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Synthesis of 2,6-diamino-3-phenyl-5-phenylazopyridine hydrochloride: An Impurity in the Process for Phenazopyridine Hydrochloride a Genito-urinary Antiseptic Drug

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Abstract— 2, 6-diamino-3-phenyl-5-phenylazopyridine hydrochloride an impurity formed in the process for the genito-urinary antiseptic drug Phenazopyridine was isolated and its structure was confirmed by independent synthesis using Suzuki reaction in a key step.

Keywords : 2, 6-diamino-3-phenylpyridine, 2, 6-diamino-3-phenyl-5-pheylazopyridine hydrochloride.

INTRODUCTION

A genito-urinary antiseptic drug[1] first introduced by Merck & Co in 1928 under the trade name pyridium is the HCl salt of the free base 2, 6-diamino-3-phenylazopyridine[2, 3] also known as Phenazopyridine hydrochloride(PPHCl). The chronology of development of the commercial process for Phenazopyridine began with the discovery by Chichibaban[4] of the reaction of pyridine with Sodamide to get 2, 6-diaminopyridine **1** followed by Diazo coupling with benzene diazonium chloride. Initially it was reported[5, 6] that the diazo coupling results in the formation of compound **3** as the major product along with side products **4** and **5** depicted in **Scheme 1.** The isomeric gamma coupled product **4** was synthesized[7] by an authentic route and found to be not formed in the coupling reaction shown in **Scheme 1**.

MATERIALS & METHODS

Analytical HPLC: Waters 2996 with PDA Detector and Pump e 2695 was used. The analysis was carried out on Intersil ODS-3 (100 mm x 4.6 mm i.d.: particle size 3 µm, 254 nm). The mobile phase consists of variable mixtures of degassed water and acetonitrile in the ratio given in Table I. The flow rate was1.0 ml/min and sample injection volume was 10 µl.

Sample preparations: Solution of PPHCl required for HPLC analysis was prepared by first dissolving 25.0 mg in 5 ml of a solvent mixture consisting of a mixture of acetonitrile and water in volume ratio 9:1 and then making it up to 25 ml.

Prep HPLC: The system consisted of a Jasco pump 2087 plus model with MD 2010 plus detector and intelligent prep-pumps. An Intersil column (250 mm x 20 mm i.d.: particle size 10 µm) flow rate of 8 ml/min and volume 1 ml was used. The mobile phase used was the same as in analytical HPLC.

The mass spectra were recorded on (QSTARXL, Applied Biosystem/MDS sciex, USA) equipped with an electron spray ionization (ESI).

1. **Isolation of impurity from PPHCl and identification:** This consists of two steps described below.

   a) Enrichment of PPHCl with the impurity

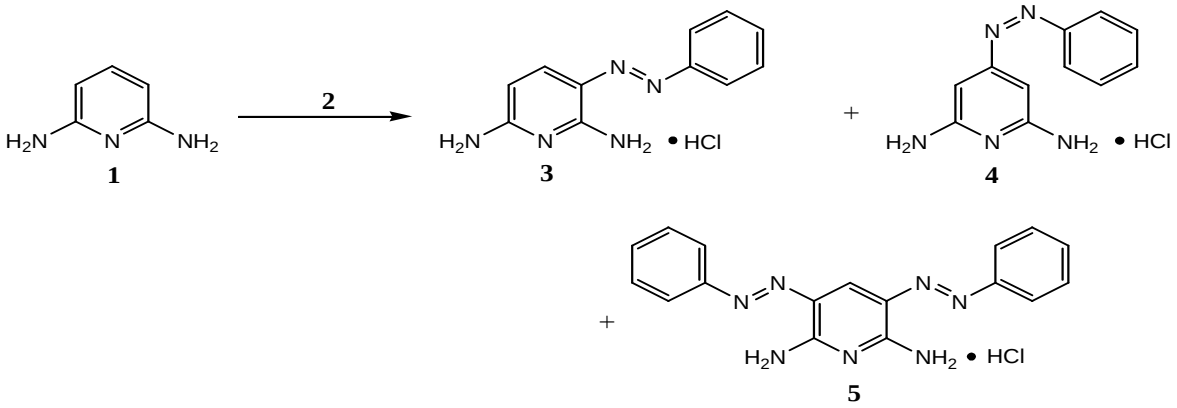
   The technical grade PPHCl isolated from the diazo coupling of benzene diazonium chloride with 2, 6-diaminopyridine contains only traces of the impurity with Rt=11.437 min. Repeated extraction of tech PPHCl with limited quantity of acetone and removal of solvent gave a product which is enriched with the impurity to the extent of 15%.

   b) Preparative HPLC

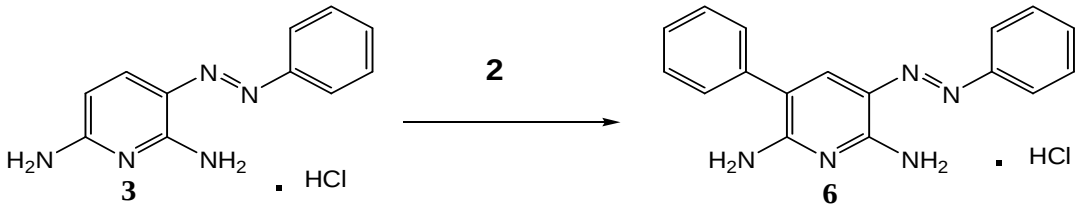
   PPHCl enriched with the impurity was subjected to preparative HPLC and the component eluting at 11.947 was collected, concentrated to dryness to get a solid. HRMS: M+1 at m/e 290.1415, calculated for $C_{17}H_{16}N_5$ 290.1405.

2. **Synthesis of 2, 6-diamino-3-phenyl-5-phenylazopyridine hydrochloride 6**

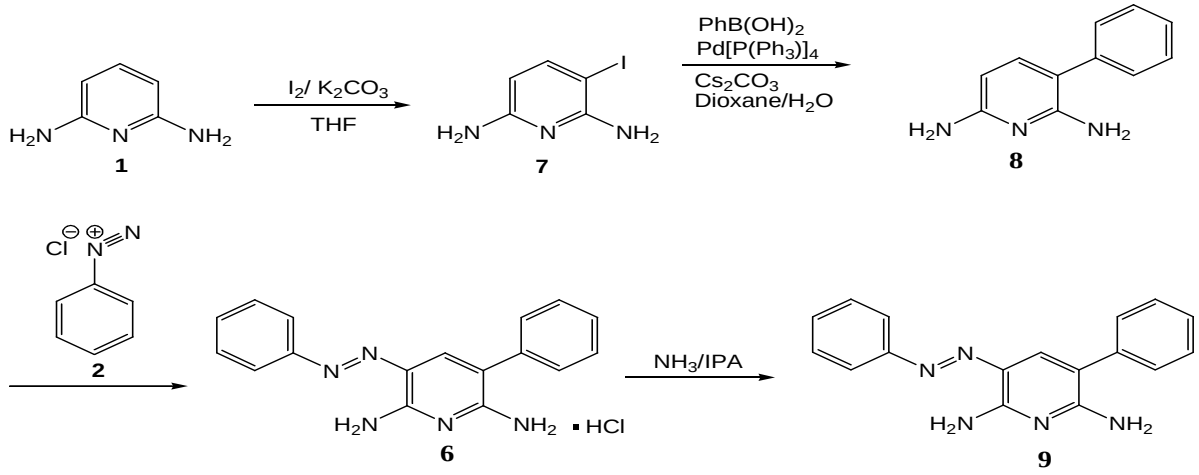
Scheme-1



Scheme 2



Scheme 3



1. *2, 6-diamino-3-iodopyridine* **7**

   The compound was prepared by iodination of 2, 6-diamiopyridine with iodine in presence of K_2CO_3 as described in literature[12].

2. *2, 6-diamino-3-phenylpyridine* **8**

The procedure for Suzuki coupling was adapted from patent literature[14]. The reaction was carried out under nitrogen atmosphere. 1, 4 dioxane (75) ml and water (75) ml were taken in a 250ml 4 necked RB flask equipped with stirrer, condenser and gas inlet tube. Nitrogen gas was bubbled for 30 min at RT. 15g (0.081 moles) 2, 6-diamino-3-iodopyridine followed by 6g phenylboronic acid, 20.8g cesium carbonate and 0.92g tetrakis (triphenylphosphine) palladium were added at RT to the stirred solvent mixture. The reaction mixture was then heated and maintained approximately at 80ºC. The reaction mixture was then refluxed for 18 hrs, concentrated under vacuum till removal of dioxane. The resulting suspension was extracted with ethyl acetate. The ethyl acetate extract washed with brine, dried over anhydrous sodium sulphate and concentrated. The solid residue was subjected to column chromatography on silica using a mixture consisting of 30% ethyl acetate in hexanes as Eluent. The fraction containing phenylboronic acid and 2, 6-diamino-3-iodopyridine elute first followed by 2, 6-diamino-3-phenylpyridinre. Yeild = 6.6g, m.p. 99-103ºC. ^{1}HNMR ($CDCl_3$, δ ppm) 5.7 (br, 4H, D2O exchangeable), 5.95 (d, 1H), 7.25(d, 1H), 7.31-7.45 (5H).

3. *2,6-diamino-3-phenyl-5-phenazopyridine hydrochloride* **6**

4.2g of 2, 6-diaminopyridine was dissolved in HCl (3.6 ml conc. HCl diluted with 15 ml water) at RT. A cold solution benzene diazonium chloride (prepared by diazotization of 3.7g aniline using 2.78g sodium nitrate and 10 ml conc. HCl at 0-5ºC). The cold solution of benzene diazonium chloride to pyridine derivative over a period of one hour. During this period the diazocoupled product gets precipitated as it is formed. After stirring 4 hrs at ambient temperature the reaction mixture was filtered, washed sequentially with water, 2% HCl, acetone and dried to get 2,6-diamino-3-phenyl-5-phenylazopyridine hydrochloride. Orange crystalline powder. Yeild-2.7g, m.p.-227-229ºC.

a) *2, 6-diamino-3-phenyl-5-phenazopyridine* **9**

2,6-diamino-3-phenyl-5-phenazopyridine hydrochloride (600 mg) was suspended in 40 ml IPA saturated with ammonia. Mixture is refluxed under stirring for 40 min. The clear solution formed was first allowed to cool to room temperature and then in ice bath. The solid formed was filtered and freed from ammonium chloride by water wash. The free base **9** was dried under vacuum. Red crystalline powder. Yeild 150 mg. m.p.-179-180ºC. ^{1}HNMR (DMSO – d6, δ ppm) 7.74 (d, 2H), 7.63 (s, 1H), 7.28-7.35 (overlapping multiplates, 2H), 7.42-7.46 (6H). The exact position of NH_2 signals could not be identified. The compound exhibits the highest mass peak at m/e 290.1414 in the High Resolution Mass Spectrum corresponding to the M+1 (calculated for $C_{17}H_{16}N_5$ 290.1405)

RESULTS & DISCUSSION

The present work is directed to closely examine the coupling reaction between benzene diazonium chloride and 2, 6-diamino-pyridine and identify all the products. The HPLC of the product (see Fig 1) shows PPHCL as the major product. There are three other components, of which two are known. The component with Rt 11.947 is a new compound and was isolated by preparative HPLC. The high resolution mass spectrum exhibited highest mass peak at m/e 290.1415 corresponding to the molecular formula $C_{17}H_{16}N_5$. This is 76 mass units higher than the molecular ion peak M+1 at m/e 214 exhibited by the free base 2, 6-diamino3-phenylazo-pyridine (PP) or its hydrochloride salt. This points out that the compound could be a phenyl derivative of PP or PPHCl. This conclusion is supported by the fact that a Gomberg type phenyl coupling reaction[8] always accompanies diazo coupling reaction of benzene diazonium salt. Separate experiments carried out by reacting PPHCl with benzene diazonium chloride had resulted in a product mixture whose HPLC analysis shows an increase in the content of the components with Rt=11.947 and the bis derivative **5** (Rt=13.342). It is known that PP forms a stable water insoluble monohydrochloride[9]. Estimation of chloride content agrees with the assignment of the molecular formula $C_{17}H_{16}N_5Cl$ to the isolated product. Coupling of the phenyl radical with PPHCl **3** can occur either in the pyridine ring or at some other position. The most favorable position for such a coupling will be the vacant position ortho to the amino group in the

pyridine ring. That indeed was the case was supported by NMR analysis the results of which are reported[10] elsewhere. **T**his compound can result via initial formation of 2, 6-diamino-3-phenyl-pyridine **8** followed by diazo coupling, or first formation of PPHCl followed by phenyl coupling. The reaction between **2** and **1** under conditions favorable for the occurrence of Gomberg reaction resulted only in the formation of PPHCl. It is therefore proposed that **6** is formed as shown in **Scheme 2**.

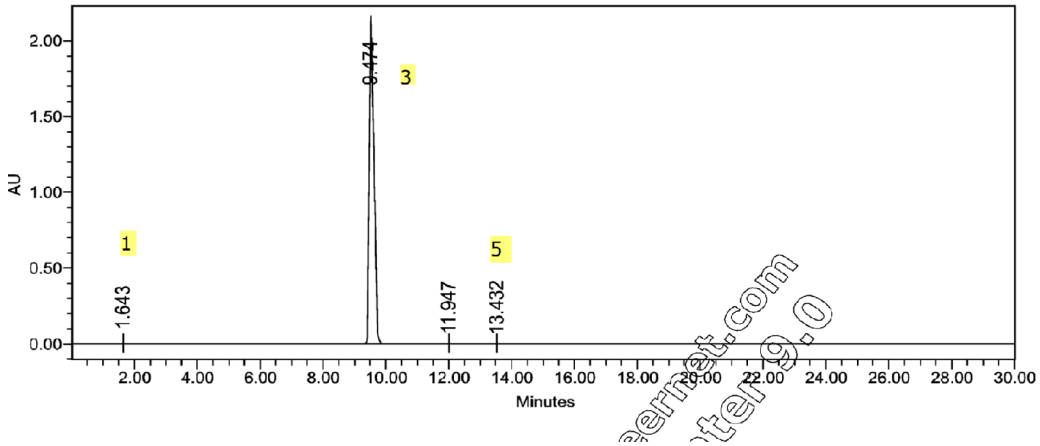


Table I – Mobile Phase

Time (minutes)	Water (%)	Acetonitrile (%)
0	90	10
0-5	50	50
5-10	5	95
10-20	40	60

The confirmation of the structure by independent synthesis was taken up next. The initial attempts of Gomberg reaction on pure PPHCl using benzene diazonium chloride has resulted in the isolation of product mixture containing unreacted starting material along with some bis compound and traces of the desired phenyl derivative. This is due to the fact that PPHCl is not soluble in the medium under which Gomberg reaction is carried out. It there appeared, that an independent synthesis of **6** should be preceded by the preparation of 2, 6-diamino-3-phenyl-pyridine. A six-step synthesis starting from benzyl cyanide via the intermediate 2-amino-6-hydroxy-3-phenyl pyridine-3-carboxylic acid is known in literature[11]. The nicotinic acid derivative referred above was synthesized but could not undergo decarboxylation to give 2-amino-6-hydroxy-3-phenyl pyridine. We found a more convenient method (**Scheme 3**) to synthesize **8** using Suzuki reaction in a key step.

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