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PREPARATION AND CHARACTERIZATION, CHROMATOGRAPHY, ANTIMICROBIAL ACTIVITIES OF SOME METAL COMPLEXES OF 1-(2^{-}-HYDROXYL-4^{-}-NITROPHENYLAZO)-2-NAPHTHOL

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ABSTRACT : A total of four new metal complexes derivatives of 1-(2^{-} - hydroxyl- 4^{-}-nitrophenylazo) -2-naphthol with the metal ions Co(II), Fe(III), Cu(II) and Zn(II) have been successfully prepared in alcoholic medium. The ligand and complexes obtained are characterized qualitatively using FTIR spectroscopy, UV- Vis spectroscopy and chromatography measurements. The ligand gave retention time 3.225 min while the complexes with Cu &Ag show retention time 3.474min ,3.774 min respectively .From the spectral study, all the complexes obtained as monomeric structure and the metals center moieties are five or six-coordinated with Octahedral geometry. The preliminary *in vitro* the biological study of ligand and their complexes against selected types of bacteria such as; *Escherichia coli*, *Streptococcus mutans*, *Proteus bacilli* and *Staphylococcus*, the antibacterial screening activity revealed that the ligand and their complexes showed moderate activity against all tested bacterial strains except *Streptococcus mutans*.

Key words : Nitrophenylazo derivatives, metal complexes, *In vitro* antibacterial activity, naphthol derivatives.

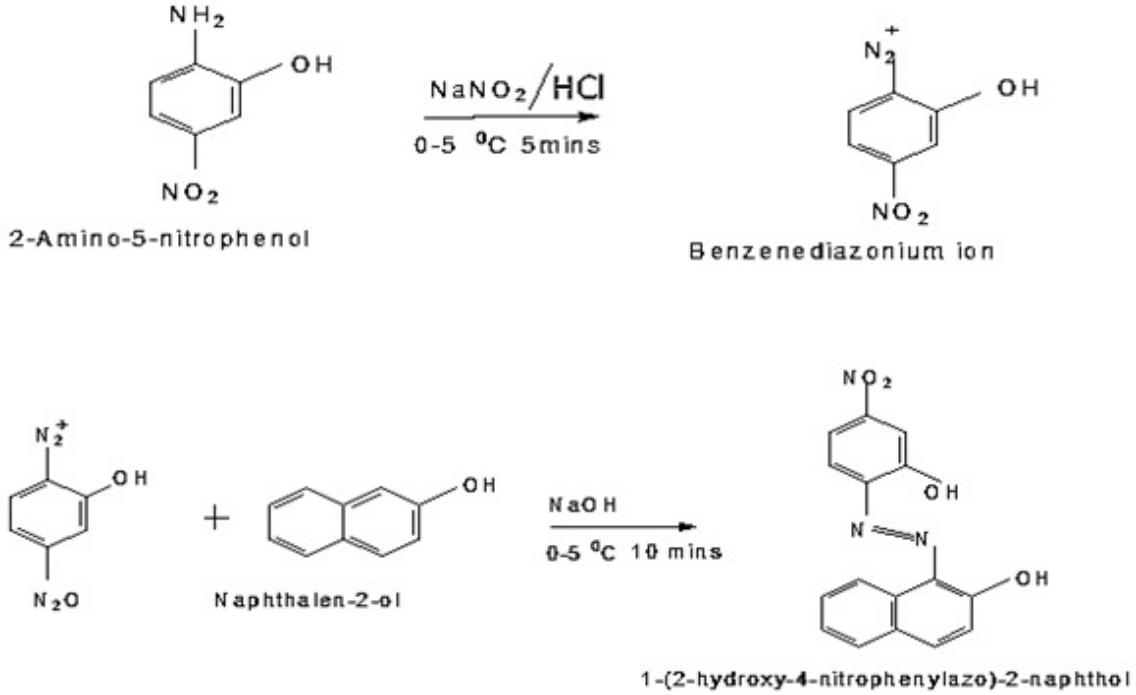
INTRODUCTION

Azo dyes are essential compounds in most organic compounds, which are not occur in nature and are produced only through chemical synthesis (Hofer and Wong, 2001). All azo dyes contain the R-N=N-R' arrangement and the nature of the aromatic substituent's on both side of the azo group controls the colors of the azo compounds as well as the water-solubility of the dyes (Chauveau *et al*, 2012). When describing a dye molecule, nucleophiles are referred to as auxochromes, while the aromatic groups are called chromophores. Together, the dye molecule is often described as a chromogen (Maynard, 1983). Theories have been developed to explain the changes in color, including resonance effects, molecular orbital explanations, electronic effects, and many more. Generally, we can say that addition of electron-withdrawing groups (such as - NO_2) shift the absorption wavelength UP, causing a darker color to appear. Addition of hydroxyl or amino groups tend to increase the color's intensity (Yildiz and Boztepe, 2001; Abd-Alredha and Jameel, 2012). These dyes are used in electro photographic or sensor applications for photoconductors (Christie, 2001). In addition, they are also preferred in high technology areas such as lasers, electro-optical devices and ink-jet printers (Karipcin *et al*, 2010).

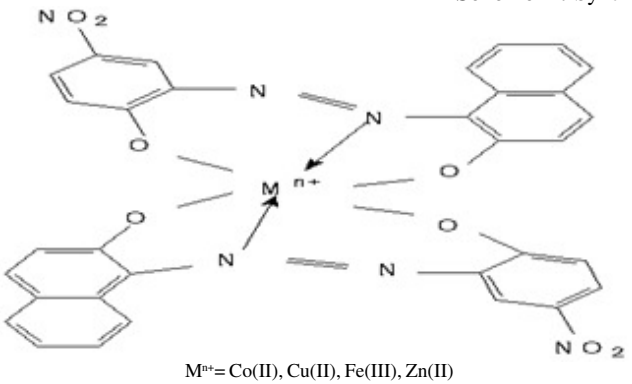
Human exposure to azo dyes occurs through ingestion, inhalation or skin contact. There is evidence that Sudan dyes have genotoxic effects and that ingestion of food products contaminated with Sudan I, II, III and IV and Para Red could lead to exposure in the human gastrointestinal tract (Hsieh, 1990; Peters and Freeman, 1991). The clastogenic effect was greatly increased when para red was metabolized, and therefore, the metabolites of this azo dye were more genotoxic than the parent compound (Gayatri *et al*, 2011). Azo dyes are aromatic compounds characterized as having one or more azo bonds ($^{-}N=N^{-}$). Diazonium salts are very important intermediates in the synthesis of aromatic compounds, and they are precursors of azo compounds which are very useful in the fields of dyes, pigment and advanced materials (Gupta, 2012).

Study of biological activity

The biological activity of our azo compound (L) and their metal complexes with Co(II), Cu(II), Fe(III) and Zn(II) against selected types of bacteria *E.coli* (G^{-}) *Staphylococcus* (G^{+}) *Streptococcus mutans* (G^{+}) *Proteus bacilli* (G^{-}) are tested to assess their potential antimicrobial agents. Nutrient agar is used as culture medium. For bacterial growth (Oltean and Nica, 2011). The plates were incubated for 24 hrs at 37^{0}C (Nischal *et al*, 2011). The stock solution is prepared by dissolving the compounds in DMSO.



Scheme 1 : Synthesis of HNPAN Ligand.



M^{n+} = Co(II), Cu(II), Fe(III), Zn(II)

Fig. 1 : Suggested structure of metal complexs of HNPAN.

MATERIALS AND METHODS

All reagents are commercially available and uses without further purification. All preparations is performed after fixing the optimum pH and molar concentration that obeyed Lambert – Beers' law in the studied pH ranges. The using substances in this work are : 2-Amino-5-nitrophenol, â–Naphthol, Sodium Hydroxide, Sodium nitrite(III), Hydrochloric acid and metal salts ($CoCl_2.2H_2O$, $CuCl_2.2H_2O$, $FeCl_3.6\ H_2O$ and $ZnCl_2$) and ammonium acetate.

Preparation of ligand

The synthesis of an azo dye requires two organic compounds- a diazonium salt and a coupling component. The diazonium salt reacts as an electrophile with an electron-rich coupling component, like a naphthol through an electrophilic aromatic substitution mechanism. The hydroxyl group (such as -naphthol) direct the aryl diazonium ion to the para position (Sanjay *et al*, 2012). The preparation of 1-(2^- - hydroxyl- 4^- -nitrophenylazo)-2-naphthol steps are following :

Weigh (0.864gm) of 2- naphthol and dissolve it into (35ml) ethanol and 10% aqueous Sodium Hydroxide solution to become pH (6-7). Cool the solution with an ice water bath. Dissolve (0.448gm) of sodium nitrite(III) in (10ml) of water. Weigh (1gm) of 2-amino-5-nitrophenol then dissolve in into (3ml) of concentrated Hydrochloric acid and (10ml) of ethanol. Cool the 2-amino-5-nitrophenol in an ice –bath. Add solution sodium nitrite(III) slowly with drop wise. The mixture should be well-stirred during addition at the temperature below 5^0C. The resulting mixture takes about 20min to ensure the diazotization goes to completion. A large amount of brick red precipitate forms during the addition .Add the benzenediazonium salt solution to the alkaline 2-naphthol solution slowly. Allow the resulting mixture for one day. Filter the mixture with cold water for several times and recrystallized by absolute ethanol.

Preparation of solutions

Solutions are prepared from metal salt in concentration of 10^{-3} M by taking a known weight of salt metals and dissolves in 100 ml of distilled water. An aqueous solution of ligand is prepared by dissolving appropriate weight of 0.31 g to reach the concentration of 10^{-3} M in 100 ml distilled water. Buffer solutions, covering the pH ranges of 4–9, were prepared as 0.01 M

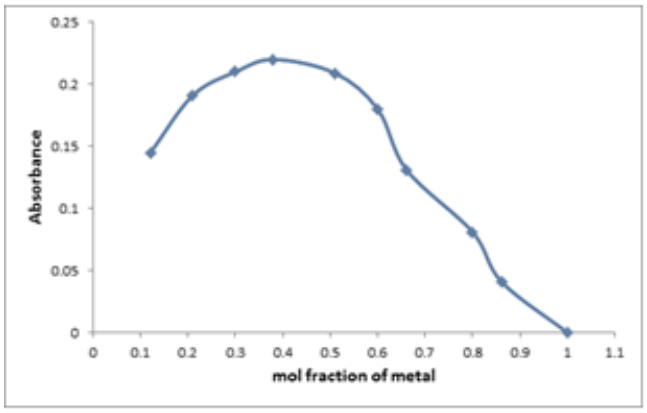


Fig. 2 : Jobs' diagram for the Cu(II)-ligand - system (ë max = 530 nm).

$FeCl_3.6H_2O$ and $ZnCl_2$ with ethanol is presented in the following reactions.

$$MCl_2.nH_2O + 2L \rightarrow ML_2.2Cl_2 + nH_2O$$

M = Co(II), Cu(II) and Zn(II)

$$FeCl_3.nH_2O + 2L \rightarrow [FeL_2.2Cl]\ Cl + nH_2O$$

RESULTS AND DISCUSSION

All preparations are performed after fixing the optimum pH and molar concentration that obeyed Lambert – Beers' law in the studied pH ranges. The structure of these complexes are deduced according to the molar ratio and Job methods depending on the spectroscopic studies of the complex solutions of the above ions. The chelating

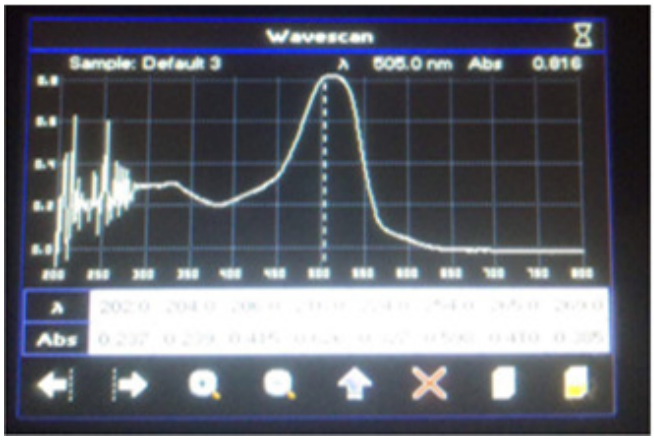


Fig. 3 : The Pigment.

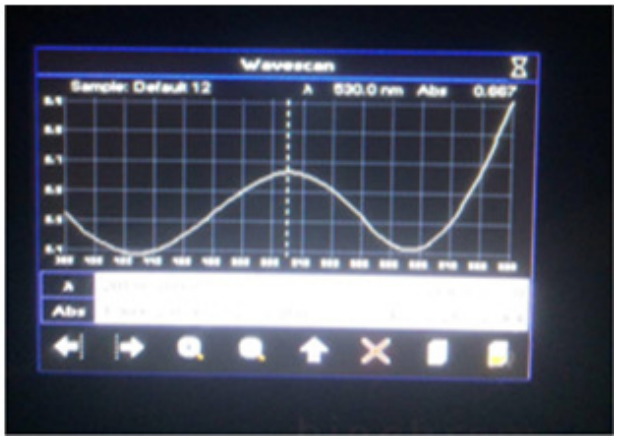


Fig. 4 : The Pigmentwith Cupper.

Table 1 : Biological activity data (zone of inhibition in mm) of ligand and its metal complexes.

Compounds	***Staphylococcus aureus*** **Gram positive**	***Streptococcus*** **Gram positive**	***Proteus*** **Gram negative**	***Escherichia coli*** **Gram negative**
LH = Ligand	–	–	++	++
$[Cu\ (L)_2].H_2O$	–	–	++	++
[Fe (L):].H_2O	+	–	++	++
[Co (L):].H_2O	–	–	++	+
$[Zn\ (Cl)_2].H_2O$	+	–	++	++

(+++) : high active – inhibition zone > 12 mm; (++) : moderate active – inhibition zone = 9-12 mm; (+) : slightly active – inhibition zone = 6-9 mm; (–) : inactive < 6mm.

solutions of ammonium acetate indistilled water. The required pH was obtained by the addition of either ammonia solution.

Preparation of Metal Complexes (general procedure)

An ethanol solution of the ligand (0.31g, 1 mmole) was added gradually with stirring to the 0.083g, 0.085g, 0.135g and 0.0682g (1 mmole) of $CoCl_2.2H_2O$, $CuCl_2.2H_2O$, $FeCl_3.6H_2O$ and $ZnCl_2$, respectively dissolved in distilled water. The mixture is stirring until dark color precipitate is formed, filtered and washed several times with (1:1) water: ethanol then with acetone. The formation of complexes of $CoCl_2.2H_2O$, $CuCl_2.2H_2O$, properties of azo ligand is studied towards Co(II), Cu(II), Fe(III) and Zn(II) ions and the spectral data revealed that the nitrogen and oxygen atoms of –N=N-and –OH groups participated in bonding with the metal ions. The chelate complexes that contain five-membered or six-membered chelate rings are the most stable complexes (Limbani and Modi, 2014).

Study of complex formation in solution

Azo complexes with metal ions are studied in solution using ethanol as a solvent (Stiborova *et al*, 2006). The stochiometry of the azo ligand –metal chelate dye is described having the metal: ligand ratio of 1:2 ML2 by

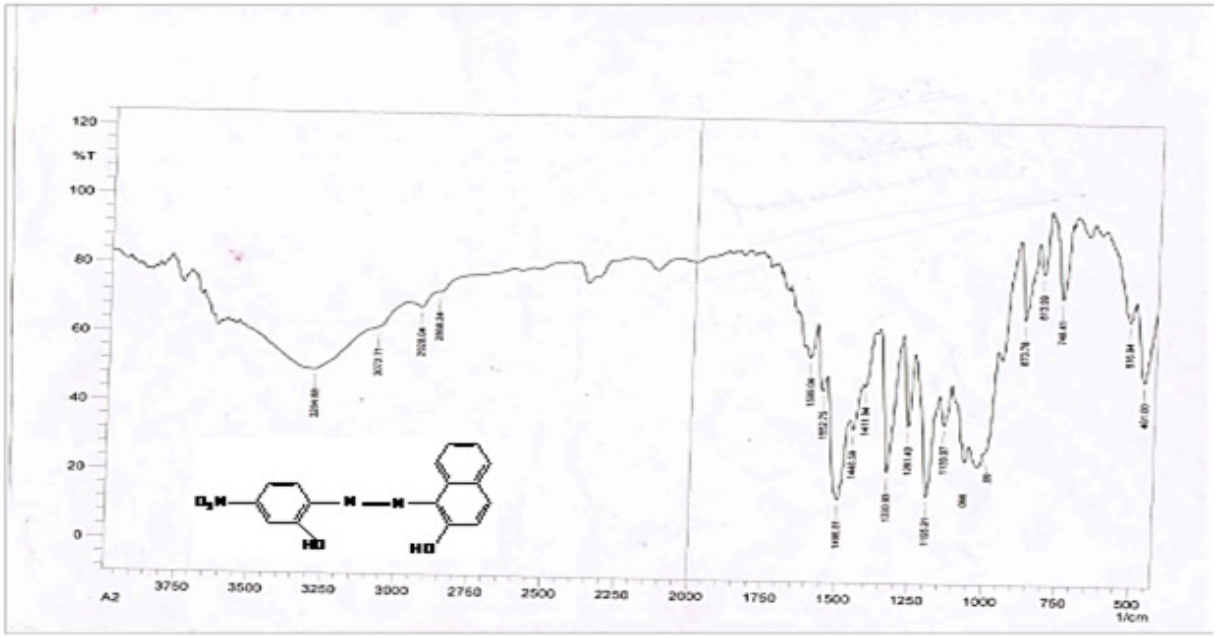


Fig. 4 : The FT-IR spectrum of azo ligand.

the spectroscopic titration method. The amount of complex ionic solution can be determined colorimetrically for various ratios of $[M^{n+}]$ to [L]; the total concentration of metal ion and ligand is kept constant.

IR Spectra

The infrared spectra of azo compound OH stretching vibration display a strong broad band at 3284.88cm^{-1}. The low value indicate that the OH group is involved in an intramolecular hydrogen bonding with the –N=N- group. The free ligand shows a band 2928.04 cm^{-1} which belongs to N-H. The strong absorption at 1496.81 cm^{-1} is typical for –N=N- while the strong absorption at 1330.93 cm^{-1} for C-N. In the region 770 - 695cm^{-1} and 450-515 cm^{-1} shows characteristic absorption bands for M ← N and M ← O, respectively.

Chromatographic study of Ligand with Complexes

Solutions of Ligand and complexes were diluted in constant concentration (1 ppm), flowed with injection of solutionsby a syringe (Hamilton) in capacity (10ml) by inert gas nitrogen (flow rate 25 ml/min) in gas chromatography, shimdzu-2014, Japan with flam ionization detector. The ligand with complexes separated according to interactions and polarity groups in terminal of ligand or complexes and their mass. The ligand separated in the first time because itsmolecular weightless thanits complexes (Figs. 5-7).

Biological activity

Metal complexes are more active than their ligand because the metal complexes may serve as a vehicle for

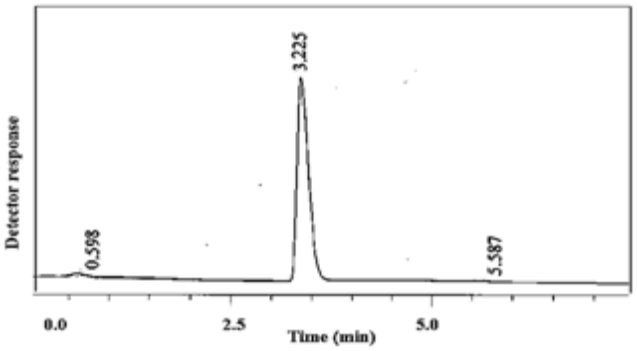


Fig. 5 : Chromotogram of Ligand.

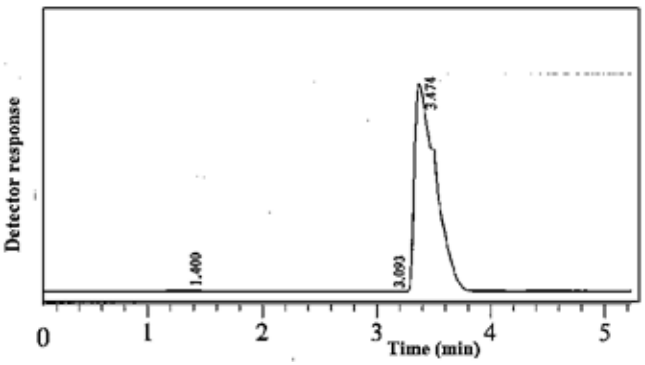


Fig. 6 : Chromotogram of Complex with Cu.

activation of ligand as the principle cytotoxic species (NagehAbotaleb *et al*, 2017). The zones of inhibition are determined at the end if an incubation period of 24 hr at 35^{0}C. During this period, the test solution diffused and the growth of inoculated microorganism is affected. The biological activity against two types of both gram positive and gram negative microorganism are studied. The

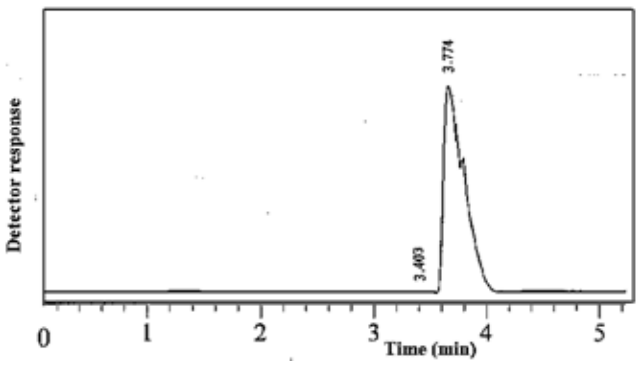


Fig. 7 : Chromotogram of Complex with Ag.

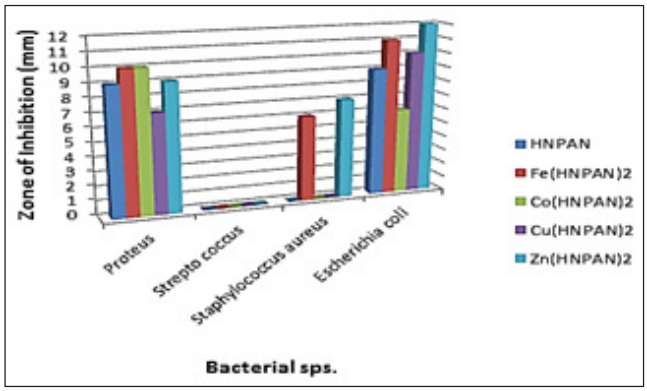


Fig. 8 : Statistical representation for biological activity of HPAN and its complexes.

bacterial inhibition zone values are summarized in Table 1.

CONCLUSION

This study confirmazo compound is prepared from reaction of 2-Amino-5-nitrophenol with â naphthol. The product characterized by FTIR and UV-Visible spectrophotometer and chromatography measurements. The antibacterial activity was studied of the ligand and their complexes indicate that the metal complexes exhibited antibacterial activity. The study showed that there is no biological activity of Streptococcus on azo dye and their complexes.

Appendices

Sample Calculation

Actual yield

a- Mass of HNPAN 1.450 gm

b- millimole of HNPAN

1.450gm HNPAN × 1 mole HNPAN / 309gm × 1000 mmol / 1 mol

= 4.692 mmol HNPAN

The optical yield

a- millimole of ANP

Photo 1 : *E. coli.*

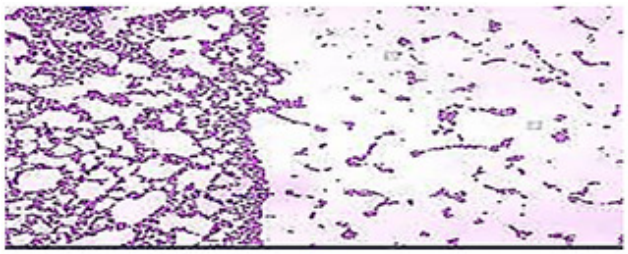


Photo 2 : *Streptococcus mutans.*

Photo 3 : *Proteus bacilli.*

1g ANP × 1mol ANP / 154gm × 1000mol / 1mol

= 6.493mmol APN

b- millimol of â-naphthol

0.864 gm × 1mol / 144gm × 1000mmol / 1mol

= 6mmol

c- millimol of HNPAN

6.493 mmol ANP × 1mmol HNPAN / 1mmol APN

= 6.493 mmol HNPAN

Percent yield

Actual yield / Theoretical yield × 100

4.692 mmol HNPAN / 6.493 HNPAN × 100

= 72.26%

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