acid which we choose.

MATERIALS AND METHODS

DL-tryptophan, D-tryptophan and L-tryptophan were obtained from S.V. Chem, Chennai. Methanesulphonic acid used was of analytical grade. Charcoal used is of commercial grade, the remaining reagent employed is of analytical grade. Sipcon polarimeter is used for measuring the optical rotation.

Preparation of DL-tryptophan methane sulphonate: 20g of DL-tryptophan and 9 ml of methanesulphonic acid are added in 100 mL of water. The solution is heated to 50°C. Clear solution is obtained, stir the solution for 30 minutes at 50°C. Then add 2 g of charcoal stir for 1 hour at 50°C. Then filter the above solution and is allowed to stand in refrigerator overnight. The crystalline precipitate thus formed is collected by filtration, washed with 50 mL of ice-water and then dried under vacuum at 50°C. Yield = 12g m.p - 218-226.

Recrystallisation of DL-Tryptophan methane sulphonate: The above sulphonate salt is recrystallized, 12 g of DL-Tryptophan methane sulphonate is dissolved in 50 mL of water. 1 g of charcoal is added and heated to 50°C and stir for 20 minutes. Cool to room temperature and stir for 30 minutes the separated solids are filtered and washed with chill water.

Optical rotation Measurement: It is necessary to take the measurement of an amino acid's optical rotation under defined conditions as its optical rotation varies with the acidity of the solvent[11]. It is necessary to specify the required path length, wavelength, temperature, solvent, and the concentration of the amino acid to measure a specific rotation. The specific rotation [α] is a measure of the optical rotation (a) at a defined concentration (c) of 1 g mL^{-1}, a path length (l) of 1 dm, wavelength of the sodium D line (598 nm) and a temperature of 25°C

$$[\alpha]_D^{25} = \alpha.100/c.l$$

Yield = 8 g m.p -224-226 $[\alpha]_D^{25} = 0.0$

Preparation of L-typtophan methane sulphonate: 10 g of L-tryptophan , 9 ml of methane sulphonic acid are added in 40 mL of water heated to 50°C clear solution obtained stir for 30 minutes at 50°C then add 1 g of charcoal stir for 30 minutes and then filter the charcoal. The filtered mL are kept in refrigerator overnight, solids are not formed.

Resolution of DL-tryptophan

Method-1: L-tryptophan methane sulphonate mL are reduced to half the volume by distillation. Then add about 8g of DL-tryptophan methane sulphonate and heated to 50°C and stirred for 30 minutes clear solution obtained then cool to 25° C and stir for 30 minutes[12]. Solids are formed. The formed solids are

filtered and washed with chill water. Then dried at 50°C under vacuum. Yield = 3.8 g, m.p -225-226, $[\alpha]_D^{25}$ = + 5.85.

Method-2: 8g of DL tryptophan methane sulphonate is dissolved in 30 mL of water and heat to 50°C clear solution obtained to the above solution 0.4g of L-tryptophan is added as such and stir for 1 hour solids are not formed so the pH is slighted adjusted to basic condition using ammonia. Solids started to form stir for 30 minutes and filter the solid. The formed solids are filtered and washed with chill water. Then dried at 50°C under vacuum. Weight of the solid = 4.2 g, m.p -225-226 $[\alpha]_D^{25}$ = + 3.25.

RESULTS AND DISCUSSION

From the above experiments results it is clear that there is an improvement in optical rotation so resolution is possible by using the L-form of the amino acid instead of using the costly resoluting agents. If we improve the process still there is scope in improving the optical rotation of the corresponding amino acid.

APPLICATIONS

L-tryptophan is considered an essential amino acid because our bodies can't make it. It is important for the development and functioning of many organs in the body. After absorbing L-tryptophan from food, our bodies convert it to 5-HTP (5-hyrdoxytryptophan), and then to serotonin. Serotonin is a hormone that transmits signals between nerve cells. It also causes blood vessels to narrow. Changes in the level of serotonin in the brain can alter mood. Most of the synthetically available amino acids are in the DL-form. So this resolution technique is useful in separation of the most useful isomer.

CONCLUSIONS

Using this technique the resolution can be carried out in different amino acids like Leucine, methionine, valine etc. The experiments can be carried out with a simple setup and with easily available chemicals. The experiment does not require any special conditions and it takes only 4 h to do the exper**i**ment except for solid isolation.

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REFERENCES

[1] Bromley et al, *ACS Chem. Biol*, **2008**, 3(1), 38-50.
[2] P. Castellino et al, *J Clin Invest*, **1987**, 80(6), 1784–1793.
[3] Wang et al, *Biochim Biophys Acta*. **2008**, 1781(11-12), 710-717.
[4] Roberts PJ Brain, *research***, 1974**, 67(3), 419-428.
[5] G Wu, *Amino acids*, **2009**, 37(1), 1-17.
[6] G. Kreil, *Science*, **1994**, 266, 996-997.
[7] P.N.V.V.L. Prameela Rani1, J. Sai Chandra, V.Parvathi, Y.Sunandamma, *Journal of Applicable Chemistry*, **2013**, 2 (2), 343-351.
[8] J.Bosch, *J. Chem. Educ.*, **1969**, 46 (10), 691.
[9] Leon Levintow, Vincent E. Price and Jesse p.Greenstein. *J. Biol.Chem.* **1950**, 184, 55-62.
[10] Sushanta Maiti, D.Rambabu, ASG Prasad, G.Venkata Rao, Mandava V.Basaveswara Rao, *Journal of Applicable Chemistry*, **2012**, 1 (4), 481-484.

[11] Ratnesh Das, Arti Saxena, *Journal of Applicable Chemistry***, 2014**, 3 (1), 426-432.
[12] Sunil Kumar T.K, Geeta Pandey, *Journal of Applicable Chemistry*, **2014**, 3 (1), 209-227.