Medical Science Sample

Case Report

Acute Appendicitis Masquerading as Distal Intestinal Obstruction Syndrome in Adult Cystic Fibrosis

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My best wishes for your success with the manuscript.

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~~Overshadowed by Sino-pulmonary infections,~~With the improved life expectancy in c~~C~~ystic f~~F~~ibrosis (CF) patients, there has been an increase in ~~commonly affects~~ gastrointestinal ~~organs~~ manifestations because of secretory and motility dysfunction. Infrequently, these changes can result in d~~D~~istal i~~I~~ntestinal ~~O~~obstruction s~~S~~yndrome (DIOS), an ~~more and more~~increasingly diagnosed gastrointestinal condition~~entity~~ in adult ~~Cystic Fibrosis~~CF patients. We present the~~a~~ case of a 22-year-old man~~le~~ who presented to our hospital with right lower quadrant abdominal pain. ~~with~~ Despite the suspicion of acute appendicitis, the patient ~~and~~ was subsequently diagnosed ~~as~~ with DIOS. Our case highlights the importance of considering DIOS as a differential diagnosis ~~of~~ for right lower quadrant abdominal pain in CF patients, especially ~~for~~ by physicians working at community hospitals that~~which~~ may not have a CF~~ystic Fibrosis~~ care program available.

1. Introduction

Cystic F~~f~~ibrosis (CF) is a genetic disease ~~of~~ that affects multiple organs. ~~With~~ Because of advancements~~ing~~ in the manage~~menting~~ of CF ~~patients~~, patients can now ~~often~~ survive ~~become~~ to adulthood~~s~~ [1]. However, the i~~I~~mproved life expectancy among adult CF patients has ~~given rise~~led to an increase in extrapulmonary, notably gastrointestinal, man~~-~~ifestations, which ~~did not happen~~ was previously uncommon. Distal ~~I~~intestinal o~~O~~bstruction ~~S~~syndrome (DIOS) continues to be a rising complication in adult CF patients, presenting as~~with~~ acute abdominal pain ~~like~~ and mimicking an acute abdominal emergency.

2. Case Report

A 22-year-old Turkish-origin man~~le~~ with a past medical history of ~~Cystic Fibrosis~~CF presented with a one-day history of right lower quadrant abdominal pain. He described a sharp periumbilical pain that continued to worsen, which then shifted to the right lower quadrant of the abdomen. Prior to the onset of the abdominal pain, he reported experiencing nausea and anorexia for three days. His last bowel movement was two days prior to admission. The patient was ~~also~~ diagnosed with CF~~Cystic Fibrosis~~ at the age of four, and the~~his~~ disease progressed to exocrine

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pancreatics insufficiency, which was being treated with pancreatic enzymes. Upon reviewing the patient's past history, it was noted that he had several episodes of pneumo–nia, for which he was appropriately treated with antibiotics.; Nnotably, no history of constipation or recurrent abdominal discomfort was reported prior to this. At home, the patient was prescribed aAlbuterol inhalationer as needed, dDornase aAlfa inhalationer, aAztreonam lysine nebulization, 500 mg aAzithromycin three times a week, lLansoprazole, lLumacaftor-ivacaftor twice a day, lLipase-protease-amylase capsule three times a day, and a multivitamin capsule once a day. The patient was also diagnosed with Cystic Fibrosis at the age of four and his disease progressed to exocrine pancreas insufficiency, which was being treated with pancreatic enzymes. On abdominal examination, he was found to havehad diminished bowel sounds and tenderness on right lower quadrant with equivocal rebound tenderness on the right lower quadrant. Lab–oratory analysis showed leukocytosis (white blood cell count,WBC 13.0 mm/K3;, nNeutrophils count, 62%) with a normal differential. He had no electrolyte imbalances. Computed tTomography (CT) of the aAbdomen revealed thickening, and edema around the termi–nal ileum, inflammatory changes in the a colon with inflammatory changes, free fluid in the right paracolic gutter adjacent to the cecum, an appendix measuring 5.3×4.6 mm, and reactive lymph nodes (Figures 1 and 2).

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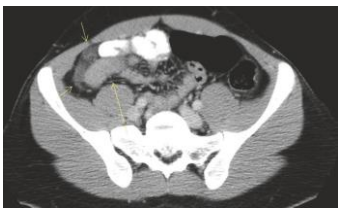

FIGURE 1: Axial abdominal computed tomography~~CAT~~ scan depicting thickening around the terminal ileum and colon (y~~Y~~ellow arrows) along with extralumi~~–~~nal fluid and reactive lymph nodes.

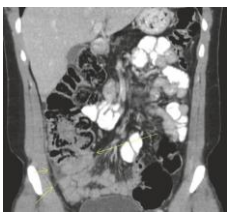

FIGURE 2: Coronal view computed tomography scan ~~with~~ showing thickening of the ileum with a distended appendix (yellow arrows).

~~measuring 5.3×4.6 mm, and reactive lymph nodes (Figures 1 and 2).~~ Due to extraluminal fluid and cecal wall edema with inflammation, early acute appendicitis could not be excluded as a possible diagnosis. Surgical intervention was ~~performed~~ required, which revealed a ruptured microperforation of a cecal diverticulum and a distended appendix in chronic adhesions, for which he required an appendectomy and partial cecectomy with an intact ileocecal ~~valve~~ (IC ~~valve~~) valve. Postoperatively, he was diagnosed with DIOS and was subsequently started on p~~P~~olyethylene g~~G~~lycol. The patient made an unremarkable recovery and was discharged. ~~home to be~~He was followed up in the outpatient clinic ~~without~~ and did not have ~~any~~ recurrence of any symptoms.

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3. Discussion

Due to the improved life expectancy of CF patients, DIOS is now being increasingly diagnosed in adult patients with CF. ~~Distal Intestinal Obstruction Syndrome (DIOS) was called a Meconium Ileus equivalent in the past, described by the collection of viscid fecal material within the lumen combined with sticky mucoid intestinal content adherent to the intesti- nal wall of the terminal ileum and cecum [1].~~ Perez-Aguilar et al. reported ~~a~~ that the prevalence of DIOS was 19.5% (mean age 20.6 years) among 46 CF patients in a retrospective analysis, while Dray et al. ~~conducted a cross-sectional study~~ report~~ed~~ing a 15.8% (mean age 28.9 years) prevalence ~~in~~ among 171 CF patients in a cross-sectional study [2, 3]. Despite the~~Though there continues to be a~~ limited assessment ~~of~~ on the prevalence of DIOS in adult CF patients, DIOS is ~~considered~~more common among adults ~~compared to~~than among children ~~due to~~because of ~~increased~~ disease progression.

~~Distal Intestinal Obstruction Syndrome (DIOS), previously known as was called a Meconium Ileus-equivalent, in the past, described~~is characterized by the collection of viscid fecal material within the lumen combined with sticky mucoid intestinal content adherent to the intesti-–nal wall of the terminal ileum and cecum [1]. Defective intestinal chloride and water secretions into the gut, luminal acidity, and loss of bile salt all contribute to the

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development of DIOS [1]. These patients characteristically present with right lower quadrant pain, nausea, abdominal distension, and failure to pass stools or flatus [1, 3]. In some patients, a palpable right lower quadrant mass ~~can~~ may be ~~appreciated~~ present that may be confirmed on abdominal radiography~~X-ray~~ [1]. Though abdominal ~~X-rays are~~radiography is recommended to aid in the diagnosis of DIOS, ~~they are~~it is inadequate in differentiating ileus from other causes of abdominal pathologies that may present in ~~Cystic Fibrosis~~CF patients [4]. Due to ~~the proximity of~~ the anatomical ~~locations~~proximity, as well as the overlapping clinical presentations, appendicitis and intussusception may mimic DIOS. This ~~which~~ further leads to diagnostic uncertainty~~. Overlap of several intra-abdominal pathologies in CF increases the risk of misdiagnosis, especially with acute appendicitis,~~ as these ~~patient's~~ underlying pathologies may be masked in CF patients with pulmonary infections~~,~~ using antibiotics, as seen in our case [5, 6].

Osmotic laxatives are the cornerstone of bowel regimens for the treatment of DIOS. The most commonly prescribed laxative is p~~P~~olyethylene ~~G~~glycol, ~~(PEG)~~ administerr~~at~~ed at a dose of 20~~-~~40 ml/k~~K~~g/h~~H,~~ ~~up t~~with~~o~~ a maximum of 1 l~~L~~/kg/h for a total of 8 hours, resulting in a~~achieving~~ fecal effluent consisting of clear fluid, along with the resolution of abdominal pain and constipation [1, 6]. If the diagnosis remains unclear, and thus, requires surgical intervention, IC~~ileocecal~~ valve resection should be considered to prevent the development and recurrence of intestinal obstruction sequalae ~~and growth~~, especially in adolescents [7].

With the increase in immigration of foreigners into~~through~~ America, inner-city and community hospitals may not be sufficiently equipped with a ~~Cystic Fibrosis~~CF care center; moreover,~~nor~~ ~~may~~ these hospitals may not have programs in provision, with expertise available to other clinicians involved in patient care. Therefore, our case highlights the significance of considering DIOS as a differential diagnosis in CF patients presenting with right lower quadrant abdominal pain, particularly in hospitals without a CF care program available.

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Conflicts of Interest

The authors declare that they have no conflicts of interest.

Authors' Contributions

All authors contributed to the revision and approval of the manuscript.

References

1. C. Colombo, H. Ellemunter, R. Houwen, A. Munck, C. Taylor, and M. Wilschanski, “Guidelines for the diagnosis and manage- ment of distal intestinal obstruction syndrome in cystic fibrosis patients,” Journal of Cystic Fibrosis, vol. 10, pp. S24–S28, 2011.

2. F. Perez-Aguilar F, J. Ferrer-Calvete J, D. Nicolas D, Berenguer J, and J. Ponce, J. “Digestive alterations in cystic fibrosis. Retrospective study of a series of 46 adult patients,”. Gastroenterología y Hepatología Gastroenterologia y hepatologia,. 1999 Feb;vol. 22, no. (2,): pp. 72–78, 1999.

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3. X. Dray, T. Bienvenu, N. Desmazes-Dufeu, et al, “Distal intestinal obstruction syn- drome in adults with cystic fibrosis,” Clinical Gastroenterology and HepatologyClin Gastroenterol Hepatol, vol. 2, no. 6, pp. 498–503, 2004.

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4. K. Nassenstein, B. Schwerger, M. Kammer, J. Status, T. Lauen- stein, and J. Barkhausen, “Distal intestinal obstruction syn- drome in the early postoperative period after lung transplan- tation in a patient with cystic fibrosis: morphological findings on computed tomography,” Gut, vol. 54, no. 11, pp. 1662– 1663, 2005.

4.

5. Y. Al Abed, Y., W. Hameed, J. W., Roy, J., and A.P.S.& Kumar, A. P. S. (2007). “Appendicitis in an adult patient with cystic fibrosis: a diagnostic challenge,”. Gut, vol. 56, no. (12), pp. 1799– 1800, 2007.

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7.6. J. M. Abraham and C. J. Taylor, “Cystic Fibrosis & disorders of the large intestine: DIOS, constipation, and colorectal cancer,” Journal of Cystic Fibrosis, supplement 2, pp. S40–S49, 2017.

7. A. Mentessidou, I. Loukou, G. Kampouroglou, A. et al, “Long-term intestinal obstruc- tion sequelae and growth in children with cystic fibrosis oper- ated for meconium ileus: expectancies and surprises,” Journal of Pediatric Surgery, vol. 2018; 53, no. (8, pp.) 1504–1508 2018.

Life Sciences Sample

Case Report

Methylmalonic Acidemia with Novel *MUT* Gene Mutations

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A 5-years-old boy presented with poor weight gain and recurrent episodes of fever, feeding problems, and lethargy, sincefrom the age of 11 months, and poor weight gain. He was admitted to our hospital and evaluated for metabolic disorders; subsequently, hecauses and was diagnosed withas methylmalonic acidemia (MMA). He was treated with vitamin B12 and carnitine supplements and has been on followed -up for the last 3 years. Mutation analysis by next generation sequencing (NGS), supplemented with Sanger sequencing, revealed two novel mutations in exon 5 and 3 ofvariants in the *MUT* gene responsible for the methylmalonic acidemiaMMA in exon 5 and exon 3. Recently, he developed dystonic movements, including orofacial dyskinesia. With advent of NGS, judicious use of Thus, next generation sequencingNGS along with Sanger sequencing can help in identification ofy causative possibly pathogenic mutations responsible for various clinical conditions and can help in early diagnosis and appropriate treatment of the conditions.

1. Case Presentation

A 5-year-old boy The child presented for the first time at the age of 11 months, presented with recurrent complaints of fever, vomiting, poor feeding, and lethargy since the age of 11 months. On examination We observed that the patient he had pallor and tachypnea and was drowsy. Laboratory tests Further evaluation suggested that the patient had was suggestive of high anion-gap metabolic acidosis with ketonuria (urine ketones 3+) and with normal electrolytes, blood sugar (94 mg/dL), vitamin B12, and homocysteine levels. Plasma ammonia and plasma lactate were was 118 units, and plasma lactate was 2.9 units, respectively. Transcranial magnetic stimulation TMS results wereas normal, but gas chromatography mass spectrometry analysis of but urine GCMS revealed elevated 3-OH propionic acid [12.39 retention time (RT)]as well asand elevated methyl malonic acid levels [16.92 RT, Suppl Figure 1, in Supplementary Material available online at https://doi.org/10.1155/2017/8984951]. Since then, theis patient child was on a low-protein diet, and carnitine, biotin, thiamine, and vitamin B12 injections;. he Child was thereafter admitted to the hospital on seven multiple occasions (7 times) with acute decompensation and managed as per protocol. Mutational analysis was sent for methylmalonic acidemia (MMA) which showed a single heterozygous missense variant c.976 A>G (p.Arg326Gly) in exon 5 of the *MUT* gene (genomic coordinates: chr 6: 49421405): as a variant of uncertain significance. Chromosomal microarray analysis done did not reveal any major deletion or duplication that which could disrupt the gene. Since exon 3 and exon 6 were not adequately covered by next generation sequencing (NGS), further evaluation by Sanger sequencing for targeted exons was performeddone, and a second 2nd

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Thanks for providing this opportunity to assist you with this manuscript. I have edited the text for language, grammar, and improved clarity. As no formatting instructions were provided, I have not looked into this aspect. I have, however, ensured that the style used predominantly by you is consistently maintained throughout the manuscript. Please check your target journal's guidelines and ensure that you comply with all the recommended guidelines. Should you have any concerns, please feel free to get back to me. My best wishes for your success with the manuscript.

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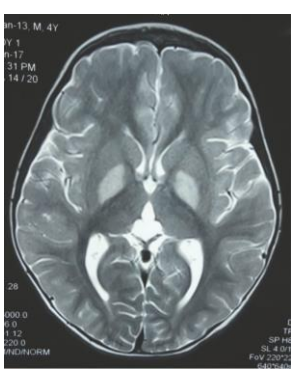
mutation in exon 3 c.753 G>A (p.=) was identified. The variants were predicted as ~~found to be~~ damaging by the ~~on~~ SIFT database ~~score~~ (Suppl data) ~~.~~ and as ~~They were also predicted to be~~ deleterious by ~~on~~ Polyphen-2 and Mutation-Taster, but they were ~~and~~ absent ~~not found~~ in the ExAC database. Brain magnetic resonance image ~~MRI brain~~ of the patient (~~done at~~from the age of four~~4~~ years) ~~was~~ showed~~ing~~ multifocal cystic encephalomalacic changes with surrounding gliosis in deep white matter predominantly in frontoparietal regions (Figure 1). During ~~In~~ the latest admission of the patient to the hospital, we observed ~~child was found to have~~ fresh neurological findings in the form of perioral tremors, generalized hypertonia, and generalized dystonia with clonus with exaggerated deep tendon reflexes. The patient ~~He~~ was treated with intravenous dextrose and sodium bicarbonate and was continued on carnitine and ~~injection of~~ vitamin B12 injections. Plasma ammonia and plasma lactate were ~~was~~ 18 units and ~~lactate level was~~ 4.9 units, respectively. Brain magnetic resonance image ~~MRI brain~~ of the patient~~was repeated~~ ~~and~~ revealed bilateral basal ganglia hyperintensities, suggestive of metabolic stroke. After the subsidence of acute crisis, he was discharged on carnitine, ~~injection of~~ vitamin B12, injections, and trihexyphenidyl. His p~~P~~arents were counseled regarding the prognosis and for prenatal diagnosis for ~~next~~subsequent pregnancy~~y~~ies.

2. Discussion

~~2.~~

MMA presents with lethargy, acidosis, hypoglycemia/ hyperglycemia, ketosis, and recurrent episodes. MMA ~~due to *MUT* gene mutations~~ usually leads to severe phenotypes due to *MUT* gene mutations, and around 35–40% of cases are due to novel~~ew~~ mutations [1, 2]. ~~There can be~~ M~~m~~issense or nonsense mutations, deletions, insertions, and so on in the *MUT* gene ~~and so on~~ can lead~~ing~~ to a clinical phenotype.



F~~F~~IGURE~~URE~~ 1: ~~The MRI B~~brain magnetic resonance image of~~in~~ the 5-year-old boy~~child~~ with MUT-related methylmalonic acidemia~~MMA~~ showing predominant frontoparietal abnormalities in the form of encephalomalacia and gliosis.

The advent of NGS technology has enabled better characterization of mutations in several populations. However, Sanger sequencing remains a useful adjunct in molecular testing in~~of~~ these cases. It is required to find mutations when there is a strong clinical suspicion for them ~~. Sometimes in NGS, due to~~because of incomplete coverage of the exons by NGS~~, Sanger sequencing is required to find mutations, if there is strong clinical suspicion~~. In this study, by

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In academic writing, the results should be clearly presented and not left at the assumption of the readers

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using both the techniques ~~By careful use of both techniques,~~ we ~~could~~ found~~ind~~ the two *MUT* variants responsible for~~-~~ MMA in the patient~~the clinical condition~~. Previously, i~~I~~n a Saudi study on 60 ~~patients of~~ MMA patients, nonsense, missense, and frameshift mutations were detected across the *MUT* gene [3]. Another study in 43 Chinese patients identified 8 recurrent mutations and 10 novel mutations in the *MUT* gene [4]. A previous Indian study in 15 patients with ~~of~~ clinically diagnosed MMA identified one novel exon 12 mutation in the *MUT* gene with predicted pathogenicity. In this case~~Here~~, we identified two novel variants, one in exon 3 and another in exon 5 of the *MUT* gene, and~~.~~ b~~B~~oth were labelled as variants of unknown significance ~~(VUS)~~. The exon 3 variant is a synonymous variant, and a different nucleotide change c.753 G>C (p.Lys251Asn) has been reported earlier in ClinVar. Some synonymous variants can also affect ~~the~~ splicing or protein function and lead to clinical phenotypes. The identified exon 5 variant is novel~~ew~~, but a~~nother~~ close variant c.977 G>A (p.Arg326Lys) has been reported in ClinVar. The variants were found to be deleterious on bioinformatic analysis and were absent ~~not found~~ in the ExAC database. Both variants identified in the present case could be responsible for ~~possibly explain the phenotype of~~ MMA phenotype in the child. *MUT*-related MMA has poor prognosis in most cases. Specializ~~s~~ed diet and supplements may not improve the outcome~~ss~~, even if MMA is diagnosed early. Early recognition and appropriate treatment of acute crises are necessary. Metabolic stroke can sometimes occur in the absence of acute metabolic decompensation; thus, ~~, so~~ meticulous neurological examination at every~~each~~ visit is important~~useful~~. The treatment options for MMA ~~for therapy~~ include early liver transplantation [5]; ~~and possibly~~ gene therapy could also be used in the future. Genetic counseling and prenatal diagnosis could help the ~~se~~ families of the patients in making reproductive decisions in the future.

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Conflicts of Interest

The authors declare that they have no conflicts of interest.

Acknowledgments

The authors would like to acknowledge Dhiti Omics Tech–nologies Pvt Ltd for assistance help in mutation analysis.

References

[1] K. Splinter, A.-K. Niemi, R. Cox et al., "Impaired health-related quality of life in children and families affected by methyl- malonic acidemia," *Journal of Genetic Counseling*, vol. 25, no. 5, pp. 936–944, 2016.

[2] A. R. R. Devi and S. M. Naushad, "Targeted exome sequencing for the identification of complementation groups in methyl- malonic aciduria: a south Indian experience," *Clinical Biochem–istry*, vol. 50, no. 1-2, pp. 68–72, 2017.

[3] F. Imtiaz, B. M. Al-Mubarak, A. Al-Mostafa et al., "Spectrum of mutations in 60 saudi patients with mut methylmalonic acidemia," *JIMD Reports*, vol. 29, pp. 39–46, 2016.

[4] L. S. Han LS, Z. Huang Z, F. Han F, et al. Ye J, Qiu WJ, Zhang HW, Wang Y, Gong ZW, Gu XF ,. "Clinical features and MUT gene mutation spectrum in Chinese patients with isolated methylmalonic acidemia: identification of ten novel allelic variants," *World Journal of Pediatrics*, 2015 vol. Nov 1;11, no. (4), pp. :358 – 365, 2015.

[5] M. Spada, P. L. Calvo, A. Brunati et al., "Liver transplantation in severe methylmalonic acidemia: the sooner, the better," *Journal of Pediatrics*, vol. 2015;167, pp.: 1173, 2015.

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Physical Science Sample

Structural ~~Prediction~~ prediction of ~~Bis~~bis(di-p-anisole)-1,4-azabutadiene-bis[triphenylphosphine]ruthenium(II) complex ~~Using~~ using ^{31}P NMR ~~Spectroscopy~~spectroscopy

Author Details

Abstract[1]

In this study, ~~The present paper reports the use of ^{31}P NMR spectroscopy to predict the isomer structures of~~ [bis-4-methoxy-phenyl-[3-(4-methoxy-phenyl)-allylidene]-amino]-bis[triphenylphosphine]ruthenium(II) complex, also known as bis(di-p-anisole)-1,4-azabutadiene-bis[triphenylphosphine]ruthenium(II)~~,~~ complex, was synthesized using~~es. The complexation reaction was carried out (di-*p*-anisole)-1,4-azabutadiene (compound 1), triphenylphosphine, and ruthenium chloride in 2:2:1 ratio~~ under reflux~~ing~~ conditions ~~of (di-p-anisole)-1,4-azabutadiene (compound 1), triphenylphosphine (PPh_3), and ruthenium chloride in the ratio of 2 : 2 : 1~~ for ~~five~~ 5 h~~ours~~. The formation of the ~~In addition,~~ ruthenium(II) complex ~~were~~ was confirmed by~~also characterized using~~ FTIR and UV–Vis spectroscopic~~ analyses to support the formation of ruthenium(II) complexes~~. ^{31}P NMR spectroscopy ~~pic study on ruthenium(II) complexes suggested~~ indicated the presence of ~~that there are~~ three isomers ~~present~~ after the complexation reaction.

Keywords: ^{31}P NMR spectroscopy; FTIR spectroscopy; UV–Vis spectroscopy; Ru complex; Isomers; Structure prediction

[1] NMR, nuclear magnetic resonance; FTIR, Fourier transform infrared; UV–Vis, ultraviolet–visible

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• ***Corresponding author.*** Clearly indicate who will handle correspondence at all stages of refereeing and publication, also post-publication. This responsibility includes answering any future queries about Methodology and Materials. **Ensure that the e-mail address is given and tha** ...

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1. Introduction

Nuclear magnetic resonance (NMR) spectroscopy is an essential instrument analytical tool in the field of chemistry as it can helps determine elucidate the structure of a molecule, identify detect the presence of impurities in a sample, and determine the rates of formation and as well as degradation of a compound. Even in 1970s, NMR has was used as early as in the 1970s already been used to determine detect the cancer formation which and was identified to be offered a simple, fast, and low-cost method to for this purpose identify cancer formation [1–3].

For Inorganic inorganic chemists commonly use, using of ^{31}P NMR spectroscopy to identify the structures of a complexes containing phosphine ligands is very common [4, 5]. ThFor example, the well-known examples is the use of ^{31}P NMR spectroscopy to determine the mechanism of Wilkinson hydrogenation was determined by ^{31}P NMR spectroscopy, mechanism based on by identifying the coupling patterns among the phosphine ligands as well as those and also the coupling constants between the phosphine ligands as well asand the rhodium(I) metal centrecenter [6].

In As part of our long-term research interest on the synthesis of in ruthenium(II) complexes synthesis, we used the (di-*p*-anisole)-1,4-azabutadiene (**1**) and triphenylphosphine (PPh_3) as the ligands to for reaction react with ruthenium trichloride under reflux conditions. The structures of theProducts resulting complexes were formed, were checked identified by using ^{31}P NMR spectroscopy, FTIR spectroscopy, and UV–Vis spectroscopyand the results found in the spectra are worth to be discussed in the present communication.
For inorganic chemist, using of ^{31}P NMR to identify the structure of a complex containing phosphine ligands is very common [4, 5]. The well-known examples is the use of ^{31}P NMR spectroscopy to determine the Wilkinson hydrogenation mechanism by identifying the coupling patterns among phosphine ligands and also the coupling constants between phosphine ligands as well as rhodium(I) metal centre [6].

2. Methodology

The ruthenium complexes were characterized using UV/Vis, FTIR, and ^{31}P NMR spectroscopy. The IR spectra were recorded using on a Thermo Scientific Nicolet iS10 spectrophotometer in using KBr discs. The ^{1}H NMR spectrum for of compound **1** and ^{31}P NMR spectrum for of the ruthenium(II) complexes were recorded usingon a JEOL JNM-ECA 500 spectrometer with TMS as an the internal standard. The absorption spectra waswere recorded with on a Jasco V-630 UV–Vis spectrophotometer.

2.1. Preparation of (4-Methoxy-phenyl)-[3-(4-methoxy-phenyl)-allylidene]-amine or (di-*p*-Anisole)-1,4-azabutadiene (**1**)

4-Methoxycinnamaldehyde (1.62 g, 10.00 mmol) was dissolved in 10 mL of ethanol, and followed by the addition of 4-methoxyaniline (1.23 g, 10.00 mmol) which was then added to solution. The rReaction mixture was stirred and to obtain a resulted in green-yellow solid, which. The solid was filtered, washed with 5 mL of ethanol, and dried *in vacuo*. The solid was purified by dissolving it in DCM and then layered with hexane via slow diffusion to yield compound **1**.: Yyield: 2.368 g (88.7%); IR (KBr, cm^{-1}) ν: 3036 (C-H stretching), 1627 (C=N- stretching), 1601 (C=C stretching, aliphatic), 1575 and 1468 (C=C stretching, aromatic), and 1110 (OCH_3 stretching);: ^{1}H NMR (500 MHz, $CDCl_3$,) δ: 8.25 (d, 1H, Hz, -CH=N-), 7.47 (d, 2H, Hz,), 7.18 (d, 2H, Hz,), 7.05 (t, 1H, Hz, H-C_α), 6.99 (m, 1H, H-C_β), 6.90 (d, 4H, Hz,), 3.83 (s, 3H, OCH_3), and 3.81 (s, 3H, OCH_3); UV-Vis (DCM, /nm): 273, 373; Anal. Calc. for $C_{17}H_{17}O_2N$ (%): C, 76.38; H, 6.41; N, 5.24; Ffound (%): C, 76.75; H, 6.31; N, 5.05.

2.2. Preparation of [Bis4-methoxy-phenyl-[3-(4-methoxy-phenyl)-allylidene]-amino}]-bis-[triphenylphosphate]ruthenium(II) or Bis(di-*p*-anisole)-1,4-azabutadiene}-bis[triphenylphosphine]ruthenium(II) Complexes

For the synthesis of bis(di-p-anisole)-1,4-azabutadiene-bis[triphenylphosphine]ruthenium(II) complex, $RuCl_3 \cdot xH_2O$ (2.070 g, 1.0 mmol) and PPh_3 (0.525 g, 2.0 mmol) were added to a round-bottom flask containing 10 mL ethanol. and tThe mixture was then refluxed. Compound **1** (0.316 g, 2.0 mmol) was then added to the round bottom flask, and the mixture was refluxed again. The resulting pPale-maroon solids were wasformed , filtered and washed with hexane, and the p. Precipitate was dried *in vacuo*: IR (KBr, cm^{-1}) ν: 3034 (C-H stretching), 1661 (C=N), 1576 (merged IR band of for aliphatic and aromatic C=C stretching from aliphatic and aromatic), 1469 (C=C stretching of aromatic ring), and 654 (Ru-C), and 577

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(Ru-N); ; ^{31}P NMR (202.5 MHz, $CDCl_3$) δ: 49.7 (d, 1P, Hz), 47.4 (d, 1P, Hz), 41.7 (d, 1P, Hz), 39.7 (d, 1P, Hz), 35.1 (s, Ph_3P=O), and 29.9 (s, 1P); UV–Vis (DCM) (): 321 and 382.

On the other hand, theThe binding of compound **1** to the ruthenium(II) metal centrecenter wasis can be confirmed using FTIR and UV–Vis spectroscopyspectroscopies. The Comparing the IR spectra spectrum between of compound **1** and the ruthenium complexes (FigureFig. 41) reveals that the , the vibrations of C=N and C=C stretching bands are shifted with respect to those in **1**, thereby bands have been shifted confirming the after binding of **1** to the ruthenium(II) metal centrecenter. The For C=N stretching band is blue-, it shifted from 1627 cm^{-1} in the spectrum of compound **1** to 1661 cm^{-1} in the spectrum of the ruthenium complex [97, 108]. In contrast, whereas the for C=C stretching, the IR band appears at 1601 cm^{-1} in the spectrum of **compound 1** but it is notcannot be clearly shown detected in the spectrum of the complex, because the IR bands of aliphatic and aromatic C=C bands for aliphatic and aromatic were mergeing into one a single broard IR band centredcentered at 1576 cm^{-1}. Nevertheless, the two additional IR peaks are present at 577 and 654 cm^{-1} in the finger print region of the spectrum at 577 and 654 cm^{-1} indicatingconfirm the formation of the respective Ru-N and Ru-C bonds [119].

FigureFig. 41: IR spectra of (a) compound **1** (a) and (b) ruthenium(II) complexes (b).

The complexation of compound **1** to the ruthenium(II) metal centrecenter is can be further supported by the UV–vVis data spectra as shown in FigureFig. 52. For In the case of compound **1**, two absorption bands were are observed at 273 and 372 nm, which are assigned to the transitions of in the benzene ring and transition of thei imine group [1210], respectively. After the complexation, both the absorption bands undergo significant bathochromic shifts to shifts to 321 and 382 nm, respectively, thereby , confirming the Significant shifts of these two absorption bands have proven compound 1 was successfully bound binding of **1** to the ruthenium(II) metal centrecenter via the nitrogen atom from in the C=N group and the carbon atom from in the aliphatic C=C aliphatic group in of the C=C-C=N moiety.

FigureFig. 52: UV–Vis spectra of (a) compound **1** (a) and (b) ruthenium (II) complex (b).

3. Results and Discussion

Characterization of the ruthenium complexes was done using UV/Vis, FTIR, and ^{31}P NMR spectroscopy. The IR spectra was found by Thermo Scientific Nicolet iS10 in KBr disc. ^{1}H NMR spectrum for compound 1 and ^{31}P NMR spectrum for ruthenium(II) complexes obtained through JEOL JNM-ECA 500 spectrometer with TMS as an internal standard. The absorption spectra recorded with Jasco V-630 spectrophotometer.

Once the complexation was confirmed, as discussed above, The the ^{31}P NMR spectrum of the ruthenium complex (Fig. 3) was analyzed for its detailed structural elucidation. The ^{31}P NMR spectrum of the product shows appearance of two pairs of doublets and one singlet, indicating in the ^{31}P NMR spectrum for ruthenium complexes (Figure 1) indicatethe formation of that there are three isomers (1:1:1 ratio) present inas a result of the the complexation reaction with the ratio of 1 : 1 : 1.

FigureFig. 13: ^{31}P NMR spectrum for of ruthenium(II) complexes.

The singlet at 29.88 ppm reveals that the two PPh_3 units are magnetically equivalent in the ruthenium(II) complex. There can be three possible structures based on this singlet. In the first case, the In this case, the two PPh_3 units are either located at the axial positions of an octahedron and are, which is trans to each other (FigureFig. 24(a)) [117], while in the other two cases, they are located on the or located at equatorial plane, which is only trans only to either one of the C atoms from in the C=C bond (Fig. 4(b)) or the N atom from in the N=C bond (FigureFig. 24(bc)).

FigureFig. 24: Postulated structures of (a) *trans*- and ((b) and (c)) *cis*-[bis(di-*p*-anisole)-1,4-azabutadiene]-bis[triphenylphosphine]ruthenium(II).

Meanwhile, a The pair of doublets at 41.84 and 39.74 ppm with a coupling constant of 21 Hz is assigned to a the cis -isomer of the ruthenium(II) complex as shown in FigureFig. 35(a). Lastly The other , another pair of doublets at 49.80 and 47.36 ppm with a coupling constant of 38 Hz is assigned to a the trans -ruthenium(II)

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complex shown in ~~(Figure~~Fig. ~~3~~5(b)~~)~~. It is evident that the ~~The~~ difference in~~-~~ the coupling constants ~~between~~ of the ruthenium(II) complexes arises~~in Figures 3(a) and 3(b) is due~~ owing to the positions of the PPh_3 ligands. The doublet with a smaller coupling constant ~~(t, namely,~~ 21 Hz~~)~~ is ~~, was~~ assigned to the cis-isomer because both the PPh_3 ligands are in the equatorial plane. The ~~The presence of~~ doublets originate ~~because the for the~~ PPh_3 ligands in the complex ~~is shown in Figure 3(a) because both PPh_3 ligands~~ are trans to different atoms~~, that is,~~ (nitrogen and carbon)~~ atoms~~. ~~For~~ In the ruthenium(II) complex shown in ~~as shown in Figure~~Fig. ~~3~~5(b), the two PPh_3 ligands are located at the axial positions and are trans to each other. The ~~Lastly, the~~ single peak ~~observed~~ at 35.14 ppm is attributed to the ~~presence of the~~ triphenylphosphine oxide [~~128~~].

~~Figure~~Fig. ~~3~~5: Postulated structures of (a) *cis*- and (b) *trans*-[bis(di-*p*-anisole)-1,4-azabutadiene~~}~~-bis[triphenylphosphine]ruthenium(II)~~}~~.

~~On the other hand, the binding of compound **1** to ruthenium(II) metal centre can be confirmed using FTIR and UV-Vis spectroscopy. Comparing the IR spectra between compound **1** and ruthenium complexes (Figure 4), the vibrations of C=N and C=C stretching bands have been shifted after binding to ruthenium(II) metal centre. For C=N stretching band, it shifted from 1627 cm^{-1} in compound **1** to 1661 cm^{-1} in ruthenium complex [9, 10], whereas for C=C stretching, the IR band appears at 1601 cm^{-1} in **compound 1** but it is not clearly shown in the complex because the IR bands of C=C bands for aliphatic and aromatic were merging into one board IR band centred at 1576 cm^{-1}. Nevertheless two additional IR peaks are present in the finger print region at 577 and 654 cm^{-1} indicating the formation of respective Ru-N and Ru-C bonds [11].~~
~~Figure 4: IR spectra of compound **1** (a) and ruthenium(II) complexes (b).~~
~~The complexation of compound **1** to ruthenium(II) metal centre can be further supported by the UV-vis data as shown in Figure 5. For compound **1**, two absorption bands were observed at 273 and 372 nm which are assigned to transition of the benzene ring and transition of the imine group [12], respectively. After the complexation, both absorption bands shifts to 321 and 382 nm, respectively. Significant shifts of these two absorption bands have proven compound 1 was successfully bound to ruthenium(II) metal centre via the nitrogen atom from C=N group and carbon atom from C=C aliphatic group in C=C-C=N moiety. The bathochromic shift of these two absorption bands was due to the backbonding of electrons from Ru to the antibonding orbitals of C=C-C=N moiety in compound **1**. This, in turn, has weakened the bond in C=C-C=N [13].~~
~~Figure 5: UV-Vis spectra of compound **1** (a) and ruthenium (II) complex (b).~~
~~In addition, the data from IR and UV-Vis revealed t~~The successful binding of ~~at~~ compound **1** ~~has bound~~ to the ruthenium(II) metal ~~centre~~center was confirmed from the IR and UV–Vis spectral data.

~~4. Conclusion~~

~~The~~ The ^{31}P NMR spectra revealed the ~~evidence from ^{31}P NMR spectrum has shown the~~ presence of three isomers of the bis(di-*p*-anisole)-1,4-azabutadiene~~}~~-bis[triphenylphosphine]ruthenium(II) complex in ~~the~~ 1:1:1 ratio ~~of 1 : 1 : 1~~. Two of the three isomers are those shown in Fig. 5, i.e., one cis and one trans isomer, while the third isomer could be any one of those shown in Fig. 4. ~~In addition, the data from IR and UV-Vis revealed that compound **1** has bound to ruthenium(II) metal centre.~~

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References

1. R. Damadian, "Tumor detection by nuclear magnetic resonance," Science, Vol. 171(, no. 3976), (1971) pp. 1151–1153, 1971. https://doi.org/10.1126/science.171.3976.1151
2. I. D. Weisman, L.H. Bennett, L. R. Maxwell Sr., D. E. Henson, Cancer detection by NMR in the living animal, J. of Res. earch of the Natl. ional Bur. eau of Stand. dards Section A: Phys. ics and Chem. istry, vol. 80(, no. 3) (1976) , pp. 439–450, 1976. https://doi.org/10.6028/jres.080a.048
3. S. Tiziani, V. Lopes, and U.-L. Günther, "Early stage diagnosis of oral cancer using ^{1}H NMR-Based metabolomics," Neoplasia, vol. 11(, no. 3) (2009) , pp. 269–276, 2009. https://doi.org/10.1593/neo.81396
4. D. G. Gorenstein, Non-biological aspects of phosphorus-31 NMR spectroscopy, Prog. ress in Nucl. ear Mag. netic Res. onance Spect. roscopy, vol. 16 (1984) , pp. 1–98, 1984. https://doi.org/10.1016/0079-6565(84)80002-3
5. P.S. Pregosin, and R. W. Kunz, ^{31}P and ^{13}C NMR Sspectroscopy of Transition Metal Complexes, Springer, Heidelberg, Germany, 1979. https://doi.org/10.1007/978-3-642-48830-6
6. P. Meakin, J. P. Jesson, and C. A. Tolman, "Nature of chlorotris(triphenylphosphine)rhodium in solution and its reaction with hydrogen," J. ournal of the Am. erican Chem. emical Soc. iety, 94(, 9) (1972) , pp. 3240–3242, 1972. https://doi.org/10.1021/ja00764a061
7. ~~N.Dharmaraj, P. Viswanathamurthi, K. Natarajan, "Ruthenium(II) complexes containing bidentate Schiff bases and their antifungal activity, Transition Metal Chemistry, vol. 26, No. 1-2, pp. 105–109, 2001.~~
8. ~~V.V. Grushin, C. Bensimon, H. Alper, Potassium complexes containing both crown ether and tertiary phosphine oxide ligands, Inorganic Chemistry, vol. 32, no. 3, pp. 345–346, 1993.~~
9. 7. N. Ahmed, M. Riaz, A. Ahmed, and M. Bhagat, "Synthesis, characterisation, and biological evaluation of Zn(II) complex with tridentate (NNO Donor) Sschiff base ligand," Int. ernational J. ournal of Inorg. anic Chem. , istry, vol. 2015 (2015), Article ID 607178, 5 pages, 2015. https://doi.org/10.1155/2015/607178
10. 8. N. Bharti, Shailendra, S. Sharma, F. Naqvi, and A. Azam, "New palladium(II) complexes of 5-nitrothiophene-2-carboxaldehyde thiosemicarbazones: Ssynthesis, spectral studies and in vitro anti-amoebic activity," Bioorg. anic & Med. icinal Chem. istry, vol. 11(, no. 13) (2003) , pp. 2923–2929, 2003. https://doi.org/10.1016/s0968-0896(03)00213-x
11. 9. N. H. Al-Sha'alan, "Antimicrobial activity and spectral, magnetic and thermal studies of some transition metal complexes of a Schiff base hydrazone containing a quinoline moiety," Molecules, vol. 12(, no 5) (2007), pp. 1080–1091, 2007. https://doi.org/10.3390/12051080
12. 10. P. Jayaseelan, S. Prasad, S. Vedanayaki, and R. Rajavel, "Synthesis, characterization, anti-microbial, DNA binding and cleavage studies of Schiff base metal complexes," Arab. ian J. Chem. , istry 9 (, 2011) S668-S677. https://doi.org/10.1016/j.arabjc.2011.07.029
13. 11. N. Dharmaraj, P. Viswanathamurthi, K. Natarajan, "Ruthenium(II) complexes containing bidentate Schiff bases and their antifungal activity, Transit. ion Met. al Chem. istry, vol. 26(, No. 1-2), (2001) pp. 105–109, 2001. https://doi.org/10.1023/a:1007132408648
14. 12. V.V. Grushin, C. Bensimon, H. Alper, Potassium complexes containing both crown ether and tertiary phosphine oxide ligands, Inorg. anic Chem. , istry, vol. 32(, no. 3) (1993) , pp. 345–346, 1993. https://doi.org/10.1021/ic00055a021
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HIGHLIGHTS

- Three isomers were detected for a phosphine-bearing Ru complex using ^{31}P NMR.
- Formation of Ru-N and Ru-C bonds were confirmed by FTIR spectroscopy.
- At least one cis isomer and one trans isomer of the complex were formed.

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