Synthesis, Characterization, and Anti-Microbial Study of Metal Complexes of Polycyclicacetal Derived from PVA & Erythro-Ascorbic Acid Derivative.

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***Abstract*—The aim of this work is the synthesis of new grafted PVA polymer with a derivative of Erythro-ascorbic acid (pentulosono-ɤ -lactone-2, 3-enedianisoate). All synthesized compounds were characterized by thin layer chromatography (TLC) and FTIR spectra and aldehyde was also characterized by (U.V-Vis), ^{1}HNMR, ^{13}CNMR and mass spectra. They were also evaluated for antimicrobial properties by dilute method against four pathogenic bacteria (*Escherichia coli* ,*Klebsiella pneumonae, Staphylococcus aureus, Staphylococcus Albus*) and two fungal (*Aspergillus Niger, Yeast*). All polymer metal complexes showed good activities against the various microbial isolates. The polymer metal complexes showed higher activity than the free polymer. The order of increasing activities was polymer < pol-Mn < pol-Ni < pol-Co < pol-Cu < pol-Hg < pol-Ag. The results provided evidence that the studied complexes might indeed be potential sources of antimicrobial agents and these would further enable us to evaluate their utility in biomedical field.**

***Index Terms*—Polycyclicacetal, polymer metal complexes, antibacterial, antifungal, activity.**

I. Introduction

Antimicrobial polymers are defined as polymers having bioactive pendant groups or bioactive repeat units in the polymer chemical structure [1]-[2], and these polymers are produced by attaching or inserting an active antimicrobial agent onto a polymer backbone via an alkyl or acetyl linker. Antimicrobial polymers may enhance the efficiency and selectivity of currently used antimicrobial agents, while decreasing associated environmental hazards because antimicrobial polymers are generally nonvolatile and chemically stable. This makes this material a prime candidate for use in areas of medicine as a means to fight infection, in the food industry to prevent bacterial contamination, and in water sanitation to inhibit the growth of microorganisms in drinking water [3].In recent years, more interests have been emphasized in the synthesis of polymers containing polyacetal segments, because of the ease of degradation of these polymers under mild conditions by treatment with a trace of acid [4]. Polymer which is Polyvinyl alcohol or known as PVA is a non-toxic, water soluble, bio-compatible and biodegradable synthetic polymer have been widely used in biomedical field. PVA has been better fiber-forming and highly hydrophilic properties and its fibers have been commercialized since the 1950s [5]. So PVA is a polymer of great interest because of its many desirable characteristics specifically for various pharmaceutical and biomedical applications [6].Vitamin C is the most important vitamin for human nutrition that is supplied by fruits & vegetables. L-ascorbic acid (AA) is the main biologically active form of vitamin (C). Currently it is best known for its antioxidant properties. Its key role however is in the prevention of scurvy, a devastating deficiency disease [7].

II. Experimental

A. Preparations of Polycyclicacetal

Melting points were determined by electro thermal Stuart melting point apparatus and are uncorrected. IR spectra (in KBr) were recorded on 8400s Shimadzu FT infrared spectrophotometer. ^{1}HNMR spectrum was recorded on Ultra Shield (300 MHz) spectrometer with tetramethyl silane as internal standard. ^{13}CNMR spectrum was recorded on a Varian Mercury plus 100 MHz spectrometer. Electronic spectrum was obtained using a (U.V-Vis) spectrophotometer type CECl 7200 England. Mass spectrum was recorded on IEOLJMS-7 high resolution instrument. Thin layer chromatography (TLC) was performed on aluminum plates coated with layer of silica gel, supplied by Merck. The spots were detected by iodine vapor. All chemical were obtained from Fluka or BDH

Manuscript received October 15, 2012; revised December 30, 2012.

B. Synthesis of 5,6-O-isopropylidene-L-ascorbic acid (2)

Dry hydrogen chloride was rapidly bubbled with stirring for 20 minutes into a (250ml) flask containing (10g, 57mmol) of powdered L-ascorbic acid (1) and (100ml) of dry acetone.

After addition of (80ml) n-hexane, stirring and cooling in an ice-water, the supernatant was decanted. The precipitate was washed four times with (154ml) of acetone-hexane mixture (4:7) (v/v), cooling in an ice-water and removal of supernatant after each addition. The last precipitate was dried under reduced pressure to give (2) (95.35%) as a white crystalline residue [8], m.p (206-208˚C). R_f (0.68) (benzene: methanol, 5:5) (v/v). FTIR (KBr, cm^{-1}): 3240 (O-H), 2993 (C-$H_{ali.}$), 2908 (C-$H_{ace.}$), 1751 (C=$O_{lac.}$), 1662 (C=C), 1431 (-CH-$_{asym}$), 1388 (-CH-$_{sym}$), 1141-900 (C-O), 767 δ (O-H) (O.O.P.) [9].

C. Synthesis of 2,3-*O-dianisoyl-5,6-O-isopropylidene-L-ascorbic Acid (3*)

To a cold solution of (2) (10g , 46mmol) in pyridine (50ml), anisoyl chloride was added as dropwise (17.5ml , 129mmol) with stirring .The resulting mixture was stirred for 2 hours , then kept in dark place at room temperature for 22 hours.

The mixture was poured into ice-water and stirred for 20 minutes, the supernatant was decanted. The oil layer was extracted with chloroform (150 ml); washed with water, dilute hydrochloric acid (5%) (2 × 100ml.), saturated aqueous sodium hydrogen carbonate (100ml) and water. Dried over anhydrous magnesium sulfate, Chloroform was evaporated to produce brown syrup and purified from chloroform: petroleum ether (60-80˚C) (1:5) (v/v) to give (3) (76.5%) as a pale yellow solid [10], m.p (102-104˚C). Rf (0.80) (benzene: methanol, 5:5) (v/v). FTIR (KBr, cm-1): 3028 (C-Har.), 2983 (C-Hali.), 2939 (C-Hace.), 2843 (OC-Hali.), 1749 (C=Olac.), 1683 (C=Oest.), 1647 (C=Cali.), 1604 (C=Car.), 1300-1107 (C-$O_{est.}$), 900-600 δ(C-H) (O.O.P.)

D. Synthesis of 2,3-O-dianisoyl-L-ascorbic Acid (4)

Compound (3) (10g , 23.6mmol) was dissolved in mixture (65%) acetic acid (30ml) and absolute methanol (10ml) and stirred for 48 hours at room temperature. The TLC showed that the reaction was complete (benzene: methanol, 6:4).

To the resulting solution a benzene (40ml) was added and evaporated (repeat this process four times) [10].The residue recrystallized from chloroform and then diethyl ether to yield (4) (77.7%) as a white crystals, m.p (130-132˚C), R_f (0.42). FTIR (KBr, cm^{-1}): 3444 (O-H), 3008 (C-$H_{ar.}$), 2972 (C-$H_{ali.}$), 2843 (OC-$H_{ali.}$), 1741 (C=$O_{lac.}$), 1681 (C=$O_{est.}$), 1647 (C=$C_{ali.}$), 1606 (C=$C_{ar.}$), 1319-1112 (C-$O_{est.}$), 900-600 δ(C-$H_{ar.}$) (O.O.P.) [10].

E. Synthesis of Pentulosono-γ-lactone2,3Enedianisoate (5)

To the stirred solution of sodium period ate (5.6g, 26mmol) in distilled water (60ml) at (0˚C), a solution of (4) (10g, 26mmol) in absolute ethanol (60ml) was added drop wise. After stirring for 15 minutes, ethylene glycol (0.5ml) was added as drop wise, stirring was continued at room temperature for 1 hour [10].The mixture was filtered and to the filtrate water (40ml) was added then the product was extracted with ethyl acetate (3×50ml), the extracts dried by anhydrous magnesium sulfate, then filtered and the solvent was evaporated and the residue recrystallized from benzene to yield the pure product of compound (5) (45%) as a white crystals, m.p (156-158˚C). R_f (0.70) (benzene: methanol, 6:4) (v/v). FTIR (KBr, cm^{-1}): 3040 (C-$H_{ar.}$), 2983 (C-$H_{ali.}$), 2843 (OC-$H_{ali.}$), 2671, 2559 (C-$H_{ald.}$), 1782 (C=$O_{lac.}$), 1749 (C=$O_{ald.}$), 1685 (C=$O_{est.}$), 1604 (C=$C_{ar.}$), 1300-1107 (C-$O_{est.}$), 900-600 δ(C-$H_{ar.}$) (O.O.P.). ^{1}HNMR (DMSO δ ppm): 12.5 (s, 1H, CHO), 7.00-7.97 (dd, 8H, aromatic), 3.86 (s, 1H, H_4), 3.82 (s, 6H, 2OCH_3) [9]. ^{13}CNMR (DMSO δ ppm): 167.50 (C=$O_{lac.}$), 163.32 (C=$O_{est.}$), 131.86 (C-4), 131.83 (C-3), 131.81 (C-2), (123.44, 114.31, 114.28, 114.26) ($C_{ar.}$), 55.90 (OCH_3). The signal of aldehydic carbonyl was disappeared due to it showed out of the scale [11]. MS, (positive ion) m/z (relative intensity): 413 [M+1, (100)], UV (λ_{max}, nm, $CHCl_3$): 300.

F. Synthesis of Polyvinyl Acetal (6)

Compound (5) was dissolved in a mixture of benzene (8ml) and ethanol (2ml) with two drops of HCl. PVA (Mw = 14000, 0.5g) was added to the mixture with vigorous stirring at (40–50) ℃ for 24 hours. The solution was poured into excess amount of methanol (100 ml) containing equimolar amount of NaOH, the product was separated by filtration and then washed with methanol and dried under vacuum. FTIR (KBr, cm^{-1}): 3448 (O-H), 3057 (C-H_{ar}), 2954 (C-H_{ali}), 1597(C=$O_{anisoate}$), 1279-1068 (-C-O-$C_{ace.}$), 923-680 δ(C-$H_{ar.}$).

G. Preparation of the Polycyclicacetal Metal Complexes

The silver nitrate ($AgNO_3$) and Mercury chloride ($HgCl_2$) were obtained from Fluka. Nickel chloride ($NiCl_2.6H_2O$), manganese sulfate ($MnSO_4.H_2O$), cobalt chloride ($CoCl_2.H_2O$) and copper chloride ($CuCl_2.2H_2O$) were obtained from Aldrich. Sabouraud agar, Blood Agar Base, MacConky Agar and Nutrient Broth were obtained from Oxoid LTD.

The general procedure for preparation of metal complex by preparing 5% from polycyclicacetal solution and mixed with equal ratios of metal solution (Cu, Co, Ni, Mn, Ag, Hg) (10 mmol), mixture was stirred for 1 hour.

H. Evaluation Test of Antimicrobial Activity

Antimicrobial susceptibility test measures the ability of an antimicrobial agent to inhibit or kill bacterial growth in vitro. This ability may be estimated by either the dilution method or the diffusion method. In this work we followed the broth dilution method. Certain bacteria and fungi isolates were chosen, *Escherichia-Coli* and *Klebsiella* Peneumoniae were representing gm-ve isolates, *Staphylococcus aureus* and *Staphylococcus albeus* were representing gm+ve isolates, two fungal (*Aspergillus Niger*, *Yeast*).Those Isolates were taken from about 50 patients at CPHL (Central Public Health Laboratory in Baghdad)

The broth dilution method: Serial twofold dilutions of an antimicrobial agent are incorporated into broth containing tubes that are then inoculated with a standard number of organisms usually 10^5-10^6 colony-forming units (CFU) per milliliter. After the culture has been incubated at 37^0C for 18 hours. The lowest concentration that prevents growth after overnight incubation is known as the minimum inhibitory concentration (MIC) of the agent, The MIC is defined as the lowest concentration of antimicrobial agent at which there is no visible growth [12]-[13].

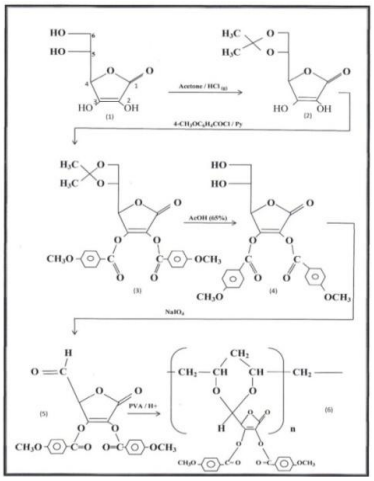
III. Results and Discussion

A. Spectroscopic Studies

In the present work the synthesis of new polycyclicacetal was achieved from pentulosono-γ-lactone-2, 3-enedianisoate (5), scheme (1). The first step employs the protection of the hydroxyl groups at C-5 and C-6 positions in L- ascorbic acid with acetal formation leading to compound (2) using dry acetone in acidic media, following Salomon [8] method. This is followed by esterification of the hydroxyl groups at C-2 and C-3 positions with excess of anisoyl chloride in dry pyridine.

The FTIR spectra for compound (2) and (3) were confirmed the formation of compound (3) by disappearance of the band for (O-H) of compound (2) and exhibited the band at (1683) cm-1 for (C=O) of the ester in compound (3) spectrum.

In order to prepare aldehyde (5), the acetal moiety was cleaved under acidic condition [14] (65% acetic acid) for compound (3) to give (4) and oxidation of the product with sodium periodate to result (5), which gave a positive Tolen's test by formation a silver mirror [15]. The FTIR spectra for compound (4) and (5) were confirmed the formation of compound (5) by disappearance of the bands for (O-H) of compound (4) and exhibited the band at (1749) cm^{-1} for (C=O) in compound (5) spectrum. The structure of (5) was confirmed by ^{1}HNMR which exhibited a signal at δ (12.5) ppm for (CHO) and was characterized by ^{13}CNMR and (U.V-Vis) spectra, which showed one peak at (300) nm (33333 cm^{-1}) assigned to (n $\longrightarrow$ π*) and (π $\longrightarrow$ π*) transitions. Finally, the mass spectrum showed a highest mass signal at [*M*+1] =413 with signal intensity 100%. The FTIR spectrum for compound (6) confirm the formation of the polycyclicacetal by disappearance of the band at (1749) cm^{-1} for ($C=O_{ald.}$) and the appearance of the band at (1279-1068) cm^{-1} for (-C-O-$C_{ace.}$).



Scheme(1): The scheme of prepared polyeyelieacetal

B. Antimicrobial Studies

Antimicrobial activity of the synthesized compound and their corresponding metal complexes was determined against two Gram-negative bacterial strains (*Escherichia coli* and Klebsiella *Pneumoniae*), two Gram–positive bacterial strains (*Staphylococcus aureus* and *Staphylococcus albus*) and two fungal (*Aspergillus niger* and *Yeast*) Table I. and Table II. respectively.

TABLE I. Antibacterial Activity of the Polyacetal and its Metal Complexes (Minimum Inhibitory Concentration).

Isolates	Gram Stain	Concentration compounds metal µg/ml						
		P-acetal	P-Cu	P-Co	P-Ni	p-Mn	P-Hg	P-Ag
Escherichia Coli	-ve	800	350	450	500	700	350	300
Klebsiella Pneumoniae	-ve	850	400	400	500	750	300	300
Staphylococcus aureus	+ve	950	400	450	550	700	400	350
Staphylococcus albus	+ve	1000	450	500	600	650	350	400

TABLE II. ANTIFUNGAL ACTIVITY OF THE POLYACETAL AND ITS METAL COMPLEXES (MINIMUM INHIBITORY CONCENTRATION).

Isolates	Concentration compounds metal µg/ml						
	P-acetal	P-Cu	P-Co	P-Ni	p-Mn	P-Hg	P-Ag
Aspergillus niger	1400	1200	1200	1250	1300	1200	1100
Yeast	1350	1300	1250	1300	1350	1200	1150

The synthesized polycyclicacetal and polymer metal complexes exhibited a good degree of inhibitory effects on the growth of different bacteria and fungi isolate. The order of increasing activities was polymer < pol-Mn < pol-Ni < pol-Co < pol-Cu < pol-Hg < pol-Ag. Antibacterial agents may affect cells in a variety of ways, many of which are poorly understood [16].

Most of the commonly used antibacterial chemotherapeutic agents act by one of the following basic mechanisms: competitive antagonism of some metabolite

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