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SYNTHESIS OF 2, 6-DIAMINO-3-PHENYLAZOPYRIDINE-1-OXIDE AND ITS HYDRO-CHLORIDE

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ABSTRACT: The compound 2, 6-diamino-3-phenylazopyridine hydrochloride salt is a genito-urinary antiseptic drug under the trade name Pyridinium. This study is directed towards the occurrence of an oxidation reaction to convert the tertiary amine present in 2, 6-diamino-3-phenylazopyridine hydrochloride to form an N-oxide derivative as an impurity in the process for its preparation. This was done by the independent synthesis of 2, 6-diamino-3-phenylazopyridine-1-oxide hydrochloride salt by two independent routes. The presence of three amine functions in the molecule required first to protect the primary amine groups by derivatisation so that oxidation occurs exclusively at the tertiary amino group of the pyridine ring. 2, 6-diamino-3-phenylazopyridine was acetylated to get 2, 6-diacetamido-3-phenylazopyridine whose structure was confirmed from ^{1}H NMR and mass spectral data. The oxidation of diacetyl derivative with peracetic acid resulted not only in the N-oxide formation but also in the cleavage of one of the acetamido group to give 2(6)-acetamido-6(2)amino-3-phenylazopyridine-1-oxide. The alkaline hydrolysis of the N-oxide form gave 2, 6-diamino-3-phenylazopyridine-1-oxide which on treatment with hydrochloric acid gave 2, 6-diamino-3-phenylazopyridine-1-oxide hydrochloride. In yet another route the N-oxide was prepared by the coupling reaction between benzene diazonium chloride and 2, 6-diaminopyridine-1-oxide in aqueous hydrochloric acid medium. This reaction resulted in the formation of 2, 6-diamino-3- phenylazopyridine-1-oxide hydrochloride as an insoluble salt. The structure of the N-oxide was confirmed from ^{1}H NMR and mass spectral data. A co-injection HPLC analysis showed the complete absence of 2, 6-diamino-3-phenylazopyridine-1-oxide hydrochloride in 2, 6-diamino-3-phenylazopyridine hydrochloride in its manufacturing process.

INTRODUCTION: The pharmacologically active compound namely 2, 6-diamino-3-phenylazo-pyridine hydrochloride [1] (PPHCl) is the active ingredient of the genito-urinary antiseptic drug [2] under the trade name Pyridinium.

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We have recently studied the impurity profile of active compound [3]. The degradation of active compound in acidic medium was reported [4]. This compound contains a tertiary amino functional group that is susceptible for oxidation to form an N-oxide. Compounds containing a N-oxide functional group [5] comes under a class of compounds likely to possess carcinogenic activity. It is therefore mandatory to verify the formation of 2, 6-diamino-3-phenylazopyridine-1-oxide hydro-chloride in the process for preparation pharma-ceutical grade PPHCl.

The present study reports the synthesis of 2, 6-diamino-3-phenylazopyridine-1-oxide hydrochloride required for its detection as an impurity in PPHCl.

MATERIALS & METHODS: Commercially available reagent grade chemicals were used as received. The reactions were monitored by TLC on E.Merck DC silikagel 60 F_{254} coated aluminium sheets. Melting points were determined by capillary method on a thermonik melting point apparatus model CPMB-2 and are uncorrected. The ^{1}HNMR spectrum was recorded on Brucker model av 400 instrument. Chemical shifts are reported in δ ppm relative to TMS. The ESI mass spectra were recorded on Applied Biosystems model API 2000/MDS Sciex Spectrometer.

HPLC: Waters with PDA detector and pump e 2695 was used. The analysis was carried out on Inertsil ODS-3 (100mmx4.6mm 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 the table 1 below. The flow rate was 1.0 ml/min and sample injection volume 10µl.

TABLE 1:

Time (min)	Solvent (vol. %)	
	Water	Acetonitrile
0	90	10
0-5	50	50
5-10	05	95
10-20	40	60

Sample preparation: A solution containing 2,6-diamino-3-phenylazopyridine 25mg and 2,6-diamino-3- phenylazopyridine-1-oxide 25 mg was dissolved in 5 ml of a solvent mixture consisting of acetonitrile and water in volume ratio 9:1 and then made up to 25 ml.

2, 6-diacetamido-3-phenylazopyridine 3: A 250ml three necked flask equipped with stirrer, thermometer and condenser was charged with acetic anhydride (55g, 0.539mol), 50g (0.234mol) 2,6–diamino-3-phenylazopyridine **2**. The mixture was heated and maintained under reflux for 3h. The RM was cooled to room temperature and the brownish syrup was slowly added to 1 lit ice cold water. The mixture is stirred for 1 hr to obtain the title compound as fine slurry which was separated by filtration under suction and washing with water till free from acid. The wet solid was dried at 100^0 to get 48g of crude product. The compound was purified by recrystallization from ethyl acetate. Orange crystalline powder. m.p : 212-214^{o}C.

MS: The mass spectrum exhibits highest mass peak at m/e 298.1 corresponding to the (M+1) ion with the base peak at m/e 256.1 due to loss of ketene fragment. The other fragment ions appear at m/e 238(10%), 214.2(13.%) and 197.1(8%).

^{1}HNMR spectrum: (DMSO-d6, δ ppm). 2.15(s,3H), 2.23 (s,3H), 7.52-7.6 (over lapping multiplet, 3H), 7.88 (d, 2H), 8.03(d, 1H), 8.08(d, 1H), 10.30(s, 1H, D_2O exchangeable) and 10.74(s, 1H, D_2O exchangeable).

2, 6-Diamino-3-phenylazopyridine-N-Oxide 5: This compound was prepared in a two-step reaction described below.

1. **2(6)-acetamido-6(2)amino-3-phenylazo-pyridine-1-oxide 4a/b**: 2,6-Diacetamido-3-phenylazopyridine **3**(5g) was dissolved in 15ml glacial acetic acid and treated with freshly prepared[6] 40% peracetic acid (15g) at ambient temperature. The reaction mixture was warmed to 50^{o}C under stirring and the progress of the reaction was followed by TLC (eluent: ethylacetate) for the disappearance of starting material. After 3h the RM was concentrated under vacuum to remove acetic acid to get a pasty solid.

   Addition of 100ml water and stirring gave slurry which was filtered, washed free from acid with water. The crude reaction mixture was dried under vacuum and purified by column chromatography over silica gel, eluent methanol: EtOAc in volume ratio (6:94). The fraction eluting third was collected and the N-oxide formed was isolated by removal of solvent under vacuum. Yield 2g. Orange crystalline powder. m.p: 215-217^{o}C. The spectral data of this compound indicate the formation of N-oxide was accompanied by the hydrolysis of one of the amide functions. The compound formed could be either 2-acetamido-6-amino-3-phenylazopyridine-1-oxide **4a** or 6-acetamido-2-amino-3-phenylazopyridine-1-oxide **4b.** The mono acetyl derivative was taken to the next step.

a. **MS**: The mass spectrum exhibits highest mass peak at m/e 272.4 corresponding to the (M+1) ion with the base peak at m/e 213.2. The other fragment ions appear at m/e 255.1(4%), 230.4(45%), 213.2 (100%), 211.8(5%), 195.1(3%), 185.4(12%), 184.3(22%), 136(35%), 108(11%), 92.2(10%), 77.1(2%).

b. **^{1}HNMR spectrum**: (DMSO-d6, δ ppm). 2.3 (s, 3H), the signals for the protons at 5 and 4 positions of pyridine ring appear as two distinct doublets at 7.73 and 7.8. The 5 protons on the phenyl ring appear as two distinct groups at 7.49-7.58 (over lapping multiplet 3H) and 7.99(d, 2H). The signal for the D_2O exchangeable protons on the amine group appear very broad centred at 8.05(2H). The lone proton on the amide nitrogen at 10.69(s, 1H, D_2O exchangeable).

2. **Hydrolysis of 4**: 1.7g of mono acetyl N-oxide derivative **4** obtained as above was taken into 5ml 10% aqueous sodium hydroxide. The mixture was heated to reflux under stirring and maintained for 3h. After cooling to room temperature the pH of the RM was adjusted to neutral using dil. HCl under stirring. The mixture was filtered, washed thoroughly with water and the solid was dried under vacuum to get 2, 6-diamino-3-phenylazopyridine-1-oxide **5**. Yield: 1.2g. Yellow crystalline powder. m.p.: 196-198^{0}C.

a. **MS**: The mass spectrum exhibits highest mass peak at m/e 230.2 corresponding to the (M+1) ion with the base peak at m/e 213.2. The other fragment ions appear at m/e 184.2(2%), 136.3 (6%) and 107.9 (2%)

b. **^{1}HNMR spectrum**: (DMSO-d6, δ ppm). The two protons in the pyridine ring appear at 6.29(d,1H) and 7.6(d,1H). The signals for the protons of the phenyl ring appear as three distinct groups at 7.38(t,1H), 7.49(t,2H) and 7.83(d,2H). The signal for the D_2O exchangeable 4 protons of the amino groups appear broad overlapping with those of the phenyl ring.

2, 6 –diamino -3-phenylazopyridine-N-Oxide hydrochloride 1: This Compound was prepared by two different routes starting from either 2,6-diamino-3-phenylazopyridine-1-oxide **5** or 2,6-diamino pyridine-1-oxide **6** as described below.

Method 1: 500mg of 2,6-Diamino-3-phenylazopyridine-N-Oxide **5** was dissolved in 60 ml methanol and treated with calculated quantity (0.25 ml 32% HCl). The mixture was stirred for 2 hrs and concentrated to dryness. The solid residue was washed with water and dried under vacuum. Yield: 420mg. Yellow crystalline powder. m.p : 230-232^0.

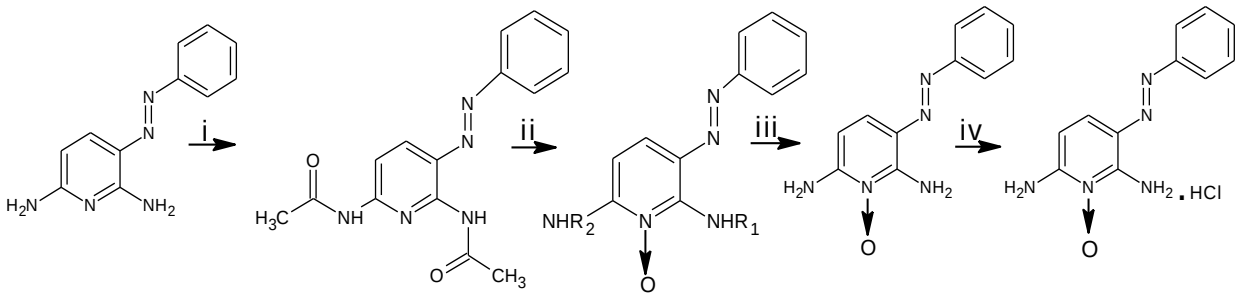
a. **Elemental analysis**: Found C(49.56%), H(4.67%), N(25.59%), Cl(13.33%). Calculated for $C_{11}H_{12}ClN_5O$, C(49.72%), H(4.55%) , N(26.36%), Cl(13.34%) .

b. **MS**: The mass spectrum exhibits highest mass peak at m/e 230.2 corresponding to the (M+1) ion with the base peak at m/e 213.2. The other fragment ions appear at m/e 184.2(20%), 136.3 (60%) and 108(41%)

c. **^{1}HNMR spectrum**: (DMSO-d6, δ ppm). Of the two pyridine ring hydrogens the one at 5 position appears at 6.24(d, 1H). The signal for the other hydrogen in the pyridine ring overlaps with the doublet of the two hydrogens ortho to the azo group in the phenyl ring and appears centred at 7.9(3H). The remaining 3 hydrogens of the phenyl ring appear at 7.41(t, 1H) and 7.50(t, 2H). The D_2O exchangeable protons appear as broad signals merging with the base line in the range 8.42, 9.16.

Method 2: Benzene diazonium chloride prepared from aniline(0.37g), sodium nitrite(.325g), con.HCl(1 ml) and water (2.5ml) was added to a solution of 2,6 diaminopyridne-1-oxide **6** (0.5g in 1 ml con.HCl and 2ml water) at 29^0. The reaction mixture was stirred for 3 hrs. The solid formed was filtered, washed with water and dried to give 2,6-diamino-3-phenylazopyridne-1-oxide hydro-chloride **1** in crude form which was purified by CC (SiO_2, eluent ethyl acetate : methanol in volume ratio 9:1). Yield: 120 mg.

RESULTS & DISCUSSION: The N-oxide are generally prepared by oxidation of tertiary amine by peracids [7, 8]. In the present case the substrate PPHCl contains two primary amino functions in a pyridine ring which leads to multiple products during the oxidation. It is therefore necessary to protect the primary amino functions in order to selectively oxidise the tertiary amino function to the corresponding N-oxide. This was achieved in a synthetic route in analogy to the preparation of 2,6-diaminopyridine-1-oxide starting from 2,6-diaminopyridine reported in literature [9,10]. The synthetic route is depicted in **scheme 1**.



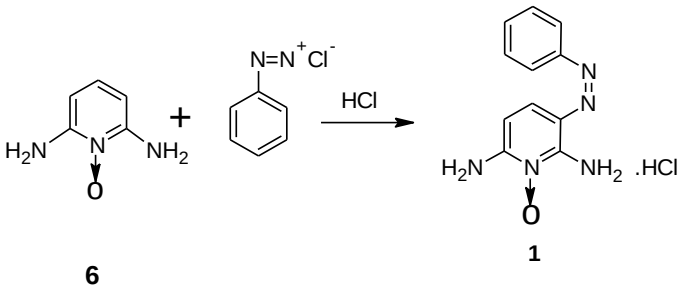
i) acetic anhydride, ii) 40%peracetic acid, iii) NaOH, iv) MeOH/dil.HCl

SCHEME - 1

The intermediates **3** and **5** were isolated in pure state and their structures were deduced based on mass and ^{1}H NMR spectral data. In the step involving the oxidation of 3 with 40% peracetic acid one of the acetamido function undergoes hydrolysis. This could result in the formation either **4a** or **4b**.

The available spectral data is not diagnostic enough to decide on the formation of **4a** or **4b** in this step. The alkaline hydrolysis of **4** gave 2, 6-dimno-3-phenylazopyridine-1-oxide which upon treatment with equimolor amount of the HCl gave 2, 6-dimno-3-phenylazopyridine-1-oxide hydrochloride **1**.

The compound **1** was also prepared by an alternative route involving diazocoupling of benzene diazonium chloride with 2, 6-diaminopyridine-1-oxide **6** (Scheme 2).



SCHEME 2

Unlike the diazocoupling at the 3 position of 2,6-diaminopyridine , the same can occurr at 3 or 4 position of pyridine ring in 2,6-diaminopyridine-1-oxide **6**.

The desired 2, 6–diamino-3-phenylazopyridine-N-Oxide hydrochloride **1** is isolated in crude form and purified by CC albeit in poor yield. The identity of the N-oxide synthesized by the two different routes given in schemes 1&2 was established by co-injection HPLC analysis.

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