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The Photolytic Cleavage of Methylpyridinecobaloxime [$Co(CH_3)(dmgH)_2(py)$] as a Mimetic of Vitamin B_{12}

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ABSTRACT

The photolytic reactions of the methylpyridinecobaloxime, $Co(CH_3)(dmgH)_2(py)$ were examined first in benzene and then in a mixture of acetonitrile, hydrogen peroxide and toluene using sunlight and 450 Watts Hanovia UV lamp. While in the first, toluene was produced, the later produced a mixture of products that is o, p, m cresol and benzaldehyde. The photolytic reaction of vitamin B_{12} (methylcobalamin) was also examined in benzene and hydrogen peroxide using sunlight. Phenol was produced. All the products formed were separated and characterized by IR and GC–MS. The formation of these products indicates that hydroxyl radicals were generated analogous to Fenton reaction and that both vitamin B_{12} (methylcobalamin) and methylpyridinecobaloxime, $Co(CH_3)(dmgH)_2(py)$ undergoes the same photolytic cleavage of the Co-C bond.

Key Words: *Methylpyridinecobaloxime, methylcobalamin, hydroxyl radicals, photolytic cleavage, Fenton reaction.*

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1. INTRODUCTION

Cobalt is a trace element in living systems and present at a concentration of only 10^{-8} M [1, 2] in humans. This low concentration does not however, indicate that cobalt is biologically unimportant. A deficiency of cobalt in the human diet leads to the fatal disorder pernicious anemia [3]. In the body, cobalt is present in the family of coenzymes called Cobalamins [4, 5]; one derivative of which is Methylcolamine or vitamin B_{12}. As the first organometallic complex found in nature, the cobalt-containing coenzyme has attracted much attention from inorganic and biological chemists. The structures of all cobalamins are similar and consist of a cobalt ion situated in a tetramacrocyclic ligand with trans imidazole and another R group (-CN, Me, OH, 5'deoxyadenosyl, etc). The structure of a general cobalamin is shown in figure 1.1 [6].

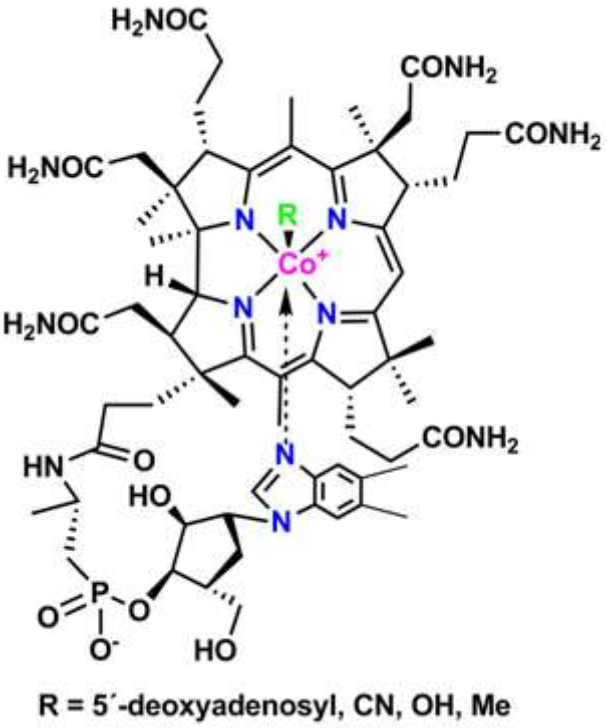
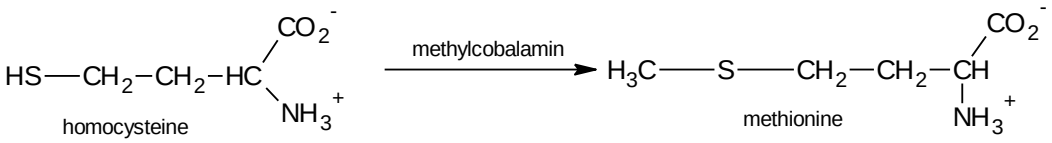
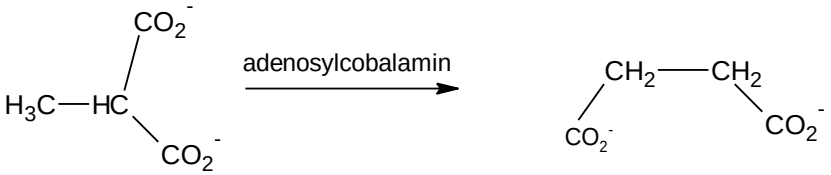


Fig. 1.1. Structure of a general Cobalamin

The most important component of the coenzyme is the cobalt-carbon bond. This bond is cleaved during the enzyme's catalytic cycle [7-9]. Cobalamin-based enzymes catalyze two kinds of transformations which are methylation and rearrangement. When the alkyl group is methyl, the enzymes are involved in methylation reactions. A specific case is the biosynthesis of the amino acid methionine from homocysteine [10].



The occurrence of the poisonous substance methyl mercury in the environment has been attributed to the action of the bacteria methylcobalamins [11, 12] on inorganic mercury salts. In the second class of cobalamin-based enzyme, the alkyl ligand is an adenosyl, which is the adenosylcobalamin [13, 14] enzyme which catalyses skeletal rearrangement. For example, one adenosylcobalamin-based enzyme catalyses the conversion of methylmalonate to succinate, a key step in the Krebs citric acid cycle.



Unlike methylcobalamine (which serves as a source of CH_3^+), adenosylcobalamin initiates reactions by means of the reversible homolysis of the cobalt-adenosyl bond. The resulting carbon-based adenosyl radical abstracts a hydrogen atom from the substrate, thereby initiating the subsequent skeletal rearrangements [14].

The molecular complexity of the cobalamin makes it difficult to study the coordination chemistry of the cobalt centre. Inorganic chemists, however, have found that properties of these enzymes can be stimulated using model compounds derived from fairly simple ligands. The most successful model complexes are known as cobaloximes [15-17]. In the cobaloximes, a pair of dimethylglyoximate ligands serves the function of the corrin macrocycle. Two dimethyloximate anions ($dmgH^-$) ligands can hydrogen bond to each other to form a planar anionic macrocycle that binds the cobalt through its four nitrogen atoms. Fig 1.2 is a typical methylcobaloxime, a model of Vitamin B_{12}.

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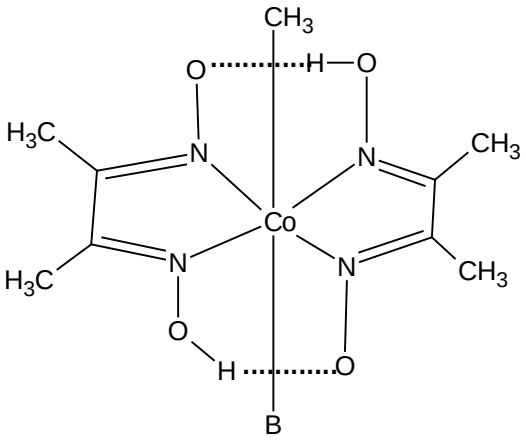

Where B = pyridine, water

Fig 1.2. Structure of a general methyl(ligand)cobaloxime

Spectroscopic studies on the model complexes have helped in establishing the basic relationship between structure and chemical properties [18, 19]. Kofod et al [20], characterized cobalt (III) compounds with classical ligands by spectroscopic techniques. The systematic analysis of the structure–property relationship has furnished useful information concerning the Co–C bond homolysis in vitamin B_{12} coenzyme [21-24]. Canpolat et al [25] reported that vicdioxime complexes of cobalt (III) complexes were the most active among the different metal complexes and may be promising for the development of new antibiotics. Halocobaloximes containing coordinated pyrazine, pyrazine carboxylic acid and pyrazine carboxamade have been found to exhibit strong antibacterial activities against *Escherichia coli*, *Proteus vulgaris*, *Proteus mirabilis* and *Pseudomonas aervugnosa* [26].

In Fenton reaction [27, 28], Ferrous iron (II) is oxidized by H_2O_2 to ferric iron (III), a hydroxyl radical and a hydroxyl anion. Iron (III) is then reduced back to iron (II), a peroxide radical and a proton by the same hydrogen peroxide

$$Fe^{2+} + H_2O_2 \longrightarrow Fe^{3+} + OH^- + OH^{\cdot}$$

$$Fe^{3+} + H_2O_2 \longrightarrow Fe^{2+} + OOH^{\cdot} + H^+$$

In the net reaction, iron reacts with two molecules of hydrogen peroxide to produce two hydroxyl radicals and water. In general alkylcobaloximes are known to undergo photolytic cleavage of the Co-C bond leading to the formation of an alkyl radical and the reduced metal, Co (II) [29, 30]. These initial products were made to react with H_2O_2 to generate the more powerful oxidant, $^{\cdot}OH$ radical analogue to the Fenton reaction.

$$Co^{III} \xrightarrow{h\nu} Co^{II}$$

$$Co^{II} + H_2O_2 \longrightarrow Co^{III} + OH^{\cdot} + OH^-$$

The aim of this work was to investigate the photolytic cleavage of Co^{III} – C in methylcobalamin (vitamin B_{12}) and methylpyridinecobaloxime [$Co(CH_3)(dmgH)_2(py)$] in sunlight and Hanovia UV lamp and also generate the hydroxyl radical ($^{\cdot}OH$) from the said photolytic reaction. The effect of the hydroxyl radical on simple organic molecules such as benzene and toluene were also investigated.

2. Experimental

2.1 General Procedure

Analytical thin layer chromatography (TLC) was used to determine the number of different components and also determine the purity of the components. Aluminum and glass plates of size 5 cm × 10 cm precoated with silica gel 60. F_{254} (Merck) with a thickness of 0.2 mm were used for TLC. The solvents that were used included various mixtures of petroleum ether (bp 60 - 80 ^{o}C),

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ethyl acetate, chloroform, hexane, acetone and methanol. Migration of the spots on the TLC plates was visualized under ultra violet (UV) light and in iodine vapour.

Melting points of pure compounds synthesized were determined using Gallenkamp Melting Point Apparatus and are uncorrected. The amount of cobalt in each complex was determined by Atomic Absorption Spectroscopy (AAS). The amounts of C, N, H, and Cl were determined using Perkin – Elmer 2400 series II Analyzer. The IR spectra were run on a Fourier Transform Infrared Spectrophotometer (FTIR-8201A). The UV spectra were run on a UV/VIS Spectrometer (T 70 UV/VIS spectrometer). 0.1% of each sample was prepared by dissolving 25 mg in 25 ml of ethanol and scanned using the T 70 UV/VIS spectrometer with silica cells in the wavelength (λ) region of 190 nm -700 nm. ^{1}H NMR and ^{13}C NMR spectra in DMSO–d6 were obtained on a Bruker Advance 300 spectrometer using TMS as an internal reference. The GC-MS used was a Varian CP 3600 GC/MS, Varian Saturn 2200. The trap, manifold and Xterline temperatures of the MS were 200 ^{o}C, 80 ^{o}C and 260 ^{o}C respectively.

Structure Elucidation and identification of synthesized compounds were done by comparing the colour, melting points and the spectra of authentic samples with those of the synthesized complexes.

2.2. Preparation of Alkylcobaloxime

2.2.1. Synthesis of Dichlorobis(dimethylglyoxime)cobalt(III) [$CoCl_2(dmgH)_2(py)$]

2.215 g of dimethylglyoxime was added to a stirred solution of 3.037 g of cobalt (II) chloride hexahydrate in 50 mL of acetone. A light blue solution was obtained. A gentle stream of air was allowed to flow over the solution until a light green solid was deposited. After 1 hour the solution was chilled in ice, filtered and washed with 2 × 10 mL of cold acetone. The yield was determined and the product characterized by elemental analysis, IR, UV, ^{1}H and ^{13}C NMR.
Green block crystals were obtained, yield 2.642 (87%), melting point mp. 336–338 ^{o}C. IR ν_{max}: 3419, 1618, 1382, 1220, 1109, 1070, UV/Vis (C_2H_5OH, nm) λ: 242, 296, 380, ^{1}H-NMR (300 MHz, DMSO) δ: 2.1 (s, 12H, CH_3-dmgH); ^{13}C-NMR (300 MHz, DMSO) δ: 10.91 (CH_3-dmgH), 150.63 (C=N-dmgH). This is in agreement with published data [31-33]

2.2.2. Synthesis of Chloropyridinebis(dimethylglyoximato)cobalt (III) [$CoCl(dmgH)_2(py)$]

1.8166 g of the light green product $CoCl_2(dmgH)_2$ synthesized above, was dissolved in 40 mL methanol. 1.0 mL of pyridine was added. The mixture was stirred until the light green solid disappeared and was replaced by an ochre crystalline solid. 60 mL of distilled water was added with stirring and then the suspension cooled in ice for 10 minutes. The product was collected by suction filtration and washed with 3 × 10 mL of 2: 1 water: methanol and 2 × 10 mL of diethyl ether. The yield was determined and the product characterized by elemental analysis, IR, UV, ^{1}H and ^{13}C NMR. Ocher coloured block crystals were obtained, yield 1.4362 (75%), melting point mp. 258–260 ^{o}C. IR ν_{max}: 3416, 1618, 1560, 1448, 1386, 1244, 1093, UV/Vis (C_2H_5OH, nm) λ: 240, 296, 376, ^{1}H-NMR (300 MHz, DMSO) δ: 2.4 (s, 12H, dmgH-CH_3), 8.20 (d, 2H, α-H py), 7.90 (t, 1H, γ-H py), 7.45 (t, 1H, β-H py); ^{13}C-NMR (300 MHz, DMSO) δ: 12.54 (dmgH-CH_3), 152.34 (dmgH-C=N), 150.14 (α-C py), 139.82 (γ-C py), 126.22 (β-C py). This is in agreement with published data [31-33]

2.2.3. Synthesis of Methylpyridinebis(dimethylglyoximato)cobalt (III) [$Co(CH_3)(dmgH)_2(py)$]

The reaction apparatus consisted of a 250 mL three–neck round bottom flask with a rubber tube to liberate the air outside. The central neck was stoppered and the third neck was equipped with an inlet to bubble nitrogen gas through the reactants. This enabled the system to be deoxygenated and allowed the later stages of the experiment to be conducted with strict exclusion of oxygen. A mixture of 100 mL methanol and 1 mL pyridine was magnetically stirred under nitrogen flow for 5 minutes. The stopper was removed briefly (the nitrogen gas flow was increased momentarily) then 2.4112 g dimethylglyoxime was added, followed by 2.3894 g cobalt (II) chloride hexahydrate. A deep brown colouration due to the presence of Co (II) was observed. 1.6734 g sodium hydroxide was dissolved in 5 mL methanol and added via a syringe. The solution was stirred for 20 minutes and then allowed to cool on ice. While the solution was cooling, 0.7894 g of sodium borotetrahydride was dissolved in 5 mL methanol. The prepared solution was then added via a syringe. The mixture was stirred and an intense blue–black solution was formed. 0.8 mL of iodomethane was added to the dark solution via a syringe. The organocobaloxime formed immediately by the dark solution turning brown. The methanol solvent was removed by rotary evaporation at 65 ^{o}C. The pasty mass was stirred with a slush of ice and 50 mL water. The orange cobalt complex was filtered using a sintered glass funnel and washed with 25 mL ice-cold water. The product was kept in the dark as it is light sensitive. The yield was determined and the product characterized by elemental analysis, IR, UV, ^{1}H and ^{13}C NMR. Orange crystals were obtained, yield 1.6914 (66%), melting point mp. 276–278 ^{o}C.

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IR ν_{max}: 3419, 2960, 1623, 1598, 1440, 1382, 1236, 1182, 1082, UV/Vis (C_2H_5OH, nm) λ: 240, 310, 400, ^{1}H-NMR (300 MHz, DMSO) δ: 0.52 (s, 3H, Co-CH_3), 2.14 (s, 12H, dmgH-CH_3), 8.40 (d, 2H, α-H py), 7.89 (t, 1H, γ-H py), 7.51 (t, 1H, β-H py); ^{13}C-NMR (300 MHz, DMSO) δ: 11.61 (dmgH-CH_3), 149.05 (dmgH-C=N), 148.73 (α-C py), 138.37 (γ-C py), 125.60 (β-C py). This is in agreement with published data [7, 31-33]

2.2.4. Synthesis of Methyl(aquo)bis(dimethylglyoximato)cobalt (III) [$Co(CH_3)(dmgH)_2(H_2O)$]

1.1216 g of the methylpyridinebis (dimethylglyoximato)cobaloxime [$Co(CH_3)(dmgH)_2(py)$] was added to 40 mL of dichloromethane followed by 30 mL of 5 M $HCLO_4$. A precipitate of $HpyCLO_4$ was formed and was filtered. Ice cold water was added to the filtrate for re–precipitation. A precipitate of $Co(CH_3)(dmgH)_2(H_2O)$ was obtained, washed with hexane and dried in a dessicator overnight. The yield was determined and the product characterized by elemental analysis, IR, UV, ^{1}H and ^{13}C NMR. Reddish orange crystals were obtained, yield 0.6384 (48%), melting point mp. 248–256 ^oC. IR ν_{max}: 3200, 2960, 2380, 1640, 1560, 1380, 1220, 1180, 1062, UV/Vis (C_2H_5OH, nm) λ: 244, 278, 310, ^{1}H-NMR (300 MHz, DMSO) δ: 1.1 (s, 3H, Co-CH_3), 1.92 (s, 12H, dmgH-CH_3), 3.2 (s, 2H-H_2O); ^{13}C-NMR (300 MHz, DMSO) δ: 11.20 (dmgH-CH_3), 147.05 (dmgH-C=N). This is in agreement with published data [31-33].

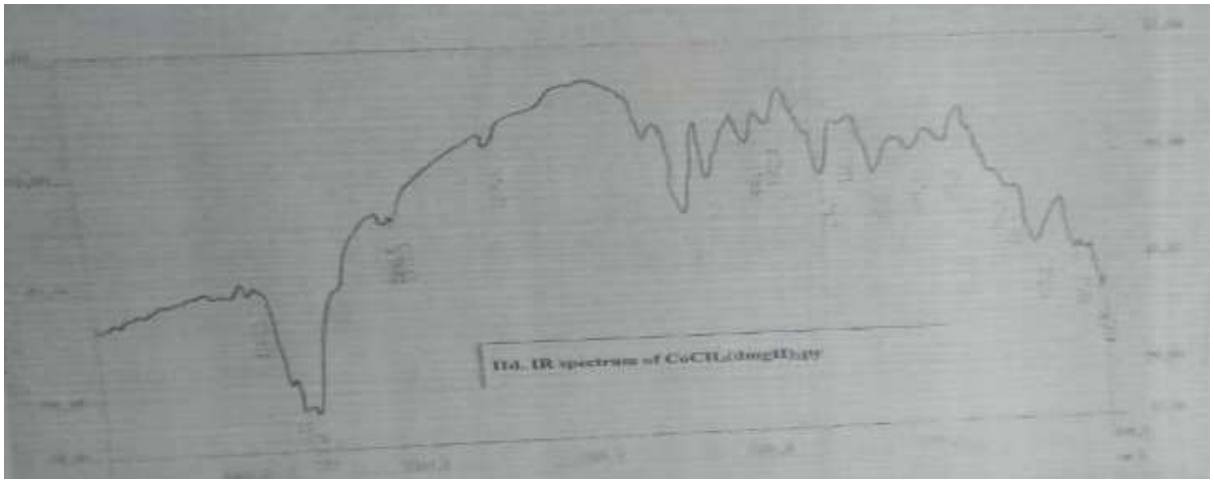

Fig. 1. Infrared spectrum of $Co(CH_3)(dmgH)_2py$

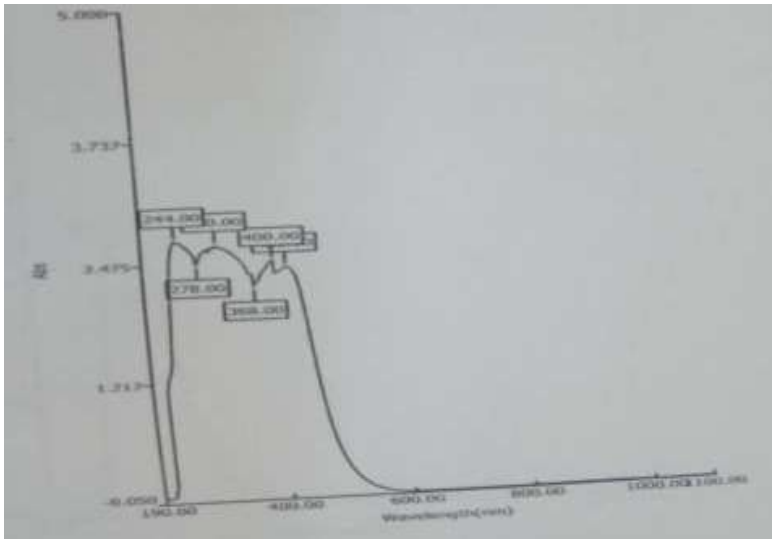

Fig. 2. UV spectrum of $Co(CH_3)(dmgH)_2py$

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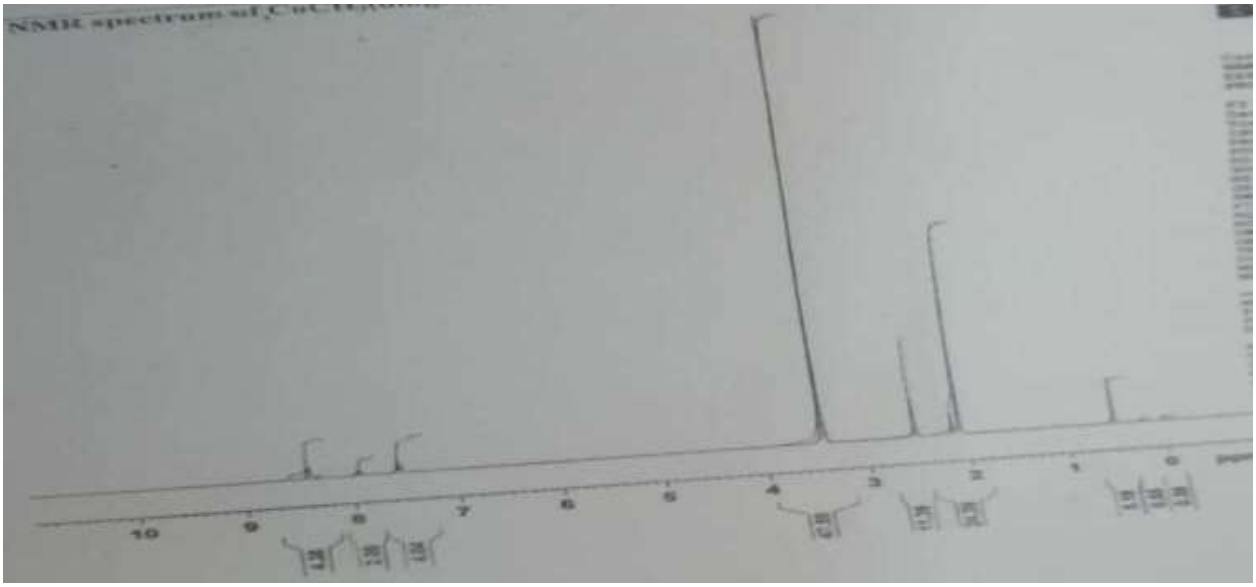

Fig. 3. ^{1}H nmr spectrum of $Co(CH_3)(dmgH)_2py$

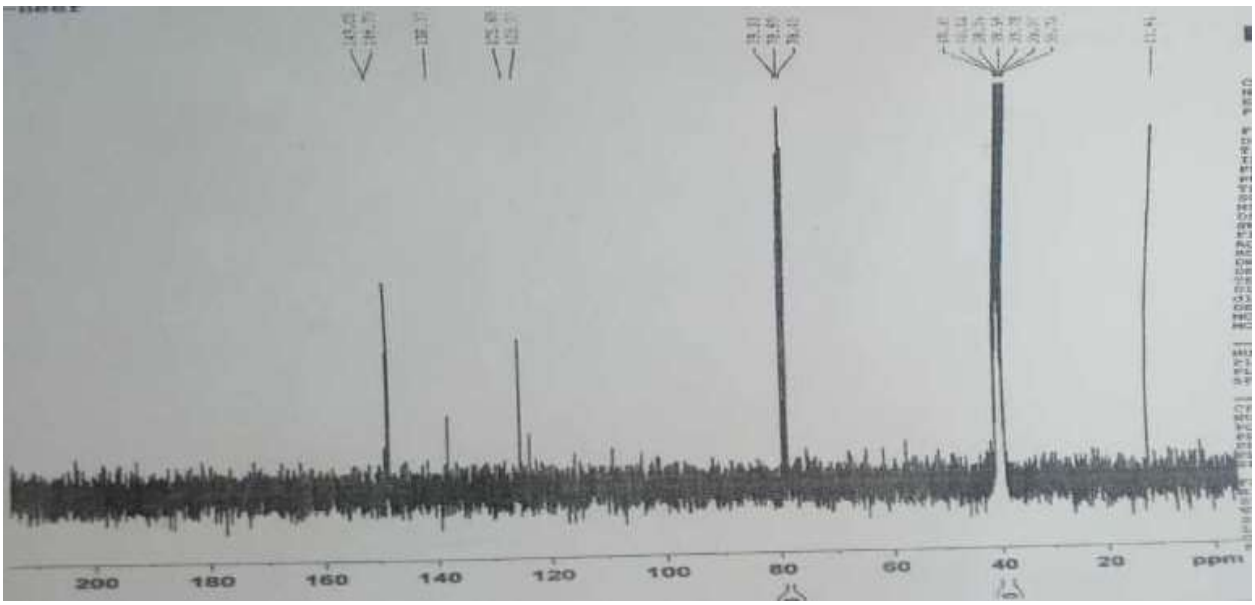

Fig. 4. ^{13}C nmr spectrum of $Co(CH_3)(dmgH)_2py$

2.3. Solubility Test

0.2 g of each of the synthesized cobaloximes was dissolved in 25 mL each of the solvents, water, methanol, hexane, chloroform, acetonitrile and dimethylsulfoxide at room temperature and on warming under stirring.

2.4. Photolytic reactions of vitamin B_{12} and alkylcobaloxime

2.4.1. Preparation of vitamin B_{12} – H_2O_2 – Benzene Mixture

0.3241 g of the powdery methylcobalamin (vitamin B_{12}) was dissolved in 30 mL acetonitrile in a beaker. After dissolving the methylcobalamin, 5 mL of 5% H_2O_2 was added using a dropping pipette. 2 mL of benzene was then added using a 1 mL syringe. The mixture was stirred while covered using a magnetic stirrer. The resulting mixture was afterwards transferred into a round bottom flask and corked. Two different spots of the resulting mixture solution were made on the TLC plates (5 cm × 10 cm) using capillary spotter. The solvent was evaporated and the TLC plate was then placed in a solvent mixture of petroleum ether–ethanol

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(3:7) in a glass tank. The migration of the spots after the solvent had risen to about 7 cm above the lower width of the TLC plate was then observed under UV light and the dark spots were circled with pencil to have a permanent record of how far the spots travelled. 3 mL of the mixture was taking into a vial, covered and kept in the dark for 7 hours. The corked round bottom flask containing the remaining mixture was then clamped and placed under sunlight for 6 hours. After removing from the sunlight, TLC analysis was carried out again for the solution kept in the dark and the one irradiated with the sunlight as previous. The placing of the mixture under sunlight was done for four weeks (6 hours each day) with TLC analysis being carried out along at intervals of two days.

2.4.1.1. Isolation and purification of organic product

The resulting mixture consisted of a deep orange coloured supernatant solution and a pale brown residue which settled on a 30 minute–centrifugation. The supernatant solution was then decanted into 250 mL beaker. The supernatant solution was then subjected to further centrifugation and decantation five more times. The resulting solution was monitored by co-TLC using a solvent mixture of pet ether-ethanol (2:8). The liquid product was characterized by IR and GC-MS. Yield was 0.0108g

2.5. Photolysis of $Co(CH_3)(dmgH)_2(py) – C_6H_6$ Mixture using UV Lamp

2.5.1. Preparation of the $Co(CH_3)(dmgH)_2(py) – C_6H_6$ Mixture

A dropper was used to measure 5 mL of benzene into a round bottom flask. 0.0312 g of $Co(CH_3)(dmgH)_2(py)$ was then added to the content in the round bottom flask. It was then stirred using a magnetic stirrer and then corked. A dropper was used to measure 5 mL of benzene into a round bottom flask. 0.0312 g of $Co(CH_3)(dmgH)_2(py)$ was then added to the content in the round bottom flask. It was then stirred for 4 hours using a magnetic stirrer and then corked. TLC was carried out on the solution. 1mL of the mixture was taking into a vial, covered and kept in the dark for 7 hours. The corked round bottom flask containing the remaining mixture was then irradiated with a 450 Watts AC Hanovia UV lamp under stirring at room temperature for 7 hours. After removing from the UV lamp, TLC analysis was re–carried out for the solution kept in the dark and the one irradiated with the UV lamp as previously done.

2.5.1.1. Monitoring of photolytic reaction

The resulting mixture consisted of a reddish orange coloured supernatant solution and a pale grey residue which settled on a 30 minutes–centrifugation. The supernatant solution was then decanted into 50 ml beaker. The supernatant solution was subjected to centrifugation and decantation one more time. The resulting solution was monitored with co-TLC using n-hexane as solvent and characterized using IR and GC–MS. Yield was 0.0075g

2.5.2. Photolysis of $Co(CH_3)(dmgH)_2(py) – H_2O_2 – C_6H_6$ mixture using sunlight

2.5.2.1. Preparation of $Co(CH_3)(dmgH)_2(py) – H_2O_2 – C_6H_6$ mixture

0.3115 g of $Co(CH_3)(dmgH)_2(py)$ was dissolved in 25 mL acetonitrile in a 250 mL beaker. The solution was stirred to ensure that the cobaloxime has completely dissolved. 5 mL of 5% H_2O_2 was added with a dropper followed by the addition of 0.5 mL toluene using a syringe. The solution was stirred for 30 minutes using a magnetic stirrer. The reddish brown solution obtained was then transferred into a round bottom flask and then corked. TLC was carried out on the solution using a solvent of pet ether – ethanol (2:8). 5 mL of the mixture was taken into a vial, covered and kept in the dark for 7 hours. The corked round bottom flask containing the remaining mixture was then clamped and placed under the sun for 7 hours. When removed from the sunlight, TLC analysis was carried out again as previous. The placing of the mixture under the sunlight was done for one week (7 hours per day) with TLC analysis being carried out after each day.

2.5.2.2. Isolation and Characterization of Product

The resulting mixture consisted of a reddish brown coloured supernatant solution and a pale brown residue which settled on a 30 minutes–centrifugation. The supernatant solution was then decanted into 250 mL beaker. The supernatant solution was then subjected to further centrifugation and decantation four more times. The solution was then analyzed using a GC–MS envisaged to contain benzoic acid, benzaldehyde and o, p,m cresol. Yield was 0.0862g

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2.5.3. Photolysis of $Co(CH_3)(dmgH)_2(py) – H_2O_2 – C_6H_6$ mixture using UV lamp

The experiment was carried out as before with sample mixture irradiated with UV lamp. The product was characterized using GC–MS. Yield was 0.0518g

3. Results and Discussions

3.1. Synthesis

The synthesized cobaloximes reported in this work are present in table 1 together with their elemental analysis. The melting points of all the synthesized cobaloximes were above 240 ^oC which were in agreement with those in literature [34]. All the cobaloximes prepared were found to be insoluble in water, hexane and diethyl ether but soluble in methanol, chloroform and dimethyl sulfoxide. However, all the complexes were found to be sparingly soluble in acetonitrile.

Table 1: Elemental analysis of the various complexes synthesized

Compound	Molecular formula (molar mass)	Elemental analysis: calculated / found (%)				
		C	**H**	**N**	**Cl**	**Co**
$CoCl_2(dmgH)_2$	$C_8H_{14}N_4O_4Co\ Cl_2$ (359.9)	26.7 (25.2)	3.89 (3.55)	15.56 (15.32)	19.72 (18.12)	16.39 (16.24)
$CoCl(dmgH)_2py$	$C_{13}H_{19}N_5O_4CoCl$ (403.4)	38.5 (38.01)	4.70 (4.64)	17.3 (16.87)	9.98 (9.55)	14.6 (13.8)
$CoCH_3(dmgH)_2py$	$C_{14}H_{22}N_5O_4Co$ (382.9)	43.87 (42.2)	5.75 (5.53)	18.28 (17.59)		15.38 (14.80)
$CoCH_3(dmgH)_2H_2O$	$C_9H_{19}N_4O_5Co$ (322.9)	33.45 (29.2).	5.89 (5.13)	17.3 (15.14)		18.24 (17.32)

3.2. Photolysis

The homolytic cleavage of the CoC bond and the effect of the $OH^{\cdot}$ generated during the said cleavage on benzene was investigated. In doing this a mixture of methylcobalamin (vitamin B_{12}), benzene and hydrogen peroxide in acetonitrile were exposed to sunlight as described above.

The expected reaction mechanism for the photolytic cleavage of the Co-C bond in methylcobalamin is shown below as scheme 1.

Scheme 1

$$\text{Vitamin } B_{12}(Co^{III}\text{-}CH_3) \xrightarrow{h\nu} \text{Vitamin } B_{12}(Co^{II}) + CH_3^{\cdot}$$

$$\text{Vitamin } B_{12}(Co^{II}) + CH_3^{\cdot} + H_2O_2 \longrightarrow \text{Vitamin } B_{12}(Co^{III}\text{-}CH_3) + {}^{\cdot}OH + OH^-$$

$$C_6H_6 + 2\,{}^{\cdot}OH \longrightarrow C_6H_5OH + H_2O$$

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From the reaction mechanism, phenol is expected to be the product. To determine the specific product, the IR spectrum (P1) of the product revealed useful information of the type of product formed. The peaks around 3080 and 3050 cm^{1} arise from aromatic C H stretching vibration and the peaks between 2000 and 1800 cm^{1} are due to overtone and combination bands. The peak at 1370 cm^{1} arises from inplane OH bending vibrations while that at 1250 cm^{1} is attributed to CO stretching vibration. Also, the peaks around 760 and 690 cm^{1} arise from outofplane C H bending vibration and those at 1670 and 1599 cm^{1} arise from sp^{2} CC vibration of the aromatic ring. Finally, the broad band occurring between 3650 cm^{1} and 3200 cm^{1} is due to O-H stretching vibration. From the IR spectrum it can be deduced that the organic product contains an aromatic ring which is mono or di-substituted and also sp^{2} CO bond is present. With this information it can be inferred that the organic product cannot be a biphenyl but rather a phenol.

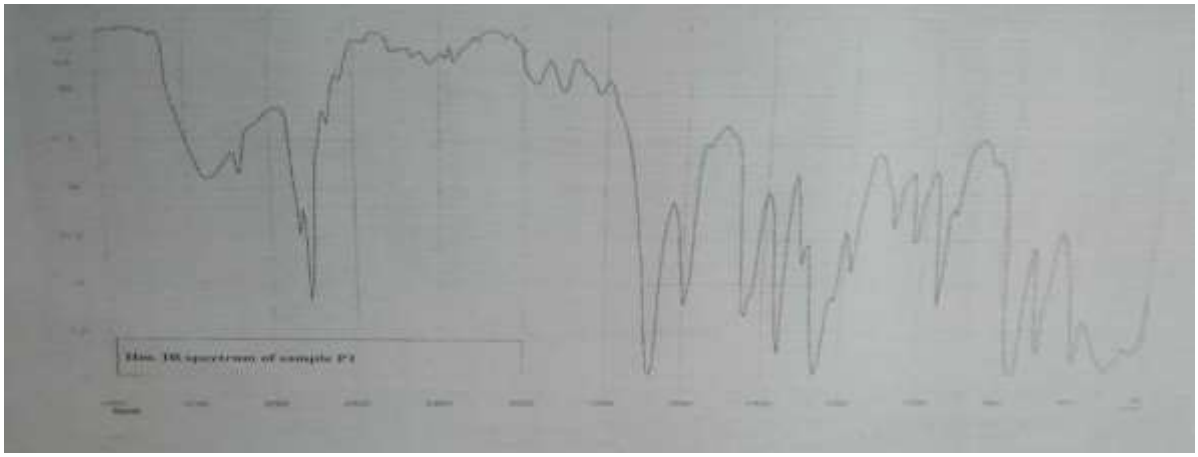

Fig. 5. IR spectrum of P1

Functional group test

Table 2: $FeCl_3$ test

Test	Observation	Inference
1 ml of the deep orange organic product + 3 drops of the prepared neutral 1% $FeCl_3$ solution	A deep blue colouration observed	Phenol and o,m,p – cresols may be present but enol absent

To confirm phenol as the only product formed during the photolytic cleavage of the Co-C bond in methylcobalamin, a GC-MS of the organic product (P1) was run and that gave very important information. The molecular mass of phenol is consistent with the molecular ion, M^{+}, of m/z = 94 (100%) depicted in the mass spectrum. Other characteristic peaks of the mass spectrum and their relative abundances occurred at m/z of 77.1(22.5%), 66.1(60%), 65.1(40%), 50.2(17.5%) and 55.1(7.5%). From the GC-MS it can be confirmed that the organic product formed was phenol and it buttresses the point that the Co-C bond in vitamin B_{12} is weak and is cleaved during the co-enzyme's biochemical activity.

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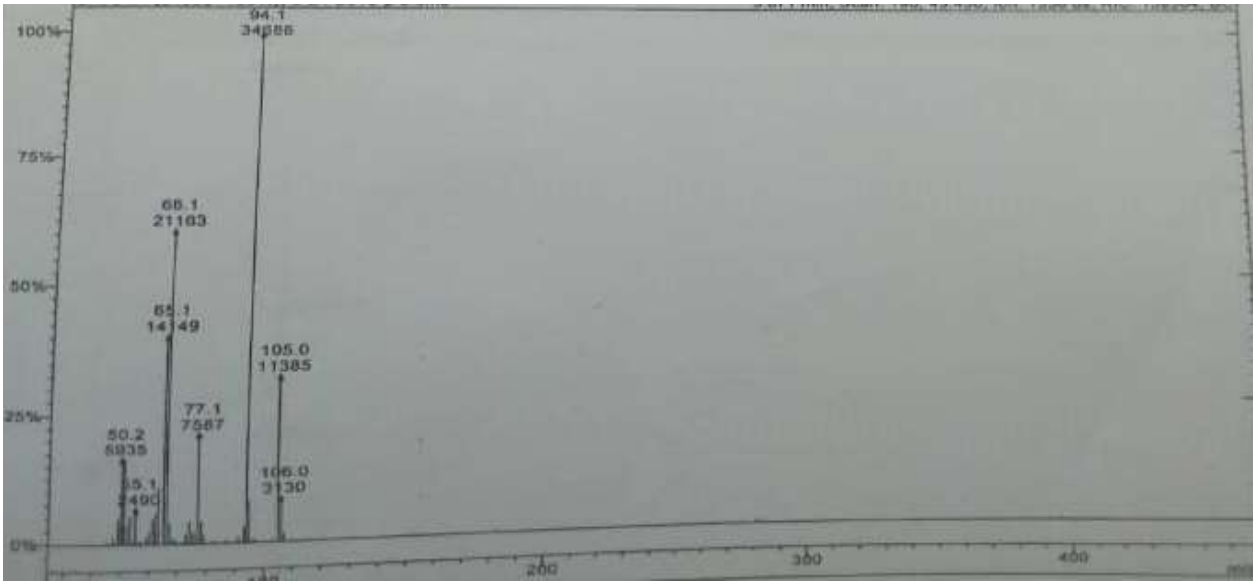
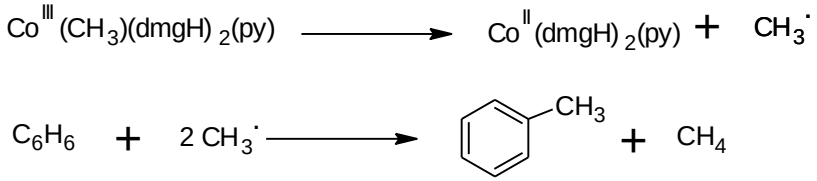


Fig. 6. Mass spectrum of P1

Cobaloximes if indeed are mimetics of vitamin B_{12} then should also undergo homolytic cleavage of the CoC bond. The photolytic cleavage of the CoC bond in methyl(pyridine)cobaloxime was also investigated first in benzene. The reaction was then carried out in acetonitrile together with toluene and hydrogen peroxide using sunlight and 450 Watts Hanovia UV lamp. The expected product from the photolytic reaction of $Co(CH_3)(dmgH)_2(py)$ in benzene is toluene. Below is a reaction scheme 2 showing the possible product to be expected.

Scheme 2



The IR spectrum (P4) gave information about the functional group present in the compound and the possible structure it will be. The presence of two weak bands around 1750 cm^{-1} to 2000cm^{-1} is due to the combination and overtone bands of the mono substituted aromatic ring. The shape of these peaks confirms the mono substitution on the benzene ring. The peak around the 1500 cm^{-1} represents the C=C stretching vibration of the aromatic ring. The peak around 3200cm^{-1} is due to sp^2 C–H stretching vibration of the aromatic ring. The peak around the 3050cm^{-1} is due to the sp^3 C–H stretching vibration of the methyl (CH_3) group on the benzene ring. From the IR spectrum, the organic product is a suspected to be mono substituted aromatic compound and is likely to be toluene. The organic product formed was confirmed by GC-MS (P4). Significant peaks appeared at m/z = 92.3 (22.5%) and 91.3(100%). These are identical to fragments of the fragmentation patterns of authentic toluene and therefore confirmed toluene as the organic product formed. The formation of toluene indicates that the Co-C is weak and can easily be cleaved. To confirm this and to also investigate the oxidative effect of the hydroxyl radical ($OH^{\cdot}$), the other photolytic reaction was carried. The reaction mechanism, scheme 3 for the photolysis of $CoCH_3(dmgH)_2py$ in acetonitrile together with hydrogen peroxide and toluene in the presence of the UV lamp and sunlight are shown below;

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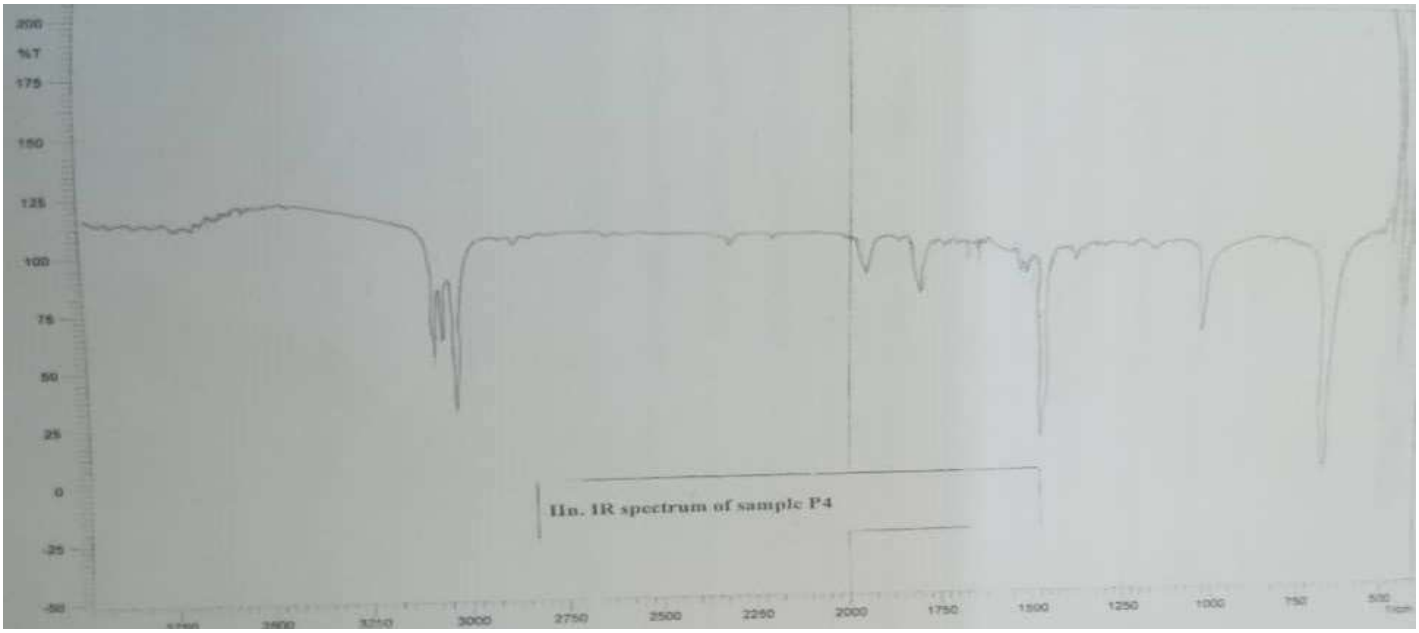

Fig. 7. IR spectrum of P4

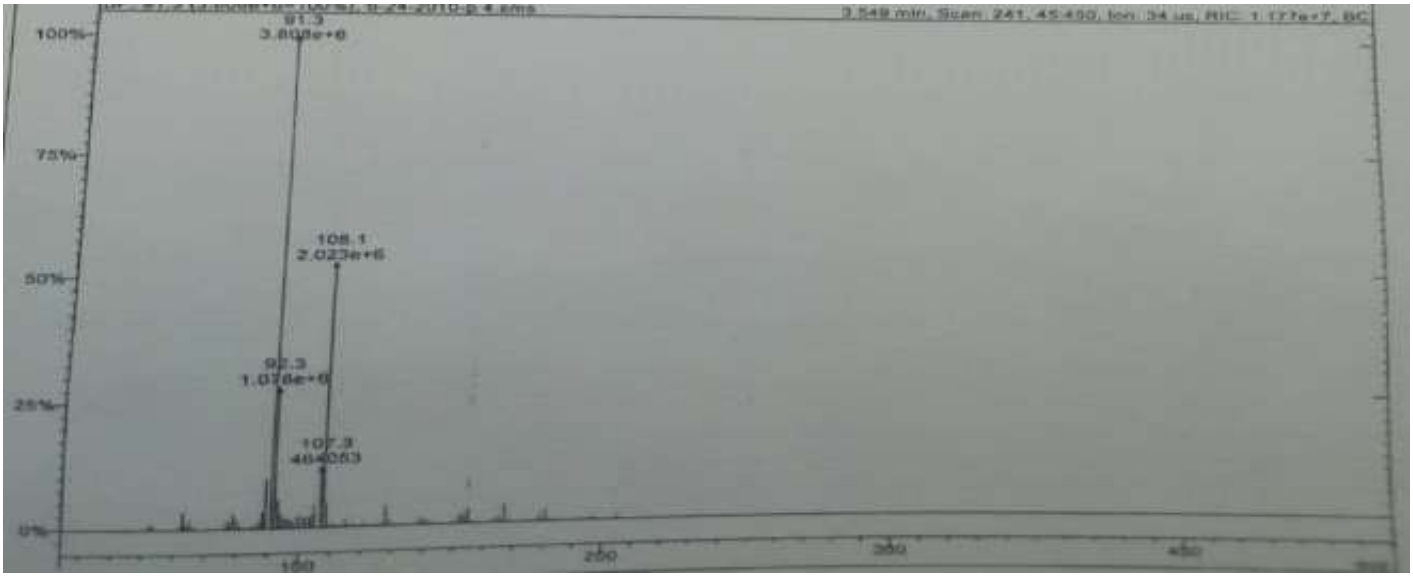

Fig. 8. Mass spectrum of P4

Scheme 3

$$Co^{III}(CH_3)(dmgH)_2(py) \xrightarrow{h\nu} Co^{II}(dmgH)_2 + {}^{\cdot}CH_3$$

$$2H_2O_2 + {}^{\cdot}CH_3 + Co^{II}(dmgH)_2 \longrightarrow Co^{III}(CH_3)(dmgH)_2(py) + 2OH^{-} + 2{}^{\cdot}OH$$

$$\underset{\text{Toluene}}{C_6H_5CH_3} + 2{}^{\cdot}OH \longrightarrow \underset{\text{o,p,m – cresol}}{C_6H_4(OH)CH_3} + \underset{\text{benzaldehyde}}{C_6H_5CHO} + \underset{\text{benzoic acid}}{C_6H_5COOH}$$

From the mechanism, the expected products are benzoic acid, benzaldehyde and o, p, m cresol. The samples irradiated with 450 Watts UV lamp produced the molecular ion, $M^{+\cdot}$, of m/z =108 which is consistent with the molecular formula of o, p, m cresol depicted in the mass spectrum (P6). Other characteristic peaks of the mass spectrum and their abundances occurred at m/z = 107 (base peak, 100%), 80 (17.5%), 77 (55%) and 51 (22.5%) which are masses from different fragments of o, p, m cresol. The mass spectrum (P8) of the sample irradiated with sunlight produced a molecular ion, $M^{+\cdot}$, of m/z =106 (100%) which is consistent with

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the molecular formula of benzaldehyde depicted in the mass spectrum. The M-1 peak at 105 (70%) is due to the loss of a hydrogen radical. Other characteristic peaks of the mass spectrum and their abundances occurred at m/z = 77.2 (22.5 %) and 51 (17.5%) which are masses of different fragments of benzaldehyde. All the other peaks are due to fragments in the main spectrum. From the GC-MS, it can be confirmed that benzaldehyde was produced during the photolytic reaction under sunlight. However, it was expected that o,p,m cresol, benzoic acid and benzaldehyde was to be generated but it was not so. The formation of o,p,m cresol using UV lamp is indicative of the photolytic cleavage of the Co-C bond and the generation of the hydroxyl radical. In using sunlight, the generated hydroxyl radical was able to oxidize the toluene to the benzaldehyde but not benzoic acid. This might be due to the duration the solution mixture was exposed to the sunlight. The formation of benzaldehyde is enough to substantiate the fact that hydroxyl radical was generated and is an oxidizing agent.

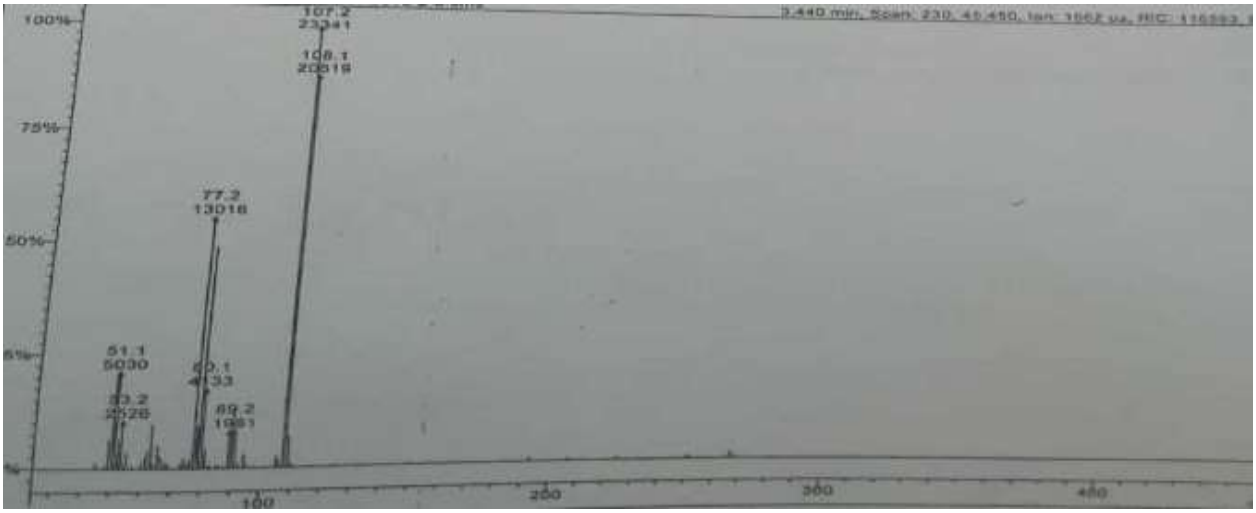

Fig. 9. Mass spectrum P6

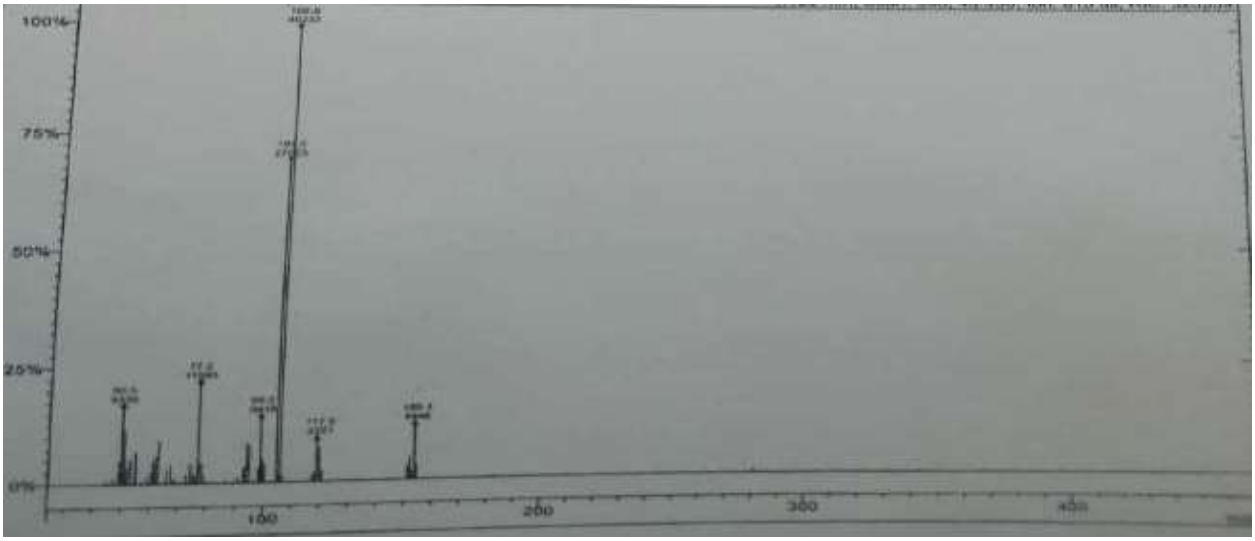

Fig. 10. Mass spectrum P8

4. CONCLUSIONS

Vitamin B_{12} is known to undergo homolytic cleavage of the Co^{III} – C bond and the biochemical reaction of vitamin B_{12} is dependent on this bond cleavage.

$$Co^{III}\ (\text{Vitamin } B_{12}) \xrightarrow{h\nu} Co^{II}$$

$$Co^{II} + H_2O_2 \longrightarrow Co^{III} + {}^{\cdot}OH + OH^-$$

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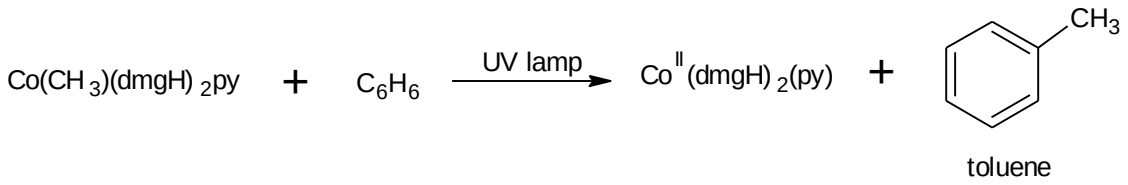
The generation of a phenol clearly indicates the ·OH radicals were generated as anticipated and so it can be confirmed there was a Co^{III} – C δ bond cleavage during the photolysis.

Methylpyridinecobaloxime $CoCH_3(dmgH)_2py$ a mimetic of vitamin B_{12} was also found to have an weak Co-C δ bond. This was observed when toluene was formed after exposing $CoCH_3(dmgH)_2py$ in benzene to UV lamp.



The formation of benzaldehyde and o,p,m cresol during the photolysis of $CoCH_3(dmgH)_2py$ using sunlight and UV lamp respectively goes to confirm that ·OH radicals were generated and was very reactive. This also confirms that the Co-C δ bond is weak in both cobalamins and cobaloximes and easily cleaves when exposed to light.

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