DNA Cleavage Activity of Copper (II) Complexes of Some Phosphonates

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Abstract- Copper (II) complexes of Diphenyl (2-hydroxyphenyl)(phenylamino) methylphos- phonate (DHPP) and Diphenyl (4-aminophenylamino)(2-hydroxyphenyl)methyl phosphonate (DAHP) were prepared and characterized by different tools such as elemental analysis, IR, ^{1}H-NMR, UV/Vis spectra and thermal studies. The IR spectral data showed that the phosphonate compounds behave as bidentate ligands coordinating to the copper ion through the P=O and NH groups. The geometry around copper ion was found to be a distorted octahedral. The activity of the phosphonate ligands and their copper (II) complexes towards DNA cleavage were investigated. The phosphonate ligands showed no activity, while the copper complexes showed DNA cleavage activity. The copper complex of Diphenyl (4-aminophenylamino)(2-hydroxyphenyl)methyl phosphonate showed strong activity towards DNA cleavage.

Index Terms- phosphonate; spectroscopic studies; DNA cleavage

I. Introduction

In recent years, many researches [1,2] have focused on interaction of small molecules with DNA. DNA is generally the primary intracellular target of anticancer drugs, so the interaction between small molecules and DNA can cause DNA damage in cancer cells, blocking the division of cancer cells, and resulting in cell death [3,4]. Survey of literature demonstrates that interest on the design of novel transition metal complexes capable of binding and cleaving DNA [5-7] increases continuously. Metal complexes that can bind to DNA are gaining considerable attention owing to their diverse applications in the field of bioinorganic chemistry viz. diagnostic agents for medical applications, development of cleavage agents for probing nucleic acid structure [8,9] and identifiers of transcription start sites [10]. The interaction of DNA with transition metal complexes is important for the design of effective antitumor drugs that exhibit different properties than those which are currently in use [11] like cisplatine. Among the metal ions regarded as coordination centers of potential anticancer agents, platinum and ruthenium ions are the most widely investigated up to now [12,13]. However, there is a growing interest in the synthesis of cheaper first-row transition metal complexes as efficient DNA binders with potential cytotoxic activity [14,15].

Phosphonates represents an important class of organophosphorus compounds. Their use in a variety of applications is well documented and their importance in a range of fields is increasing. Phosphonates have a lot of industrial applications as effective chelating agents, corrosion inhibitors in cooling systems [16-18] and water softeners[19]. In addition, the phosphonate functionality has been incorporated into a range of clinically useful drugs. cyclic nucleoside phosphonates have shown potential as therapeutics for pathogenic species [20], anti-parasitic agents. [21,22] and HIV protease inhibitors [23]. Phosphonate containing protease inhibitors also have shown great potential for the treatment of Hepatitis C virus [24]. Some have shown potential as cancer therapies [25] to inhibit growth of malignant cell lines [26,27] and also as Inhibitors for Urokinase-Type Plasminogen Activator [28].

In this study, small phosphonate molecules were prepared and characterized. The copper (II) complexes of the phosphonate ligands were also prepared and fully characterized. The ability of the phosphonates and their copper (II) complexes to cleave the DNA was investigated.

II. Experimental Section

2.1. Materials.

Perchloric acid, acetaldehyde, aniline, *p*-phenylenediamine and acetonitrile were purchased from Sigma-Aldrich Chemical Co.

2.2. Synthesis of phosphonate ligands

2.2.1. Diphenyl (2-hydroxyphenyl) (phenylamino)methylphosphonate (DHPP)

$HClO_4$ (0.201 g, 2 mmol) was added to a solution of the salcylaldehyde (1.22g, 0.01 mol) and aniline (0.93 g, 0.01 mol) in acetonitrile. The mixture was stirred for 15 min and then triphenyl phosphite (3.1 g, 0.01 mol) was added. After completion of the reaction (6 h), the reaction mixture was quenched with aq. saturated $NaHCO_3$ followed by brine solution and then extracted with CH_2Cl_2, dried over Na_2SO_4, and concentrated under vacuum. The crude mixture was purified by washing with mixture of ether and pet. ether to afford the product.

2.2.2. Diphenyl (4-aminophenylamino)(2-hydroxyphenyl)methylphosphonate (DAHP)

$HClO_4$ (0.201 g, 2 mmol) was added to a solution of the salcylaldehyde (1.22g, 0.01 mol) and *p*-phenylenediamine (1.08 g, 0.01 mol) in acetonitrile. The mixture was stirred for 15 min and then triphenyl phosphite (3.1 g, 0.01 mol) was added. After completion of the reaction (6 h), the reaction mixture was quenched with aq. saturated $NaHCO_3$ followed by brine solution and then extracted with CH_2Cl_2, dried over Na_2SO_4, and concentrated under vacuum. The crude mixture was purified by

washing with mixture of ether and pet. ether to afford the product.

2.3. Synthesis of metal complexes

Copper (II) chloride (0.01 mol) dissolved in about 50 ml absolute ethanol was added to the ethanolic solution of the selected ligand (0.01 mol). Small amount of solid sodium acetate was added to the solution with heating and contentious stirring. The precipitate was obtained, filtered off and washed many times with ethanol and then dried in an oven at 80 ^{o}C.

2.4. Physical methods

Carbon, hydrogen and nitrogen contents were determined at the Microanalytical Unit, Cairo University, Egypt. IR spectra of the ligand and its solid complex were measured in KBr on a Mattson 5000 FTIR spectrometer. The electronic spectra were performed using Varian Cary 4 Bio UV/VIS spectrophotometer. ^{1}H-NMR spectrum of the ligand was recorded on Joel-90Q Fourier Transform (200 MHz) spectrometers in [D_6] DMSO. The mass spectra of the phosphonate ligands were recorded on a Shimadzu GC-S-QP 1000 EX spectrometer using a direct inlet system. Thermal analysis measurement (TGA) was recorded on a Shimadzu thermo-gravimetric analyzer model TGA-50 H, using 20 mg sample. The flow rate of nitrogen gas and heating rate were 20 cm^3 min^{-1} and 10°C min^{-1}, respectively. The magnetic susceptibility measurements for the copper (II) complexes were determined by the Gouy balance using $Hg[Co(NCS)_4]$ as a calibrant at room temperature.

2.5. Nuclease-like activity assay (DNA cleavage)

Genomic DNA extracted from mammalian blood by salting out method was used to examine the DNA cleavage activity of the examined ligands and their copper (II) complexes. DNA purity and concentration were examined spectrophotometrically at 260/280 nm. The cleavage reactions were carried out in a total volume of 15 µl containing 5 µl genomic DNA, 5 µl of ligand or complex (from 1.0 nM to 1mM), and 5 µ l TE buffer (25.0 mM Tris–HCl containing of 50.0 mM NaCl pH 7.2). Genomic DNA alone or genomic DNA in the buffer was used as a control. The reactions were carried out at 37 ˚C at different time points (0, 0.5, 1, 2, 6, 12 hours). A solution of loading dye (0.05% bromophenol blue, 5% glycerol, and 2 mM EDTA) was added to the reactions mixtures prior to running the gel. Dose dependent and time dependent experiments were carried out for each ligand and its complex used in this study. The prepared compounds were run on 1.0% agarose slab gel at a constant voltage of 100 V for 30 min in TBE (Tris– Borate-EDTA) buffer. Gels were stained with ethidium bromide and visualized under UV trans-illuminator (Syngene gel documentation system with digital camera).

III. Results and Discussion

The phosphonate compounds were prepared as mentioned previously [29] by stirring the mixture of the aldehyde, amine and triphenyl phosphite as one-pot reaction.

3.1. IR spectra

IR spectroscopy is very important technique for the characterization of the organic compounds and their metal complexes. The IR spectral data (Figure 1) of the prepared ligands (DHPP) and (DAHP) exhibit peaks at 3440 cm^{-1} due to νOH. The bands appear at 3320 and 3430 cm-1 are corresponding to νNH for (DHPP) and (DAHP), respectively. The bands corresponding to ν P=O appear at ~1215 cm^{-1}[30]. The bands attributed to NH_2 are observed at 3320 and 3280 cm^{-1}. The spectral data show bands at ~1640 cm^{-1} and 1435 cm^{-1} attributed to NH and OH bending, respectively.

^{1}H-NMR spectrum (Figure 2) of the ligand (DHPP) shows δ = 4.68 (d, 1H, CH), 6.78-7.78 (m, 19H, Ar-H), 5.41 (brs, 1H, OH) and 8.43 (brs, 1H, NH). At the same time the ^{1}H-NMR spectrum of DAHP shows δ = 4.76 (d, 1H, CH), 6.79-7.78 (m, 19H, Ar-H) and 8.33 (s, 1H, NH). 5.61 (s, 1H, OH), 2.73 (s, 2H, NH_2).

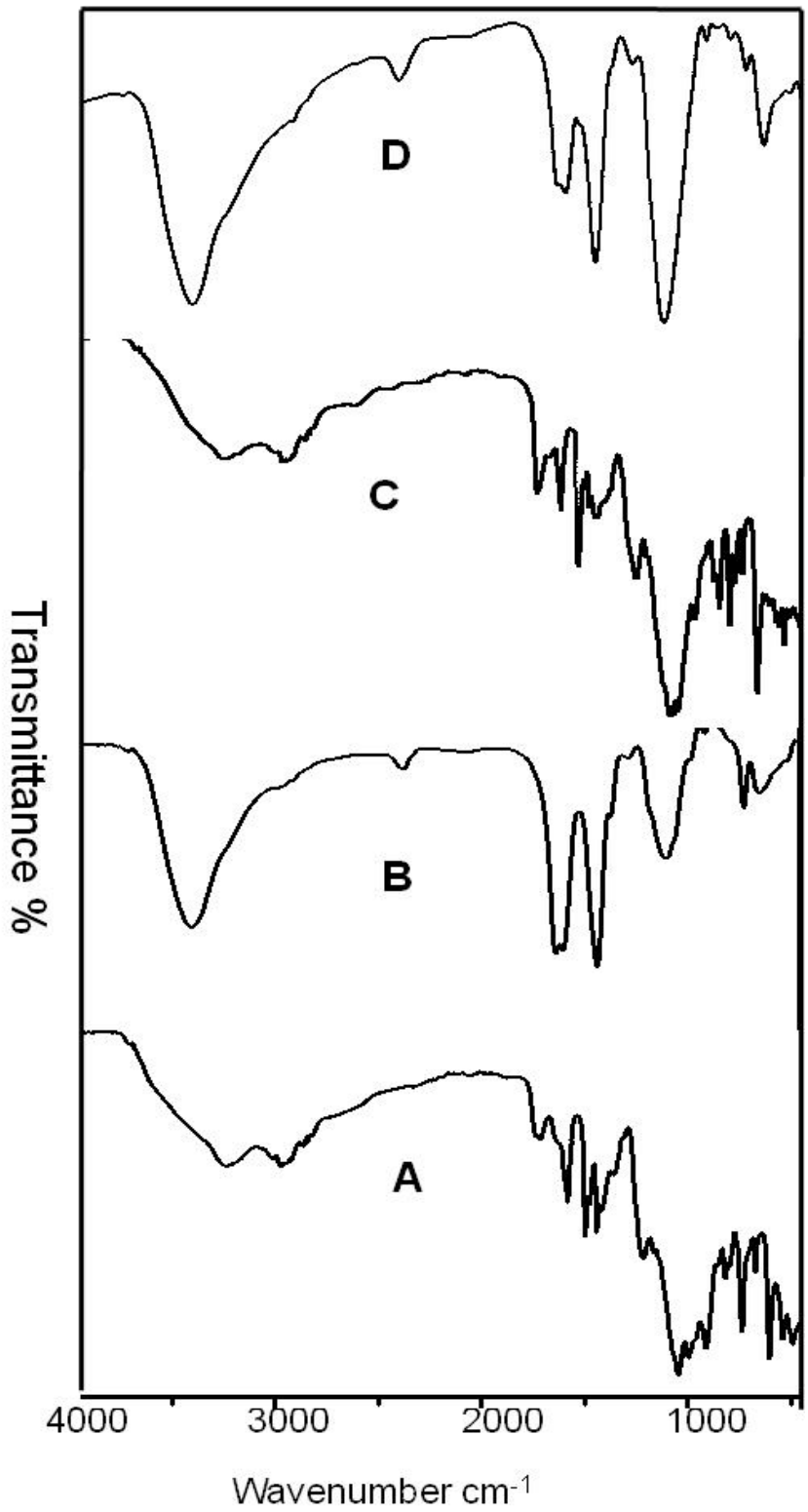


Figure 1. IR spectra of the ligands and their copper (II) complexes DHPP (A), Cu^{II}-DHPP (B), DAHP (C) and Cu^{II}-DAHP (D).

3.2. Mass spectroscopy

The mass spectrum of the ligand (DHPP) shows ion peak at m/e = 431 as the molecular peak. The ion peak at m/e = 339.23 is due to M^+($C_{19}H_{16}O_4P$). The ion peak at m/e = 338.28 corresponds to M^+($C_{19}H_{17}NO_3P$). The ion peak at m/e = 265.14 is due to M^+($C_{13}H_{13}O_4P$), while the ion peak at m/e =111.08 corresponds to $M^+(CH_6NO_3P)$ and the ion peak at m/e = 71.09 (100%) corresponds to M^+(CNOP) which is the base peak. The fragmentation patterns of DHPP are shown in Scheme (1); Figure3.

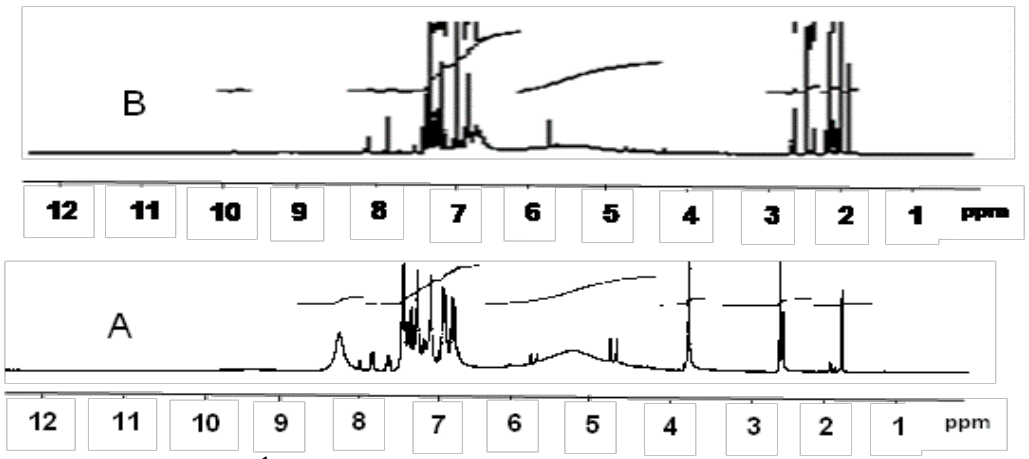
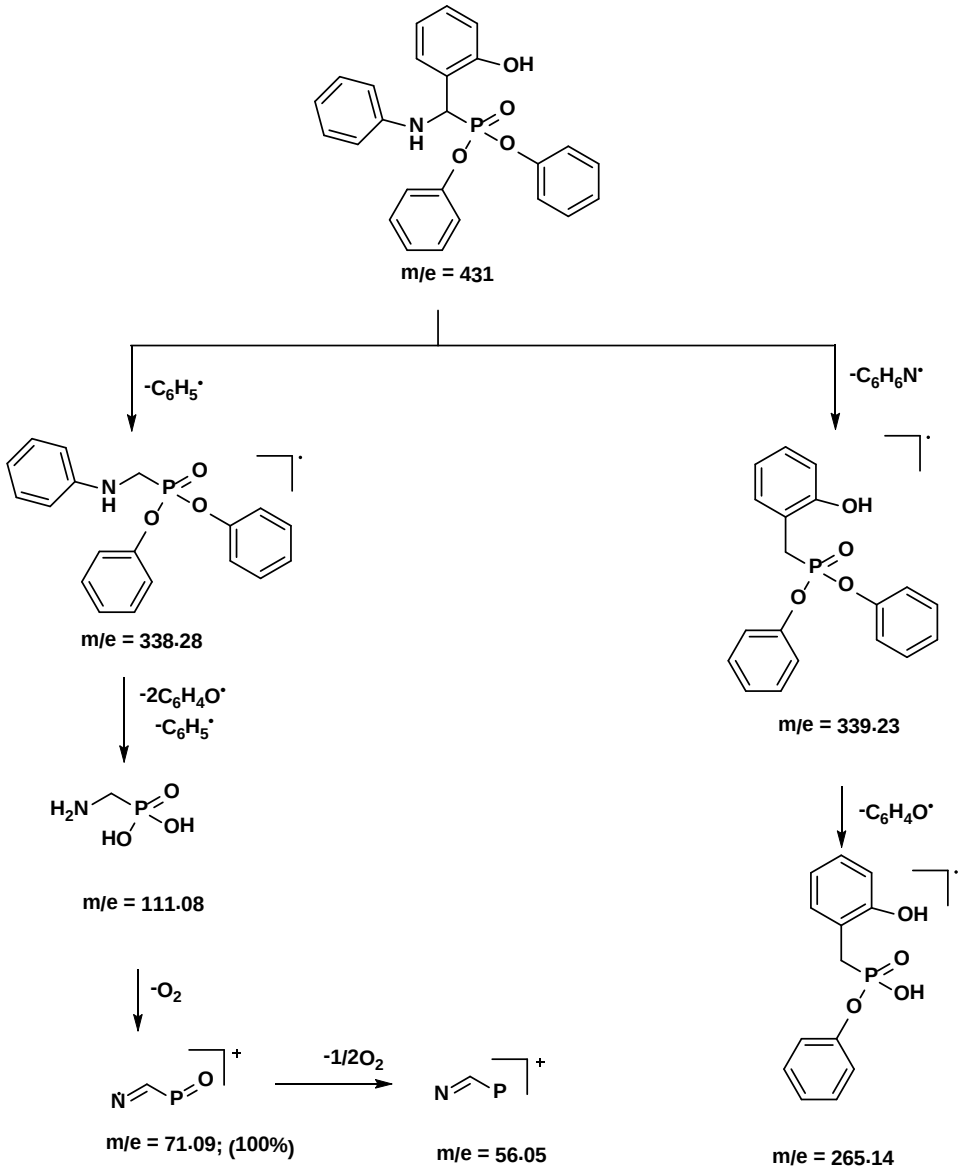
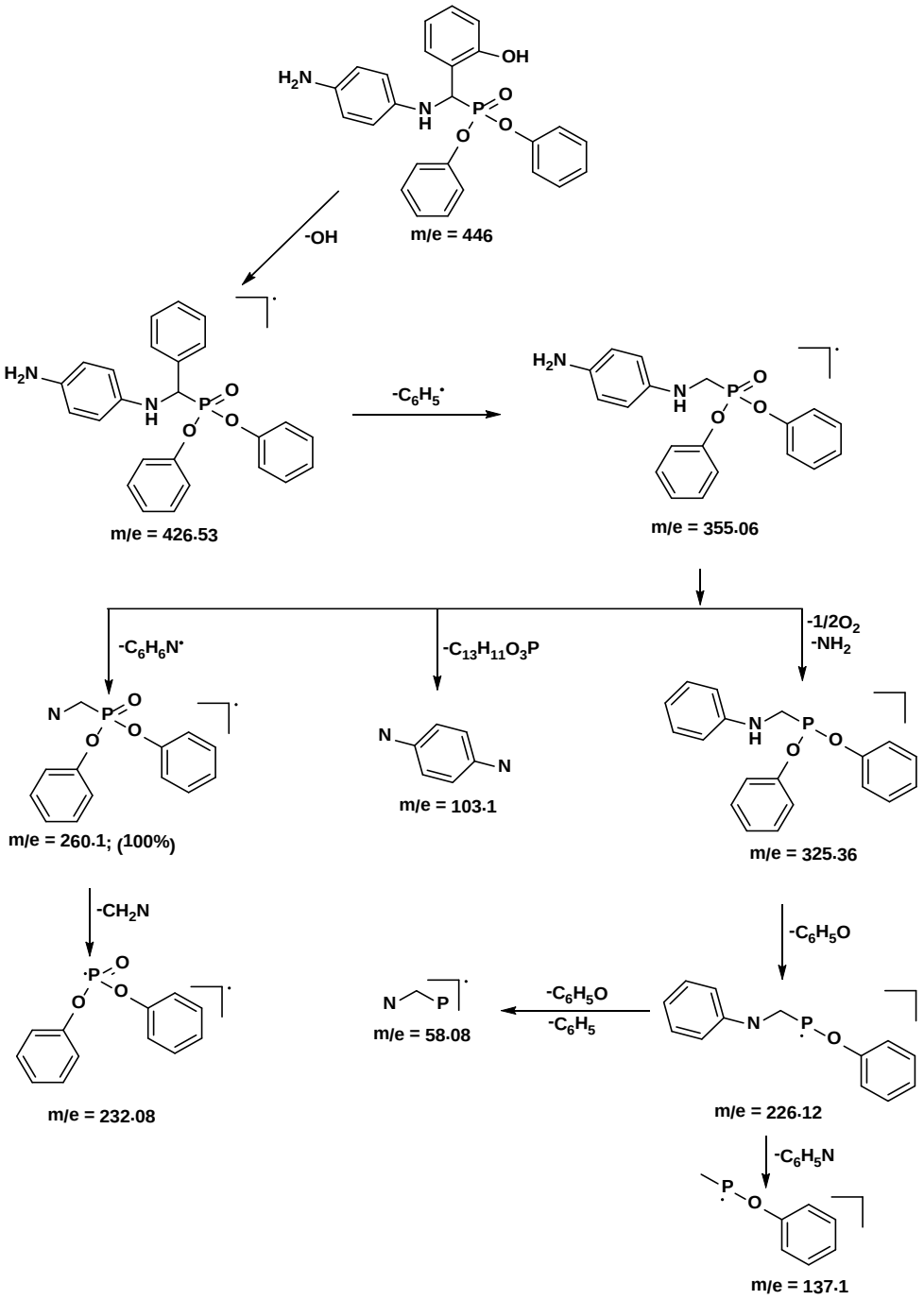


Fig. 2; ^{1}H-NMR of DHHP (A) and DHAP (B) in d6 DMSO



Scheme 1; Mass fragments of DHPP



Scheme2; Mass fragments of DAHP

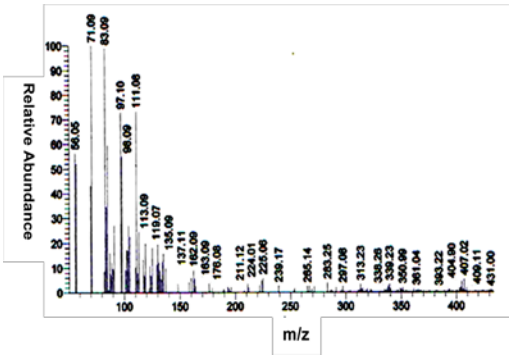


Fig. 3; Mass spectrum of DHPP

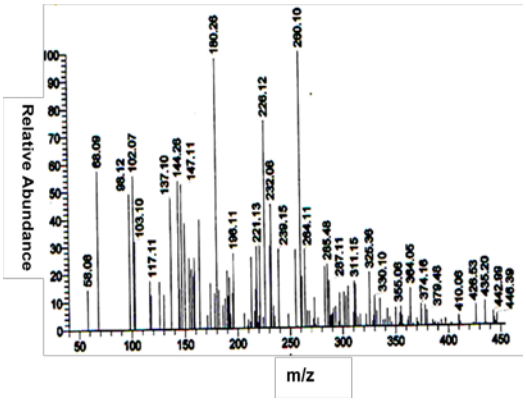


Fig. 4; Mass spectrum of DAHP

The mass spectrum of the ligand (DAHP) shows ion peak at m/e = 446 as the molecular peak. The ion peak at m/e = 426.53 is due to M^+($C_{25}H_{20}N_2O_3P$). The ion peak at m/e = 355.06 corresponds to $M^+(C_{19}H_{18}N_2O_3P)$. The ion peak at m/e = 325.36 is due to $M^+(C_{19}H_{18}NO_2P)$, while the ion peak at m/e =260.1 (100%) corresponds to $M^+(C_{13}H_{11}NO_3P)$ which is the base peak, while the ion peak at m/e = 232.08 corresponds to $M^+(C_{12}H_{10}O_3P)$. The ion peak at m/e = 226.12 corresponds to $M^+(C_{13}H_{12}NOP)$. The ion peak at m/e = 137.1 corresponds to $M^+(C_7H_6OP)$. The ion peak at m/e =103.1 corresponds to $M^+(C_6H_4N_2)$ and the ion peak at m/e = 58.08 corresponds to $M^+(CH_2NP)$. The fragmentation patterns of DAHP are shown in Scheme (2); Figure 4.

The IR and ^{1}H-NMR and mass spectral data suggest the structure of the phosphonate ligands as shown in Figure 5.

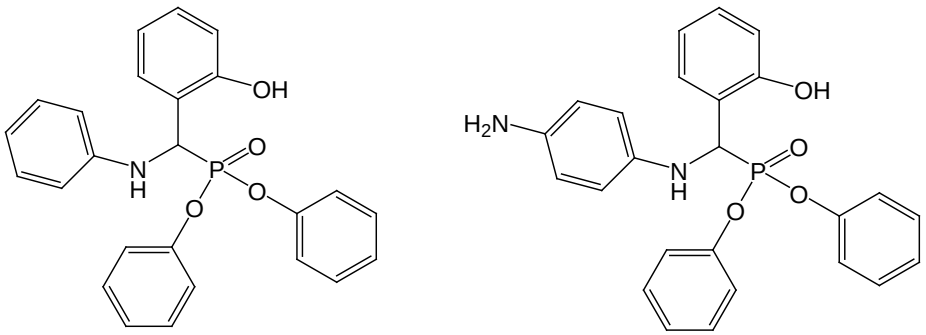


Figure 5. Structure of the phosphonate ligands

IR spectra of the copper (II) complexes were recorded to give more information about their structures. The vibrational mode assignments of the metal complexes were compared with the vibrational frequencies of the free ligands (Figure 1). By comparing the IR spectral data of the prepared ligands with their copper (II) complexes, it is found that the phosphonate ligands are coordinating with the copper ion by P=O oxygen and NH nitrogen atoms. This suggestion is supposed by shifting in the position of ν P=O band from1215 cm^{-1} in the ligands to 1229-1254 cm^{-1} in the Cu^{II}-DHPP and Cu^{II}-DAHP, respectively. At the same time, the position of νNH was shifted to 3260 cm^{-1} and 2930 cm-1 in Cu^{II}-DHPP and Cu^{II}-DAHP, respectively. The binding with NH is supported by shifting in the position of NH bending from to 1641 to 1629 cm^{-1} in the copper (II) complexes.

3.3. Electronic spectra

The electronic spectra of the prepared ligands DHPP and DAHP were recorded in ethanolic solution and showed two bands at ~335 and ~ 490 nm assigned to $\pi \rightarrow \pi*$ and $n\rightarrow\pi^*$ electronic transitions, respectively. The magnetic moment (1.93 and 186 BM) of the Cu(II) complexes Cu^{II}-DHPP and Cu^{II}-DAHP, respectively at room temperature corresponds to one unpaired electron [31]. The copper(II) complexes with d^9 configuration are expected to experience Jahn-Teller distortion which leads to further splitting of the ^2E_g and $^2T_{2g}$ levels. Moreover, they give rise to the $^2B_{1g}\rightarrow{}^2A_{1g}$ (ν_1), $^2B_{2g}$ (ν_2) and ^2E_g (ν_3) transitions which are expected to be close in energy and generally appears as a broad band. The electronic spectra (not shown) of the copper complexes Cu^{II}-DHPP and Cu^{II}-DAHP in ethanolic solution, show a broad band at ~700 nm. The a broad band centered at ~700 nm is assigned to the envelope of $^2B_{1g}\rightarrow{}^2A_{1g}$, $^2B_{2g}$ and ^2E_g transitions [32]. This band with the magnetic moment value, support a distortion octahedral geometry around copper (II) in the complex.

3.4. Thermal analysis

The thermodynamic activation parameters of decomposition processes of the copper (II) complexes, namely activation energy (E*), entropy (ΔS) and Gibbs free energy change of the decomposition (ΔG) were evaluated graphically by employing Coats–Redfern method [33]. The results obtained by applying this method (Table 1) give some information about the stability of the copper (II) complexes. The high values of the activation energies for Cu^{II}-DHPP and Cu^{II}-DAHP reflect the thermal stability of these complexes [34] due to their covalent bond character [35]. The entropy ΔS gives information about the degree of disorder of the

Table 1; Thermodynamic parameters for copper complexes.

Cu^{II}-DHPP				
	Ea	ΔH	ΔS	ΔG
53-150 ^oC	12861	9926	-35.62	22503
163-270 ^oC	16345	12279	-36.67	30213
820-990 ^oC	26288	16328	-42.22	66909
Cu^{II}-DAHP				
	Ea	ΔH	ΔS	ΔG
41-103 ^oC	23129	20277	-33.59	31802
167-318 ^oC	13942	10134	-37.67	27391
679-915 ^oC	21882	12753	-40.05	56732

system. According to the kinetic data the entropy of activation was found to have negative values in all decomposition steps for all complexes. The negative ΔS values indicate that all studied complexes are more ordered in their activated states [36] and the decomposition reactions proceed with a lower rate than the normal ones. It also reveals that the complexes are formed spontaneously [34,37]. The enthalpy ΔH gives information about the total thermal motion. Gibbs or free energy gives information about the stability of the system. The positive sign of ΔG for the investigated complexes reveals that the free energy of the final residue is higher than that of the initial compound, and all the decomposition steps are non spontaneous processes.

The thermal analyses of the prepared complexes were investigated in order to give fully characterization of their chemical structure by measuring and confirming the solvent inside/or outside the coordination sphere. At the same time the thermal analyses give more information about the stability of the metal complexes. The correlations between the different decomposition steps of the complexes with the corresponding weight losses are discussed in terms of the proposed formula of the complexes. The TGA curves are given in Figure 6 . The weight losses for each complex are calculated within the corresponding temperature ranges

The copper complex Cu^{II}-DHPP is thermally decomposed in four stages of mass loss from 25-1000 ^oC. The first stage at the temperature range of 53-150 ^oC with weight loss of (Calcd. 5.98%, found 5.57%) corresponding to two water molecules. The second stage in the temperature range of 163-270 oC with weight loss of (Calcd.19.15%, found 19.49%) corresponding to PhOH + NH_3. The third and fourth stages are corresponding to removal of one Ph molecule with one molecule of HCl. with weight loss of (Calcd. 19.03 %, found 19.17%). The total weight loss of the Cu(II) complex Cu^{II}-DHPP is 44.23% and the remaining weight is 55.77%, indicating that the complex is stable till 1000 ^oC. The TGA of Cu^{II}-DAHP complex (Figure 7) shows four degradation stages. The first stage in the temperature range of 41-103 ^oC is assigned to removal of two water molecules (Calcd. 5.83%, found 5.93%). The second stage in the temperature range of 167-318 ^oC is assigned to removal of two NH_3 molecules with weight loss of (Calcd. 5.50%, found 5.63%). The third stage in the temperature range of 345-522 ^oC with weight loss of (Calcd. 11.82%, found 11.59%) is corresponding to removal of two molecules of HCl. The fourth decomposition stage in the temperature range of 679-915 ^oC is related to removal of two Ph molecules with weight loss of (Calcd. 25.49%, found 25.45%). The remaining weight is 51.40%, indicating that the complex is stable till 1000 ^oC.

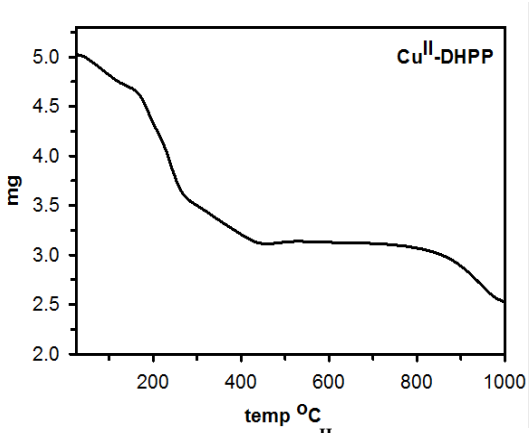


Fig. 6; TGA for Cu^{II}-DHPP.

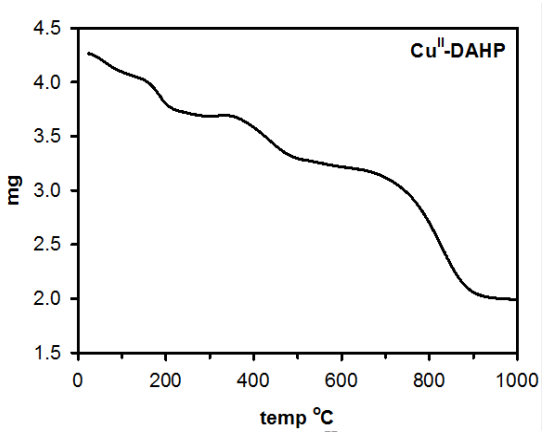


Fig. 7; TGA for Cu^{II}-DAHP.

The elemental analysis, IR and electronic spectral data with thermal analyses support the chemical structure of the prepared copper (II) complexes as $[CuL(H_2O)_2Cl_2]$ where L = DHPP and DAHP respectively, (Figure 8).

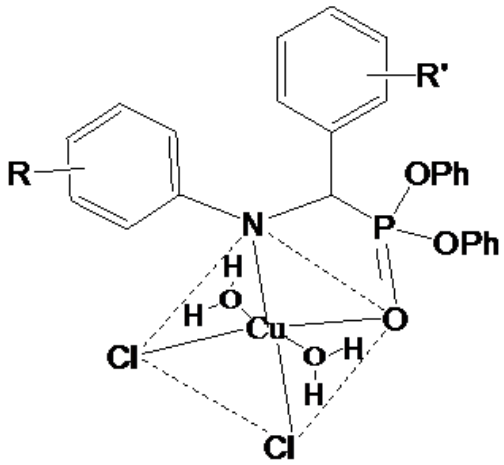


Fig. 8; The chemical structure of Cu^{II}-DHPP (R = H, R' = OH), and Cu^{II}-DAHP (R = NH_2, R' =OH).

3.5. Gel electrophoresis

The interactions of DNA which is considered as an important target of antitumor drugs with transition metal complexes are important for the design of effective drug entities [38], Particular attention has been devoted to transition metal complexes endowed with planar aromatic side groups, which can bind with DNA by both metal ion coordination and intercalation of the aromatic moiety [39]. The activity of the prepared ligands and their copper (II) complexes towards the DNA cleavage was determined by incubating the prepared compounds in different concentration with 100 ng genomic DNA for 24 hours at 37 ºC. The samples were run in 1.0 % agarose gel in TBE (tris– Borate-EDTA) buffer of pH 7.4 at 2 V/cm for 30 min.

The gel was stained with EtBr and photographs were taken in a syngene gel documentation system. It was observed (Figure 9) that complex Cu^{II}-DAHP exhibits potent nuclease activity at concentration range from 1nM to 1mM, followed by the complete conversion of supercoiled DNA to its open circular form[40] after 24 h incubation. On the other hand when the genomic DNA was incubated with 100 µM copper complex Cu^{II}-DAHP for interval times from 30 mins to 12 h (Figure 10), the complex revealed strong cleavage showing the supercoiled DNA band. In this time course the suercoiled DNA hasn't been converted into its open circular form and the linear form doesn't appear. It was found that the more concentration of the copper (II) complex the more strongly cleavage effect on the genomic DNA. It is clear that the cleavage of the genomic DNA is dependent on the number of metal ions as well as the presence of an aromatic ring.

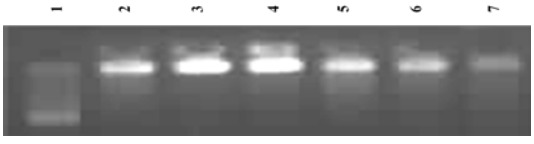


Fig. 9; Various concentrations of complex Cu^{II}-DAHP (0, 1 nM,10 nM,100 nM, 1 **m**M, 10 **m**M,100 **m**M) were incubated with genomic DNA (100 ng) for 24 hours at 37 ºC. DNA was electrophoresed in 1% agarose gel stained with ethedium bromide for 30 minutes and photocopied on Syngene gel documentation system.

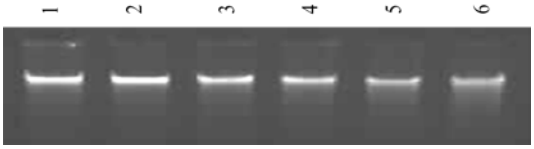


Fig. 10; Time dependant effect of the complex Cu^{II}-DAHP on DNA binding activity. Cu^{II}-DAHP (100 mM) was incubated with genomic DNA for 0, 30 min, 1, 2, 6 and 12 hours for lanes 1-6, respectively, at 37 ºC. DNA was electrophoresed in 1% agarose gel stained with ethedium bromide for 30 minutes and photocopied on Syngene gel documentation system.

IV. Conclusion

The phosphonate ligands DHPP and DAHP were synthesized and characterized by different physicochemical tools. The copper (II) complexes of these phosphonates were also synthesized and characterized. the mode of binding was studied and the thermal analysis of these complexes showed that the Cu^{II}-DAHP is more stable than Cu^{II}-DHPP. The activity of these complexes towards the DNA cleavage was investigated and showed that Cu^{II}-DAHP reveal the highest ability to cleavage the genomic DNA.

Acknowledgments

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