*Asian Journal of Chemistry* Vol. 20, No. 5 (2008), 3502-3506

# Bioconversion of Mandelonitrile to Mandelic Acid using Plant extracts from Barley, Cabbage and Radish

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Bioconversion of mandelonitrile to R-mandelic acid, an α-hydroxy phenyl acetic acid, using crude plant extracts reported here. Chemical process of conversion of mandelonitrile yields both R- and S-enantiomers of mandelic acid. The objective was to convert it to only R- enantiomers. Nitrilases are capable of synthesizing only R-enantiomers. Some plants have been reported to be rich source of nitrilases. Based on the screening results of plants containing nitrilases; cabbage, radish and barley extracts were used for this purpose. The conversion rate was maximum (15 g) with barley leaf extract at 28 ºC; cabbage and radish converted 3.23 and 2.45 g, respectively at 32 ºC. The preliminary results indicated plants can be used for conversion of mandelonitrile to mandelic acid; though lot of optimization and various parameters need to be studied further.

**Key Words: Mandelic acid, Mandelonitrile, Nitrilase, Barley, Cabbages, Radish.**

## INTRODUCTION

Bioconversion or biotransformation is mostly being tried using microbes as source of enzyme[1]. However plants have not been considered as source of nitrilase for this conversion. Since nitrilase is not readily available, crude plant extracts as the source of enzyme was tried. Thimann and Mahadevan[2] have extracted and purified nitrilase from plants.

Mandelic acid, an α-hydroxy acid named after the German mandel (almond) and derived from the hydrolysis of an extract of bitter almonds has been studied extensively for its anti-aging and antimicrobial effect. It is prepared by a chemical method from benzaldehyde. This reaction is not specific and results in the formation of both R (-) mandelic acid and S (+) mandelic acid which are enantiomers of each other (**Scheme-I**).

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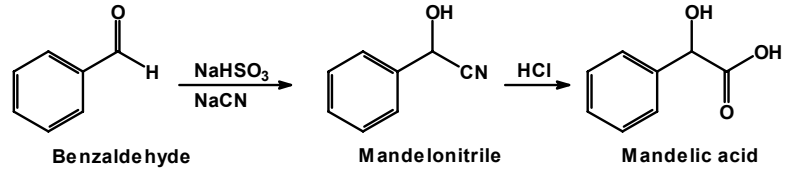



**Scheme-I**. Production of mandelic acid from benzaldehyde

This conversion requires harsh conditions and generates significant hazardous waste. In contrast enzymatic hydrolysis of nitriles can be performed under mild conditions. To acquire only R (-) mandelic acid (which has a great demand in skin care industry)[3,4], racemization of the S (-) mandelic acid is carried out to form R (-) mandelic acid. Nitrilase enzyme is specific for the production of R (-) mandelic acid. Unfortunately, there is no nitrile-hydrolyzing enzyme preparation commercially available at present. Therefore, a process using extracts of different plants as source of nitrilase for converting mandelonitrile to mandelic acid is presented here.

## EXPERIMENTAL

Mandelonitrile and mandelic acid was provided by R.L. Chemicals, Ambernath, India.

**Plants as enzyme source:** The leaves of young seedlings of barley, cabbage and radish were taken as the source of enzyme nitrilase.

**Extraction of enzyme from plants:** Leaves were cleaned with distilled water and cut in to small pieces. 10 g of cut leaves of each plant material was taken separately in a pre-cooled mortar to which 5 mL of potassium phosphate buffer (to control the pH of the extract and stabilize the protein), 2 mL of $1 \times 10^{-3}$ M EDTA (for chelating metal ions), 0.2 g potassium chloride (to maintain the standard cellular environment), 1 mL of $1 \times 10^{-3}$ M cysteine (for preventing oxidation of proteins), 5 mL of 5 % glycerol (to reduce the polarity) and a pinch of phenyl methyl sulfonyl fluoride (as protease inhibitor) was added. The mixture was grinded using mortar and pestle, filtered through muslin cloth and then centrifuged at 10000 rpm at 4 ºC for 15 min. The extract obtained was used as a source of enzyme. Activity of nitrilase was assessed using conversion of acetonitrile to acetic acid and detected using paper chromatography. The mobile phase was 10:1:1 *iso*-propanol:ammonia:water and indicator was 0.4 % bromo-cresol purple in ethanol.

**Bioconversion of mandelonitrile to mandelic acid:** 2 mL of plant extract was added to 2 mL 0.1 M potassium phosphate buffer of pH 8.0 in each test tube and to this mixture 2 mL of neutralized mandelonitrile was added. The samples were incubated at 28, 30 and 32 ºC for 24 h. After the

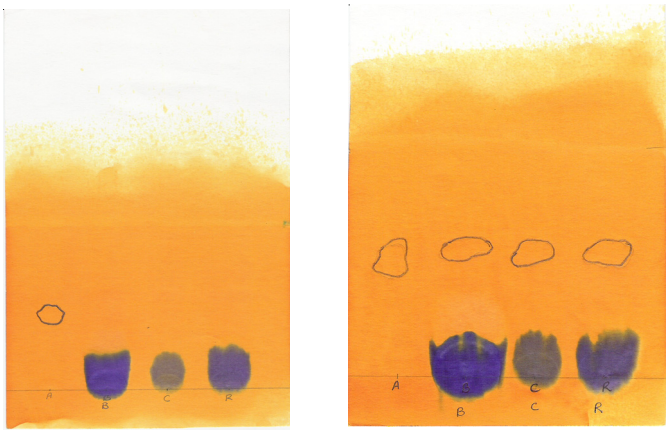
appropriate incubation period, presence of mandelic acid was analyzed using thin layer chromatography (to detect the presence of mandelic acid) and high performance liquid chromatography (to quantify the mandelic acid formation).

**Thin layer chromatography:** Silica gel F254 plates were activated at 110 ºC for 20-30 min. A mobile phase of toluene:dioxane:acetic acid (90:25:4) was used. The sample (0.1 g) was dissolved in 5 mL of methanol. The sample solution (5 µL) along with the reference solution (5 µL) was loaded on the TLC plates. Chromatogram was run till the solvent reached 3/4th of the TLC plate. The plates were dried and observed under UV light

**High performance liquid chromatography:** $C_{18}$ (Octyl decylsilinazized) column was used. The mobile phase had 0.01 M phosphoric acid: acetonitrile:methanol (70:27:3). 300 mg of the sample was dissolved in 25 mL mobile phase and filtered. The sample solution (20 µL) was injected. A UV detector of 240 nm was used. Flow rate was kept at 1.0 mL/min. 1 mg/mL standard mandelic acid was used as the reference sample.

## RESULTS AND DISCUSSION

Presence of enzyme nitrilase in all the three tested plants was confirmed by conversion of acetonitrile to acetic acid detected by paper chromatography (Fig. 1). Moreover, Pace and Brenner[3] have demonstrated that nitrilase and nitrile hydralase (in combination with amidases) are the two enzymes, which are mainly involved in the hydrolysis of nitrile compounds in plants, animals and microorganisms. They carry out either one step or two-step enzymatic reaction. Nitrilase catalyzes the mild hydrolytic conversion of organonitriles directly to the corresponding carboxylic acids.




Before reaction After reaction

Fig. 2. Paper chromatography for acetic acid; A- Authentic acetic acid, B-Acetonitrile treated with barley extract, C- with Cabbage extract and R- with Radish extracts showing presence of acetic acid)

The $R_f$ values obtained also confirmed (Table-1) conversion of acetonitrile to acetic acid by plant extract suggesting nitrilase activity.

TABLE-1
$R_f$ VALUES OF CONVERTED ACETONITRILE TO ACETIC ACID WHICH CONFIRMED THE ACTIVITY OF NITRILASE

| Sample | $R_f$ of Spots |
|---|---|
| Standard acetic acid | 0.48 |
| Barley leaf extract | 0.49 |
| Cabbage extract | 0.48 |
| Radish extract | 0.49 |

TLC data ($R_f$) of mandelonitrile treated with plant extracts showed presence of mandelic acid. Nitrilases from the different plant extracts showed optimum conversion at different temperatures. It was 32 ºC for cabbage and radish, whereas 28 ºC for barley. Since at other tried temperatures (as mention in the material and methods) no conversion was observed. it should be mentioned here that conversion of mandelonitrile to mandelic acid was highly temperature specific.

TABLE-2
TLC $R_f$ VALUES OF MANDELIC ACID FORMED FROM MANDELONITRILE TREATED WITH DIFFERENT PLANT EXTRACTS CONFIRMING IT DUE TO NITRILASE IN THE PLANT EXTRACTS

| Source of nitrilase | $R_f$ value | Incubation temp. (ºC) |
|---|---|---|
| Authentic sample | 0.20 | – |
| Cabbage extract | 0.20 | 32 |
| Radish extract | 0.19 | 32 |
| Barley extract | 0.21 | 28 |

HPLC also showed peak for mandelic acid. However three more distinct peaks for mandelonitrile, benzaldehyde and benzoic acid were observed in all the samples. Mandelonitrile peak must have been due to presence of left over or unconverted mandelonitrile.

Since, mandelonitrile provided for the present experiment was synthesized from benzaldehyde, there are chances of having residual benzaldehyde in the sample, accounting for a benzaldehyde peak. Moreover at neutral pH mandelonitrile has a tendency to deteriorate in to benzaldehyde, which may get oxidized to benzoic acid. Thus, a benzoic acid peak was also observed.

TABLE-3
MANDELONITRILE CONVERSION TO MANDELIC ACID BY ENZYME EXTRACT'S FROM DIFFERENT PLANTS

| Crude enzyme extracts from | Conversion of 100 g mandelonitrile to mandelic acid (g) by enzyme extract |
|---|---|
| Barley | 15.00 |
| Cabbage | 3.23 |
| Radish | 2.45 |

Using the HPLC data amount of mandelic acid formed was calculated. Barley was found to be a better source of nitrilase. Cabbage and radish were more or less the same.

## ACKNOWLEDGEMENTS

The authors wish to acknowledge Dr. Naresh Chandra, Principal Birla College and Dr. Mrs. Anita Phatak for their support.

## REFERENCES

1. K. Yamamoto, K. Oishi, I. Fujiasmstsu and K. Komatsu, *Appl. Environ. Microbiol.*, **57**, 3028 (1991).
2. K.V. Thimann and S. Mahadevan, From the Biological Laboratories, Harvard University, Cambridge, Massachusetts, U.S.A, Occurrence, Preparation and General Properties of the Enzyme, 9 September (1963).
3. H.C. Pace and C. Brenner, *Genome Biol.*, **2**, 1.1 (2001).
4. M. Daniel, Basic Biophysics for Biologists, Anees Offset Press, New Delhi, Chromatographic Techniques.

(*Received*: 20 June 2007; *Accepted*: 4 February 2008) AJC-6280