

Medical Science Sample

Case Report

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

Sushant M. Nanavati ¹ Hiren Patel ² Gabriel Melki,¹ Vinod Kumar,¹ Edward Milman,³ Patrick
Michael,¹ and Ariy Volfson²

*1 Department of Internal Medicine, St. Joseph's University Medical Center-New
York Medical College, USA 2Department of Gastroenterology, St. Joseph's
University Medical Center-New York Medical College, USA 3Department of
Radiology, St. Joseph's University Medical Center-New York Medical College, USA*

Correspondence should be addressed to Sushant M. Nanavati;
snanav2@gmail.com

Commented [A1]: 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.

Overshadowed by ~~S~~ino-pulmonary infections, ~~c~~Cystic ~~f~~Fibrosis (CF) commonly affects gastrointestinal organs because of secretory and motility dysfunction. Infrequently, the ~~resulting~~ changes ~~can result~~ ~~in~~cause ~~d~~istal ~~i~~ntestinal ~~O~~bstuction ~~s~~ndrome (DIOS), an ~~more-and-more~~increasingly diagnosed gastrointestinal ~~condition~~entity in adult ~~Cystic-Fibrosis~~CF patients. We present ~~thea~~ 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 ~~thatwhich~~ may not have a ~~C~~Fystic Fibrosis care program available.

Commented [A2]: The meaning of this phrase and how it is related to the rest of the sentence are not completely clear. Do you mean that cystic fibrosis is not a primary sinopulmonary infection? Please clarify so that I can suggest a suitable revision.

1. Introduction

Cystic ~~F~~ibrosis (CF) is a genetic disease ~~of-that affects~~ multiple organs. ~~With-Because of~~ advancements ~~in the~~ management ~~ing of CF-patients~~, patients ~~can~~ now ~~often-survive become-to~~ adulthood [1]. ~~However, the i~~Improved life expectancy among adult CF patients has ~~given-riseled~~ to ~~an increase in~~ extrapulmonary, notably gastrointestinal, manifestations, which ~~did-not-happen-was~~ previously ~~uncommon~~. Distal ~~i~~ntestinal ~~o~~bstuction ~~s~~ndrome (DIOS) continues to be a rising complication in adult CF patients, presenting ~~aswith~~ acute abdominal pain ~~like-and~~ ~~mimicking~~ an acute abdominal emergency.

Commented [A3]: Please include the relevant citation here.

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. 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. Notably, no history of constipation or recurrent abdominal discomfort was reported prior to this. At home, the patient was prescribed aAlbuterol inhalation~~er~~ as needed, dDornase aAlfa inhalation~~er~~, 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 FibrosisCF at the age of four, and thehis disease progressed to exocrine pancreat~~ics~~ insufficiency, which was being treated with pancreatic enzymes. On abdominal exam~~ination~~, 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).

Commented [A4]: Please consider specifying the dosage and form of intake for all drugs to ensure consistency.

Commented [A5]: I have deleted this abbreviation as has not been subsequently used in the main text. Figure legends generally require abbreviations to be spelt out separately.

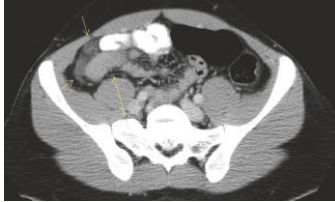


FIGURE 1: Axial abdominal computed tomography scan depicting thickening around the terminal ileum and colon (yellow arrows) along with extraluminal fluid and reactive lymph nodes.

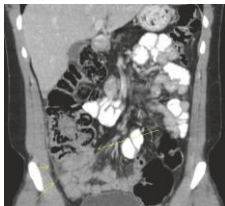


FIGURE 2: Coronal view computed tomography scan with showing thickening of the ileum with a distended appendix (yellow arrows).

measuring 5.34.6 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, 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). Postoperatively, he was diagnosed with DIOS and was subsequently started on pPolyethylene glycol. The patient made an unremarkable recovery and was discharged. He was followed up in the

Commented [A6]: The use of "in" is a little unclear. Do you mean to say distended appendix with/caused by chronic adhesions instead?

outpatient clinic ~~without and did not have any~~ recurrence of any symptoms.

3. Discussion

~~Distal Intestinal Obstruction Syndrome (DIOS,)~~ ~~was previously called known as~~ 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 intestinal wall of the terminal ileum and cecum [1]. Perez-Aguilar et al. reported ~~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-reporting~~ 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.

Defective intestinal chloride and water secretions into the gut, luminal acidity, and loss of bile salt all contribute to the

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, which further leads to diagnostic uncertainty. ~~Overlap of several intra-abdominal pathologies in CF increases the risk of misdiagnosis, especially with for acute appendicitis,~~ as these ~~patient's~~ underlying pathologies may be masked ~~in patients~~ with pulmonary infections² ~~using~~ antibiotics [5, 6].

Commented [A7]: This sentence and the previous sentence are redundant and provide repeated information (i.e. overlapping pathologies between appendicitis and DIOS leading to misdiagnosis). Therefore, please consider deleting this.

Osmotic laxatives are the cornerstone of bowel regimens for the treatment of DIOS. The most commonly prescribed ~~laxative is p~~Polyethylene Glycol, (PEG)-administered at a dose of 20–40 ml/~~kKg/hH~~, ~~up to with~~ a maximum of 1 ~~L/kg/h~~ for a total of 8 hours, ~~resulting in 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, ~~ICileocecal~~ valve resection should be considered to prevent the ~~development and~~ recurrence of intestinal obstruction sequelae ~~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.

Commented [A8]: The relationship between the two parts of this sentence was not clearly established, as it was unclear how immigration affected care centers' ability to manage patients. I have added this term to make this association clearer.

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 management 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.

Commented [A9]: I have ensured consistency among the references, maintaining the predominant style used. I have also ensured correspondence between the in-text citations and references.

Commented [A10]: Please ensure you include the supplement number here.

2.

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

Commented [A11]: Please include the names of all authors here.

3.

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.

5.

6.

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.

Commented [A12]: Please ensure you include the volume number here.

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.

Commented [A13]: Please include the names of all authors here.

Life Sciences Sample

Case Report

Methylmalonic Acidemia with Novel *MUT* Gene Mutations

Inusha Panigrahi, Savita Bhunwal, Harish Varma, and Simranjeet Singh

Department of Pediatrics, Advanced Pediatric Centre, PGIMER, Chandigarh, India

Correspondence: Inusha Panigrahi; inupan@yahoo.com

A 5-year-old boy presented with recurrent episodes of fever, feeding problems, and lethargy, ~~from since~~ the age of 11 months, and poor weight gain. He was admitted to our hospital and evaluated for metabolic disorders; subsequently, he ~~because and was~~ diagnosed with 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 variants in ~~exon 5 and exon 3 of~~ the *MUT* gene responsible for ~~the methylmalonic acidemia MMA in exon 5 and exon 3~~. Recently, he ~~had~~ developed dystonic movements including orofacial dyskinesia. ~~With the~~ advent of NGS, judicious use of NGS with Sanger sequencing can help identify causative ~~and~~ possibly pathogenic mutations.

1. Case Presentation

~~A 5-year-old~~ The child boy presented ~~for the first time at the age of 11 months,~~ with complaints of fever, vomiting, poor feeding, and lethargy ~~for the first time at the age of 11 months.~~ We observed that the patient ~~he~~ had pallor and tachypnea and was drowsy. Further evaluation ~~was~~ suggested ~~due to~~ 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 ~~were~~ normal, ~~but gas chromatography mass spectrometry analysis of but urine GCMS~~ revealed elevated 3-OH propionic acid [12.39 retention time (RT)] ~~as well as and 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, ~~the this child patient~~ was on a low-protein diet, ~~and~~ carnitine, biotin, thiamine, and vitamin B12 injections. The 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 ~~performed done~~, and a ~~second 2nd~~ 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~~

Commented [A 1]: Dear Authors, 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.

Formatted: Right: 0.25 cm, Space Before: 0.35 pt, Line spacing: single

Commented [A2]: From what age was this observed? Please clarify.

Commented [A3]: As a general rule, an abbreviation should only be introduced if it's used a minimum of three times in each standalone section of the paper.

Commented [A 4]: I would suggest that you revise the highlighted part as "NGS along with Sanger sequencing can help identify pathogenic mutations responsible for various clinical conditions" to reduce wordiness.

Formatted: Font: Italic

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 showing~~ 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, generaliz~~ed~~ed hypertonia, and generaliz~~ed~~ed 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~~Parents were counseled regarding ~~the~~ prognosis and for prenatal diagnosis ~~for next subsequent pregnancies~~.

2. Discussion

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~~ mutations [1, 2]. ~~There can be Missense or nonsense mutations, deletions, insertions, and so on in the MUT gene and so on can~~ leading to a clinical phenotype.

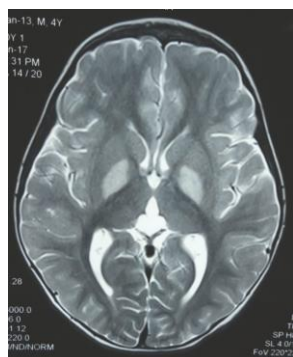


FIGURE Figure-1: ~~The Brain magnetic resonance image MRI brain of in~~ the -child -with MUT-related ~~methylnalonic acidemia~~MMA showing -predominant -frontoparietal abnormalities -in ~~the~~ form -of encephalomalacia and gliosis.

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 using both the techniques ~~By careful use of both techniques, we could found~~ the two ~~MUT~~.

Formatted: Font: Bold

Formatted: Font color: Auto

Formatted: Font: Bold

Formatted: Indent: Left: 7.25 cm, No bullets or numbering

Commented [A5]: Please clarify whether you mean recurrent episodes of lethargy, acidosis, hypoglycemia/hyperglycemia, and ketosis.

Formatted: Font: Italic

Formatted: Font: Italic

Formatted: Font: Italic

variants responsible for- MMA in the patientthe clinical condition. In 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 [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 caseHere, we identified two novel variants, one in exon 3 and another in exon 5 of the *MUT* gene. Both 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 novelnew, but another 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 possibly explainbe responsible for the phenotype of MMA phenotype in the child. *MUT*-related MMA has poor prognosis in most cases. Specialized diet and supplements may not improve the outcomes, 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, so meticulous neurological examination at every each visit is useful. The treatment options for therapy include early liver transplantation [5] and possibly gene therapy in the future. Genetic counseling and prenatal diagnosis could help these families of the patients in making reproductive decisions.

Commented [A 6]: I would suggest that you establish in the previous section that the patient was diagnosed with MMA in the Case presentation, as it has not been concluded there. The section seems incomplete.

Commented [A 7]: I would suggest that you use MM. instead of clinical condition here.

Commented [A 8]: Do you mean in a study on Saudi patients? If so, please changed to "In a study on 60 Saudi patients with MMA."

Formatted: Font: Italic

Commented [A9]: Do you mean a study on 15 Indian patients? If so, please rephrase as "A previous study involving 15 Indian patients."

Formatted: Font: Italic

Formatted: Font: Italic

Commented [A 10]: Please provide a reference at all instances where previous studies are cited.

Commented [A 11]: In the current context, "novel" is better-suited than "new." Please ensure that you agree with this change.

Formatted: Font: Italic

Formatted: Space Before: 0.35 pt

Conflicts of Interest

The authors declare that they have no conflicts of interest.

Acknowledgments

The authors would like to acknowledge Dhiti Omics Technologies Pvt Ltd for 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 Biochemistry*, 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 methylmalonic acidemia," *JIMD Reports*, vol. 29, pp. 39–46, 2016.
- [4] L. S. Han, Z. Huang, F. Han, 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*, vol. 2015 Nov 11, no. 11(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.

Commented [A 12]: As no specific instructions for the format of references were provided, I have edited the references only to ensure consistency in format. Please check your target journal's guidelines for the required format and ensure that your references follow it.

Formatted: Space Before: 0 pt, Line spacing: single

Formatted: Indent: Hanging: 0.5 cm, Space Before: 0 pt, Line spacing: single

Formatted: Font: Italic

Formatted: Font: Italic

Formatted: Font: Italic

Physical Science Sample

Structural Prediction prediction of Bisbis(di-p-anisole)-1,4-azabutadiene-bis[triphenylphosphine]ruthenium(II) Using ³¹P NMR Spectroscopy

Author Details

Abstract¹

The present paper reports the use of ³¹P NMR spectroscopy to predict the isomers structures of [bis-4-methoxy-phenyl-[3-(4-methoxy-phenyl)-allylidene]-amino]-bis[triphenylphosphine]ruthenium(II), also known as bis(di-p-anisole)-1,4-azabutadiene-bis[triphenylphosphine]ruthenium(II), complexes. The complexation reaction was carried out using (di-p-anisole)-1,4-azabutadiene (compound 1), triphenylphosphine, and ruthenium chloride in 2:2:1 ratio under refluxing conditions of (di-p-anisole)-1,4-azabutadiene (compound 1), triphenylphosphine (PPh₃), and ruthenium chloride in the ratio of 2:2:1 for five 5 hours. The formation of the In addition, ruthenium(II) complexes were was further confirmed by also characterized using FTIR and UV-Vis spectroscopy- analyse to support the formation of ruthenium(II) complexes. The results of ³¹P NMR spectroscopy pie study on ruthenium(II) complexes suggested indicated the presence of that there are three isomers present after the complexation reaction.

Keywords:

¹ NMR, nuclear magnetic resonance;

Commented [A1]: Thanks for providing this opportunity to assist you with this manuscript. I have edited the text for language, grammar, and improved clarity. I have also checked the manuscript for conformance with the formatting guidelines for Inorganic Chemistry Communications provided at

<https://www.elsevier.com/journals/inorganic-chemistry-communications/1387-7003/guide-for-authors#25000>

In the cases where additional information is required from you, I have added comments to bring them to your attention.

Should you have any questions, please feel free to get back to me.

My best wishes for your success with the manuscript.

Commented [A2]: To ensure completeness of the name of the compound, this should be "Bis(di-p-anisole)-1,4-azabutadiene-bis[triphenylphosphine]ruthenium(II) complex" or "Bis(di-p-anisole)-1,4-azabutadiene-bis[triphenylphosphine]ruthenium(II) species." Please choose the one that you find the most suitable and incorporate the change.

Commented [A3]: Please clearly indicate the given name(s) and family name(s) of each author and check that all names are accurately spelled. You can add your name between parentheses in your own script behind the English transliteration. Present the authors' affiliation addresses (where the actual work was done) below the names. Indicate all affiliations with a lower-case superscript letter immediately after the author's name and in front of the appropriate address. Provide the full postal address of each affiliation, including the country name and, if available, the e-mail address of each author.

• **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

Commented [A4]: This is a more concise term. Moreover, this will ensure consistency in the text.

Formatted: Font: Italic

Formatted: Font: Bold

Commented [A5]: Both *refluxing conditions* and *reflux conditions* are acceptable in this context, but I have used the latter consistently in the text.

Commented [A6]: The journal requires that an abbreviation be spelled out at its first occurrence in the text, followed by the abbreviation in parentheses. (Exception: If the abbreviation is on the journal's list of permitted abbreviations, this need not be done.) Thereafter, only the

Commented [A7]: Please add a maximum of 6 keywords, using American spelling and avoiding general and plural terms and multiple concepts (avoid, for example, 'and', 'of'). The journal states that only abbreviations firmly established

Commented [A8]: **Highlights** are optional yet highly encouraged for this journal, as they increase the discoverability of your article via search engines. Highlights should be submitted in a separate editable file in the online submission system. Please use 'Highlights' in the file name and include 3 to 5 bullet points (maximum 85 characters,

1. Introduction

Nuclear magnetic resonance (NMR) spectroscopy is an essential instrument-analytical tool in the field of chemistry as it can help 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 been 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].

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 (PPh₃) as the-ligands to-for reaction react-with ruthenium trichloride under reflux conditions. The resulting pProducts were-formed, were checked-analyzed by using ³¹P NMR spectroscopy, and the spectral observations-results found-in-the spectra are worth-to-be-discussed in the present communication.

For-Inorganic inorganic chemists commonly use, using-of ³¹P NMR spectroscopy to identify the structure of a complex containing phosphine ligands is very-common [4, 5]. ThFor example, this technique has been usede well-known-examples-is the use of ³¹P NMR spectroscopy to determine-elucidate the mechanism of Wilkinson hydrogenation 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-as-and the rhodium(I) metal centre-center [6].

2. Methodology

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

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

4-Methoxycinnamaldehyde-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 layered with hexane via slow diffusion. Yield: 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 (OCH₃ stretching); ¹H NMR (500 MHz, CDCl₃): 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, OCH₃), and 3.81 (s, 3H, OCH₃); UV-Vis (DCM, /nm): 273, 373; Anal. Calc. for C₁₇H₁₇O₂N (%): C, 76.38; H, 6.41; N, 5.24; Found (%): C, 76.75; H, 6.31; N, 5.05.

To prepare 2.2. Preparation of [Bis-(4-methoxy-phenyl)-[3-(4-methoxy-phenyl)-allylidene]-amino]-bis-[triphenylphosphine]ruthenium(II) or Bis-(di-*p*-anisole)-1,4-azabutadiene)-bis[triphenylphosphine]ruthenium(II) Complexcomplex, es

RuCl₃·xH₂O (2.070 g, 1.0 mmol) and PPh₃ (0.525 g, 2.0 mmol) were added to a round-bottom flask containing 10 mL ethanol, and the 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-was-formed, 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 (Ru-N); ³¹P NMR (202.5 MHz, CDCl₃): δ: 49.7 (d, 1P, Hz), 47.4 (d, 1P, Hz), 41.7 (d, 1P, Hz), 39.7 (d, 1P, Hz), 35.1 (s, Ph₃P=O), and 29.9 (s, 1P); UV-Vis (DCM) (λ): 321 and 382.

3. Results and Discussion

The Characterization of the-ruthenium complexes were characterized was-done-usingby UV-Vis, FTIR,

Commented [A9]: I have removed all the section headings as the journal guidelines state: "Communications should be written in the correct format (i.e. the Introduction, Experimental, Results and Discussion sections combined into a single untitled section)"

Commented [A10]: I made this change as spectroscopy is a method/tool, and not an instrument.

Commented [A11]: The in-text citations are in the style prescribed by the journal.

Formatted: Font: Italic

Formatted: Font: Bold

Commented [A12]: Please check if you mean "constants" here as well.

Formatted: Font: Bold

Formatted: Font: Italic

Formatted: Font: Bold

Formatted: Font: Italic

Formatted: Font: Italic

Commented [A13]: The information here may be a little difficult to follow; I recommend presenting this in the form of a table to improve readability.

Formatted: Font: Italic

Formatted: Font: Bold

Formatted: Font: Italic

Formatted: Font: Italic

Commented [A14]: Please update the missing information.

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

The ^{31}P NMR spectrum of the ruthenium complexes (Fig. 1) shows appearance of two pairs of doublets and one singlet, indicating in the ^{31}P NMR spectrum for ruthenium complexes (Figure 1) indicate the presence of that there are three isomers (1:1:1 ratio) present induring the complexation reaction with the ratio of 1:1:1.

FigureFig. 1: ^{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. The In this case, the two PPh_3 units are either located at the axial position and are, which is trans to each other (FigureFig. 2(a)) [7], or located at in the equatorial plane, which is only trans only to either one of the C atoms from in the $\text{C}=\text{C}$ bond or the N atom from in the $\text{N}=\text{C}$ bond (FigureFig. 2(b)).

FigureFig. 2: 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. 3(a). Lastly, A, 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) complex (FigureFig. 3(b)). The difference in-coupling between the ruthenium(II) complexes in FiguresFig. 3(a) and 3(b) is due to the positions of the PPh_3 ligands. The smaller coupling constant of τ , namely, 21 Hz is, was assigned to the *cis*-isomer because both the PPh_3 ligands are in the equatorial plane. Fig. 3(a) shows The presence of doublets, which are for assignable to the PPh_3 ligands in the complex is shown in Figure 3(a) because both the PPh_3 ligands are trans to different atoms, that is, (nitrogen and carbon) atoms. For In the ruthenium(II) complex (as shown in FigureFig. 3(b)), the two PPh_3 ligands are located at the axial position 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 [8].

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

On the other hand, theThe binding of compound 1 to the ruthenium(II) metal eentrecenter is can be confirmed using FTIR and UV-Vis spectroscopy. Comparison of the IR spectra between of compound 1 and the ruthenium complexes (FigureFig. 4) reveals that, the vibrations of $\text{C}=\text{N}$ and $\text{C}=\text{C}$ stretching bands are have been shifted after binding to the ruthenium(II) metal eentrecenter. The For $\text{C}=\text{N}$ stretching band, it shifted from 1627 cm^{-1} in the spectrum of compound 1 to 1661 cm^{-1} in the spectrum of the ruthenium complex [9, 10]. In contrast, whereas the for $\text{C}=\text{C}$ stretching, the IR band appears at 1601 cm^{-1} in the spectrum of compound 1 but it is not clearly shown detected in the spectrum of the complex because the IR bands of aliphatic and aromatic $\text{C}=\text{C}$ bands for aliphatic and aromatic were merging into one a single broad IR band eentrecentered 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} indicating confirm the formation of the respective Ru-N and Ru-C bonds [11].

FigureFig. 4: IR spectra of (a) compound 1 (a) and (b) ruthenium(II)- complexes (b).

The complexation of compound 1 to the ruthenium(II)- metal eentrecenter is can be further supported by the UV-Vis data spectra as shown in FigureFig. 5. For In the case of compound 1, two absorption bands were are observed at 273 and 372 nm , which are assigned to the transition of the benzene ring and -transition of the imine group [12], respectively. After the complexation, both absorption bands show significant shifts to 321 and 382 nm , respectively, demonstrating the Significant shifts of these two absorption bands have proven compound 1 was successfully bound-binding of 1 to the ruthenium(II) metal eentrecenter via the nitrogen atom from in the $\text{C}=\text{N}$ group and the carbon atom from in the aliphatic $\text{C}=\text{C}$ aliphatic group in of the $\text{C}=\text{C}-\text{C}=\text{N}$ moiety.

Commented [A15]: Although I have edited this section, I would like to note that the text repeats information presented in an earlier paragraph. Therefore, I recommend deleting this one.

Commented [A16]: Since you talk about axial position, this needs to be "at the equatorial position,..."

Commented [A17]: Changes were made here to improve the clarity and readability of this part. Please check whether the revised part retains the intended meaning.

Formatted: Font: Italic

Formatted: Font: Italic

Formatted: Font: Italic

Formatted: Font: Italic

Formatted: Font: Italic

Formatted: Font: Italic

Formatted: Font: Bold

Formatted: Font: Bold

Formatted: Font: Bold

Formatted: Font: Bold

Formatted: Font: Bold

Commented [A18]: I used this word as the fingerprint region is unique to each compound and the peaks in this region are typically used for confirming the structure of a compound.

Formatted: Font: Bold

Formatted: Font: Bold

Formatted: Font: Bold

Formatted: Font: Bold

Figure 5: UV-Vis spectra of (a) compound 1 and (b) ruthenium (II) complex.

Formatted: Font: Bold

4. Conclusion

Based on ^{31}P NMR spectral evidence, we confirmed 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. In addition, the data from IR and UV-Vis spectral data revealed the successful binding of at compound 1 has bound to the ruthenium(II) metal centre.

Formatted: Font: Italic

Formatted: Font: Bold

References

1. R. Damadian, "Tumor detection by nuclear magnetic resonance," *Science*, **Vol.** 171(**no.** 3976), (1971) pp. 1151–1153, 1971.
2. I.-D. Weisman, L.H. Bennett, L.-R. Maxwell Sr., D.-E. Henson, "Cancer detection by NMR in the living animal," *J. of Research of the National Bureau of Standards Section A: Physics and Chemistry*, **vol.** 80(**no.** 3) (1976), pp. 439–450, 1976.
3. S. Tiziani, V. Lopes, and U.-L. Günther, "Early stage diagnosis of oral cancer using ^1H NMR-based metabolomics," *Neoplasia*, **vol.** 11(**no.** 3) (2009), pp. 269–276, 2009.
4. D.-G. Gorenstein, "Non-biological aspects of phosphorus-31 NMR spectroscopy," *Progress in Nuclear Magnetic Resonance Spectroscopy*, **vol.** 16 (1984), pp. 1–98, 1984.
5. P.S. Pregosin, and R.-W. Kunz, ^{31}P and ^{13}C NMR Spectroscopy of Transition Metal Complexes, Springer, Heidelberg, Germany, 1979.
6. P. Meakin, J.-P. Jesson, and C.-A. Tolman, "Nature of chlorotris(triphenylphosphine)rhodium in solution and its reaction with hydrogen," *Journal of the American Chemical Society*, 94(**no.** 9) (1972), pp. 3240–3242, 1972.
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), (2001) 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) (1993), pp. 345–346, 1993.
9. N. Ahmed, M. Riaz, A. Ahmed, and M. Bhagat, "Synthesis, characterisation, and biological evaluation of Zn(II) complex with tridentate (NNO Donor) Schiff base ligand," *International Journal of Inorganic Chemistry*, **vol.** 2015 (2015), Article ID 607178, 5 pages, 2015.
10. N. Bharti, Shailendra, S. Sharma, F. Naqvi, and A. Azam, "New palladium(II) complexes of 5-nitrothiophene-2-carboxaldehyde thiosemicarbazones: Synthesis, spectral studies and in vitro anti-amoebic activity," *Bioorganic & Medicinal Chemistry*, **vol.** 11(**no.** 13) (2003), pp. 2923–2929, 2003.
11. 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.
12. P. Jayaseelan, S. Prasad, S. Vedanayaki, and R. Rajavel, "Synthesis, characterization, anti-microbial, DNA binding and cleavage studies of Schiff base metal complexes," *Arabian J. Chemistry* (2011).

Commented [A19]: Please include the DOI for all references.

Commented [A20]: Please update the correct page number range here and remove the article ID.

Commented [A21]: Please add the volume number and page number range.