

**NOTE****Antibacterial Agent from Ninhydrin**

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Ninhydrin, a well known reagent for both the detection and quantitative estimation of amino acids, is a non-bioactive compound against some bacterial organisms. On heating with concentrated sulfuric acid-nitric acid mixture (1:1; v/v) for prolonged period on water bath, ninhydrin gave a compound which showed high activity against some bacterial organisms (*Bacillus subtilis*, *Escherichia coli* and *Salmonella* sp.). Infrared spectral analysis, <sup>1</sup>H NMR study and ultimate X-ray studies showed the prepared compound was phthalic acid.

**Key Words: Ninhydrin, Bacterial organism, Antibacterial agent.**

Ninhydrin (indane-1,2,3-trione hydrate) is a well known compound which is used as a reagent (spraying) for the detection as well as quantitative estimation<sup>1</sup> of amino acids and its detection limit for amino acids is very high<sup>2</sup>. But there is no evidence to use ninhydrin as an antibacterial agent in literature. On prolonged heating with concentrated sulfuric acid-nitric acid mixture (1:1, v/v), it produced a compound which showed considerable activity against some bacterial micro-organisms (both gram positive and gram negative organisms) like *Bacillus subtilis*, *Escherichia coli* and *Salmonella* sp. This is a part of our programme to convert some common non-bioactive compounds to bioactive agents by synthetic way.

Ninhydrin was obtained from Sigma Chemical Co., USA. Sulfuric acid and Nitric acid were obtained from Qualigens, India and E. Merck, India, respectively.

**Synthesis of compound A:** Ninhydrin (solid) was heated with concentrated sulfuric acid-nitric acid mixture (1:1, v/v) for prolonged period (3 h) on water bath. A white crystalline compound was obtained at the bottom of the flask and that compound was initially named as **A**. Compound **A** was filtered off, washed well with cold water and again recrystallized from acetone. Then inhibitory tests was performed with this compound.

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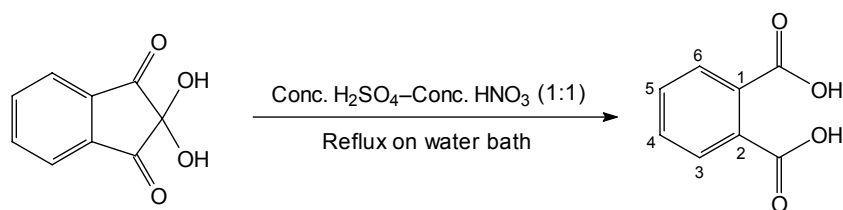
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**Inhibitory test with compound A:** Bacterial organisms (gram positive and gram negative microorganisms) taken for the experiment were *Bacillus subtilis*, *Escherichia coli* and *Salmonella* species. Each of the solutions of compound A and ninhydrin in acetone (concentration: 2 mg/mL in each case) was given separately to observe its activity against the aforementioned micro-organisms. The cup bore method<sup>3</sup> was used to perform susceptibility test<sup>4</sup>. The zones of inhibition were estimated visually and data were placed in Table-1.

TABLE-1  
ANTIBACTERIAL ACTIVITY OF COMPOUND A AND  
NINHYDRIN ON SOME MICROORGANISMS

Name of the compound	Inhibition zone (mm)		
	<i>Escherichia coli</i> gram negative	<i>Bacillus Subtilis</i> gram positive	<i>Salmonella sp.</i> gram negative
Compound A	15	15	15
Ninhydrin	–	–	–

Cup diameter = 9.0 mm; Sample used : 0.1 mL/cup; Concentration of sample : 2 mg/mL; Medium used for assay : Nutrient agar.



**Identification of compound A:** The compound A is white crystalline compound having melting point 200 °C and soluble in acetone. Spectral analysis of the compound A was done in the following way to determine its structure. Infrared spectral analysis of the compound was done by FT-IR of Perkin Elmer Pvt. Ltd., Singapore (Model No. Spectram RX-1) in our department (Department of Chemistry, University of Burdwan, Burdwan, India).

**IR Spectral analysis:** (KBr,  $\nu_{\max}$ ,  $\text{cm}^{-1}$ ): 3087-2528 (strong and multiple; OH str.- a part of -COOH group), 1689 (C=O str.), 1586, 1496 (aromatic group frequencies), 1281 (C-O str.).

$^1\text{H}$  NMR spectral analysis:  $\delta_{\text{H}}$  (200 MHz, acetone- $d_6$ ) 7.59-7.68 (m, 2H, H-3, H-6,  $J = 1.00$ ), 7.73-7.79 (m, 2H, H-4, H-5,  $J = 1.05$ ).

**X-ray crystallography:** From the above spectral analysis and picture of X-ray crystallography (Fig. 1), it may be concluded that compound A is phthalic acid. From the Table-1 it may be noted that the product obtained

from ninhydrin showed profound activity against the test microorganisms and the product **A** *i.e.*, phthalic acid was the first time synthesized from ninhydrin by the following procedure.

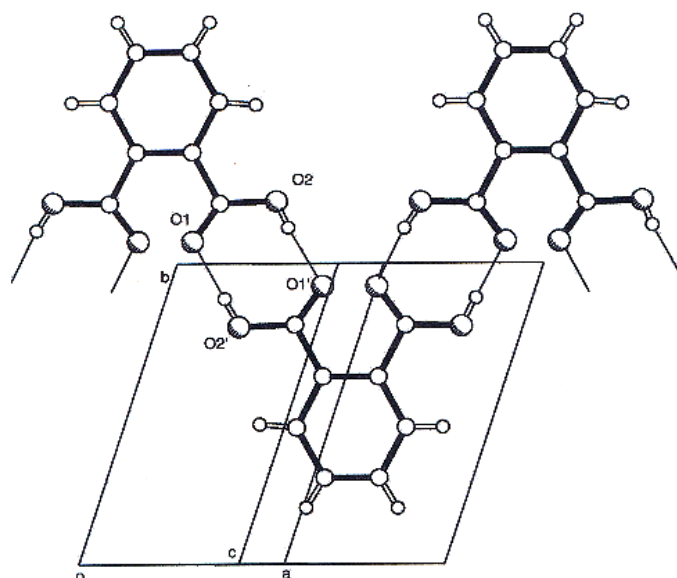


Fig. 1. X-Ray spectral analysis of compound **A**

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