Research Article **CODEN: AJPAD7** **ISSN: 2321 - 0923**

Asian Journal of Pharmaceutical Analysis and Medicinal Chemistry

Journal home page: www.ajpamc.com

https://doi.org/10.36673/AJPAMC.2022.v10.i02.A08

STEREOSELECTIVE SYNTHESIS OF (R)-2-AMINOPROPAN-1-OL USING TRANSAMINASE ENZYMES AND ITS COMPARATIVE STUDIES USING PLUG FLOW REACTOR

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ABSTRACT

A novel enzymatic process for amination of 1-hydroxypropan-2-one for the synthesis of (R)-2-aminopropan-1-ol (Figure No.1) was identified. (R)-2-aminopropan-1-ol can serve as a key intermediate of many pharmaceutically active compounds and it has been used as a derivatizing agent for gossypol during the analysis of gossypol enantiomers in cottonseed by high-performance liquid chromatography[1]. Single step complete conversion was achieved using Transaminase enzymes with high enantioselectivity and better yield in comparison with chemical processes. 100% chiral purity has been obtained for the enantiomerically pure intermediate, confirmed by both TLC and Chiral HPLC. Performed same reaction in flow reactor and observed lesser time for same reaction.

KEYWORDS

Transaminase enzymes, 1-hydroxypropan-2-one, (R)-2-aminopropan-1-ol, Chiral purity, Flow reactor and Gossypol analysis.

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INTRODUCTION

Chiral synthesis[2] (enantioselective synthesis, also called asymmetric synthesis) is the synthesis of a compound by a method that favors the formation of a specific enantiomer or diastereomer. It is a key process in modern chemistry and is particularly important in the field of medicinal chemistry, as the different enantiomers or diastereomers of a molecule often have different biological activities.

Chiral purity or enantiomeric excess of a pharmaceutical active intermediates can be

achieved using different methods like Asymmetric catalysis[3], Enantioselective organo-catalysis, Chiral pool synthesis, Chiral resolutions, and Bio catalysis[4]. Generally chiral synthesis involves costly organo-metallic reagents[5] and environmentally hazardous by products of metallic reagents. This reaction conditions involves multiple steps and extreme reaction conditions and high catalytic loading. There is a high need for an alternative process that could address these issues. However, bio catalysis is the only method which have environmentally friendly reaction conditions[6].

Bio catalysis can be defined as the use of natural substances that include enzymes from biological sources or whole cells that can speed up chemical reactions. Enzymes have pivotal role in the catalysis of hundreds of reactions that include production of alcohols from fermentation and cheese by breakdown of milk proteins. Recent advances in the field of scientific research have helped to understand the structure and functional activities of enzymes[7], which has in turn led to an increase in their stability, activity, sustainability, and substrate specificity. Currently, there are hundred different biocatalytic processes that have been implemented in various pharma, chemical, food, and agro-based industries.

Transaminases[8] catalyze the transfer of an amino group from a primary amine to a prochiral ketone or aldehyde. Transaminases are highly enantioselective, so they can be used for resolving racemic amines or for synthesizing chiral amines from inexpensive ketones. The ketone compound can be directly reductively aminated to our required (R)-amine compound in a single step with better enantiomeric purity than the chemical process.

By using biocatalysis technique and transaminase, we have converted the 1-hydroxypropan-2-one to of (R)-2-aminopropan-1-ol in presence of triethanolamine buffer and environmentally friendly conditions.

Flow chemistry explains chemical processes that occurs in a continuous flowing stream instead of a regular batch mode. Flow chemistry relies on concept of pumping reagents to a tubular reactor and allow the reagents to react by the pressure applied through pumps. In the field of chemical synthesis, they are used mostly in pharmaceutical chemistry for efficient synthesis of small amounts of active substances. The main concept of this work is to show the overlapping of development trends in the design of instrumentation and various ways of the utilization of specificity of chemical operations under flow conditions, especially for synthetic and analytical purposes, with a simultaneous presentation of the still rather limited correspondence between these two main areas of flow chemistry[9].

Plug flow reactors are alternatives to regular batch chemical reactors, which are evolved from flow chemistry concepts. Flow reactors widely accepted as green chemistry approach towards reducing waste generation from chemical and pharmaceutical industries. Continuous or flow process chemistry was widely incorporated in Petrochemical industry. In enzymatic synthesis damage of enzymes are major disadvantages during stirring or shaking of reaction mass which eventually leads to lesser conversion rates of desired products and high surface are in flow reactions reactors controls temperatures. To overcome these concerns, we have applied tubular column by packing the enzymes in tubular columns and circulated the liquid medium of reaction mass through pumps. As expected, enzyme was not damaged and leads to complete the reaction in shorter time cycle when compared to regular batch mode reaction[10].

MATERIAL AND METHODS

Reagents and Chemicals

In the experimental section, unless and otherwise stated, all reagents and solvents used in this study are commercially obtained. Transaminase enzymes were purchased from Iosynth, Bangalore, India and Enzyme works, China.

Methodology

Experimental Section

Experimental procedure using Batch reactor:

Step I: Preparation of Buffer Solution

In a flask, secondary butylamine (0.2g) Water (1.8ml) Triethanolamine (0.05g) and Pyridoxal phosphate (0.70mg) were taken at 25°C and cooled

to 10-15°C. pH was adjusted to 8 using Conc. HCl and the temperature was raised to 20-25°C and Water (0.5ml) was added.

Step II: Preparation of (R)-2-aminopropan-1-ol

To the buffer solution (2.69ml), Transaminase enzyme (12mg) was added. 1-hydroxypropan-2-one (2.0mg) and Dimethyl sulfoxide (0.3 ml) were charged. Temperature was raised to 40°C and maintained at same for 96 hrs. Reaction was monitored by TLC. After completion of the reaction, to the reaction mixture, 10% sodium hydroxide solution (0.2ml) was added and stirred for 30 mins at 20-25°C. The resulting reaction mass was extracted with ethyl acetate and distilled under vacuum to yield (R)-2-aminopropan-1-ol (1.7mg), which was further confirmed by sending the sample for chiral purity.

Experimental procedure using plug flow reactor:

Step I: Preparation of Buffer Solution

In a flask, secondary butylamine (2.g) Water (18ml) Triethanolamine (0.5g) and Pyridoxal phosphate (70mg) were taken at 25°C and cooled to 10-15°C. pH was adjusted to 8 using Conc. HCl and the temperature was raised to 20-25°C and Water (5ml) was added.

Step II: Preparation of (R)-2-aminopropane-1-ol

To the buffer solution (26.9ml), Transaminase enzyme (Enzyme EW-ATA-R123) (60.0mg) was added. 1-hydroxypropan-2-one (120.0mg) and Dimethyl sulfoxide (3ml) were charged. Pass the reaction mixture into flow reactor through flow pump at 40°C for 27 hrs at flow rate 0.2ml /min. Reaction was monitored by TLC, reaction was completed after three cycles. After completion of the reaction, filtered the reaction mass through hyflow, to the reaction mixture added 10% sodium hydroxide solution (0.2ml) and stirred for 30 mins at 20-25°C. The resulting reaction mass was extracted with ethyl acetate and dried over anhydrous Sodium Sulfate and distilled under vacuum to yield (R)-2-aminopropan-1-ol (95.0mg).

TLC Conditions

To the TLC plate, were applied spots of our keto compound and final product which was immersed in a mobile phase of following composition- Dichloromethane: Methanol = 9:1 respectively. The plate was then viewed under UV light of 254nm after sprayed with Ninhydrin (1% in ethanol).

RESULTS AND DISCUSSION

Several variants of transaminase enzymes were screened in the following study out of which the below 6 variants showed significant product formation and the results were observed as follows in Table No.1.

Product conversion observed by TLC (Figure No.2) for our required (R)-amine compound with 100% chiral purity is shown below for the mobile phase conditions (MDC (9): Methanol (1)) previously described.

The chiral HPLC data in column chiralpak 1G (250X4.6) mm 3micron for the intermediate prepared through synthetic route is as follows

For 100% Chiral purity in column chiral pak 1G (250X4.6) mm 3micro our desired (R)-amine compound prepared using transaminase enzymes, the Chiral HPLC data is as follows:

Table No.1: Screening data of different transaminase enzymes used for the synthesis of (R)-2-aminopropan-1-ol

S.No	Enzyme Variant	Product	Time taken for reaction completion	Chiral Purity	Enzyme Loading with respect to 1-hydroxypropan-2-one
1	ML-TA-228	(R)-2-aminopropan-1-ol	96 HRS	100%	6 TIMES
2	ML-TA-231	(R)-2-aminopropan-1-ol	96 HRS	100%	6 TIMES
3	ML-TA-244	(R)-2-aminopropan-1-ol	96 HRS	100%	6 TIMES
4	ML-TA-254	(R)-2-aminopropan-1-ol	96 HRS	100%	6 TIMES
5	EW-ATA-6	(R)-2-aminopropan-1-ol	96 HRS	100%	6 TIMES
6	EW-ATA-R123	(R)-2-aminopropan-1-ol	96 HRS	100%	6 TIMES
7	CN102-ATA-LP016	(R)-2-aminopropan-1-ol	96 HRS	51.3%	6 TIMES
8	CN102-ATA-LP049	(R)-2-aminopropan-1-ol	96 HRS	56.27%	6 TIMES
9	CN102-ATA-LP016	(R)-2-aminopropan-1-ol	96 HRS	51.53%	6 TIMES
10	CN102-ATA-LP019	(R)-2-aminopropan-1-ol	96 HRS	51.73	6 TIMES
11	CN102-ATA-LP021	(R)-2-aminopropan-1-ol	96 HRS	51.71%	6 TIMES

Table No.2: Comparison of transamination reaction in batch reactor and plug flow reactor for the synthesis of (R)-2-aminopropan-1-ol

S.No	Enzyme Variant	Reactor	PRODUCT	Time taken for reaction completion	Chiral Purity	Enzyme Loading with respect to 1-hydroxypropan-2-one
1.	EW-ATA-R123	Batch reactor	(R)-2-aminopropan-1-ol	96 HRS	100%	6 TIMES
2.	EW-ATA-R123	Flow reactor	(R)-2-aminopropan-1-ol	27 HRS	100%	6 TIMES
3.	EW-ATA-R123	Flow reactor	(R)-2-aminopropan-1-ol	27 HRS	90.09	0.5 TIMES
4	EW-ATA-R107	Flow reactor	(R)-2-aminopropan-1-ol	27 HRS	100%	0.5 TIMES
5	EW-ATA-R132	Flow reactor	(R)-2-aminopropan-1-ol	27 HRS	72.24	0.5 TIMES
6	CN102-ATA-LP016	Flow reactor	(R)-2-aminopropan-1-ol	27 HRS	61.3	0.5 TIMES
7	CN102-ATA-LP049	Flow reactor	(R)-2-aminopropan-1-ol	27 HRS	56.8	0.5 TIMES
8	CN102-ATA-LP016	Flow reactor	(R)-2-aminopropan-1-ol	27 HRS	52.15	0.5 TIMES
9	CN102-ATA-LP019	Flow reactor	(R)-2-aminopropan-1-ol	27 HRS	51.26	0.5 TIMES

Reaction scheme for the synthesis of (R)-2-aminopropan-1-ol

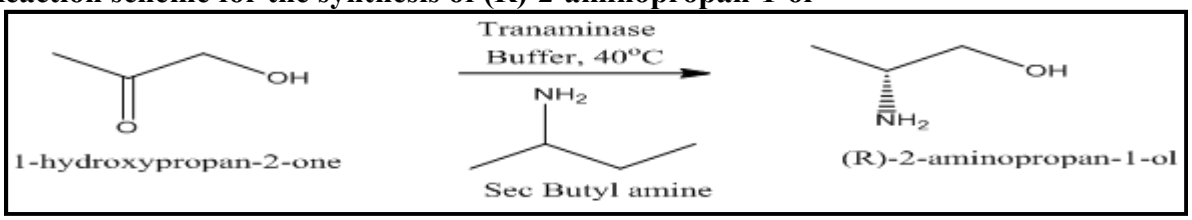

Figure No.1: Reaction scheme for the synthesis of (R)-2-aminopropan-1-ol

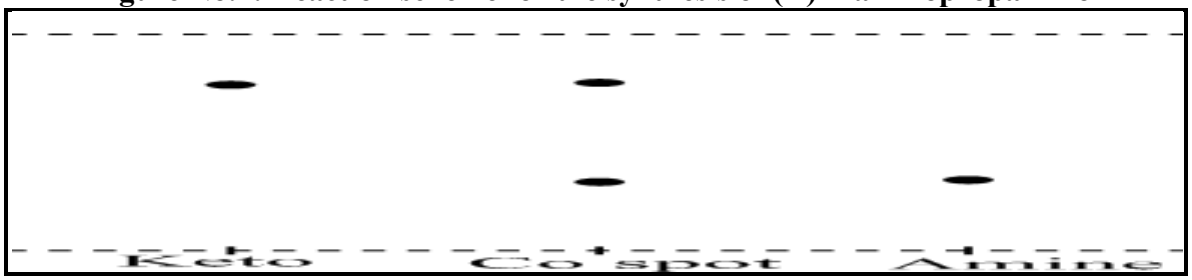

Figure No.2: TLC of starting material and product conversion

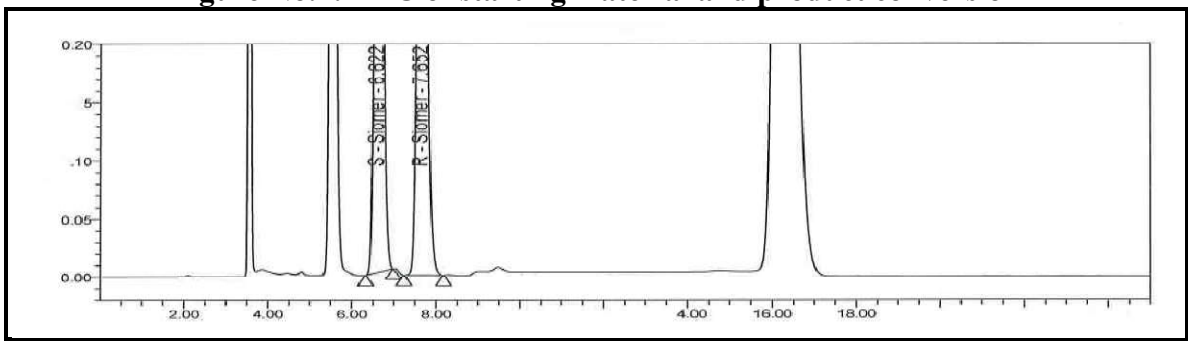

Figure No.3: Chiral HPLC chromatogram of 2-aminopropan-1-ol by using synthetic route

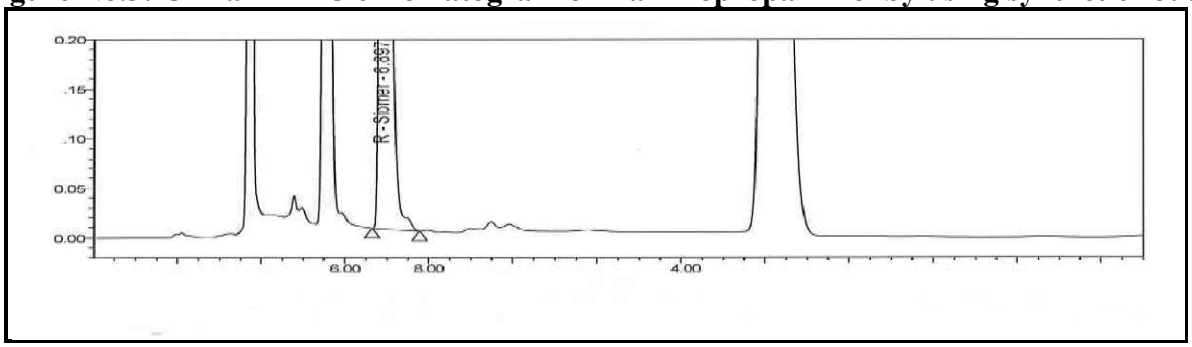

Figure No.4: Chiral HPLC chromatogram of (R)- 2-aminopropan-1-ol by using transaminase enzyme

CONCLUSION

It can be determined from the above study that, (R)-2-aminopropan-1-ol which can serve as key chiral intermediate of the many active pharmaceutical ingredients.(R)-2-aminopropan-1-ol can be prepared using transaminase enzyme with 100% chiral purity and 100% yield in a single step opposed to conventional chemical processes involving multiple steps with poor yields and low enantioselectivity. Time for the conversion of 1-hydroxypropan-2-one to (R)-2-aminopropan-1-ol was less in flow reactor (27 hours and Enzyme loading is 0.5 times) as compared with Batch reactor (96 hours) and enzyme loading is 6.0 times with respect to 1-hydroxypropan-2-one.

ACKNOWLEDGEMENT

Our group is thankful to Department of Scientific and Industrial Research India, Sanjeev Sethi (COO and Chief Scientific Office Mylan Inc); Dr. Abhijit Deshmukh (Head of Global OSD Scientific Affairs); Jyothi Basu {Head - Global API (Active Pharmaceutical Ingredients)}; Dr Chandra Has Khanduri (Head of Global API R and D and Scientific Affairs, R and D); Dr Arvind Kumar (Head of Analytical Dept. MLL API R and D) as well as analytical development team of Mylan Laboratories Limited for their encouragement and support. We would also like to thank Dr. Narahari Ambati (AGC- India IP), his Intellectual property team for their support.

CONFLICT OF INTEREST

We declare that we have no conflict of interest.

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Please cite this article in press as: Madhuresh Kumar Sethi *et al*. Stereoselective synthesis of (r)-2-aminopropan-1-ol using transaminase enzymesand its comparative studies using plug flow reactor, *Asian Journal of Pharmaceutical Analysis and Medicinal Chemistry,* 10(2), 2022, 58-63.