Synthesis and characterization of PVA cyclic acetal derivative and study of its biological activity.

تحضيروتشخيص الاسيتال الحلقي للبولي فنيل الكحولي ودراسة فعاليته البايولوجية.

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Abstract:

The aim of this work is the synthesis of new grafted PVA polymer with aderivative of L-ascorbic acid (pentulosono-ɣ-lactone-2,3-enedibenzoate).

To obtain this polymer, the 5,6-O-isopropylidene –L-ascorbic acid (2)was chosen , which was prepared from the reaction of L-ascorbic acid (1)as starting material with dry acetone in the presence of hydrogen chloride .The esterification of hydroxyl groups at C-2 and C-3 positions with excess of benzoyl chloride in dry pyridine was obtained compound(3). Hydrolysis for compound (3)in acetic acid(65%) gave the compound (4). Peroxidation of the product (4) with sodium periodate results an aldehyde (5), which was reacted with PVA polymer to give acetal derivative of PVA (6).

All these compounds were characterized by Thin layer chromatography (TLC) and FTIR spectra and some were characterized by (U.V-Vis) spectra, ^{1}HNMR spectra and ^{13}CNMR spectra.

The polymer & polymer metal complex were found to posses' good antibacterial activity.

الخلاصة:

يتضمن هذا البحث تحضير مشتقات جديدة من البولي فنيل الكحولي لحامض D-ارثرواسكوربيك.للحصول على هذه المشتقات تم اختيار5,6-*O*-ايزوبروبيليدين-L-حامض الاسكوربيك (2) والتي حضرت من تفاعل L- حامض الاسكوربيك (1) كمادة اولية مع الاسيتون الجاف بوجود غاز كلوريد الهيدروجين. تمت استرة مجاميع الهيدروكسيل في المواقع C-2 و C-3 باستخدام زيادة من كلوريد البنزويل بوجود البيريدين الجاف حيث تم الحصول على المركب (3). التحلل المائي للمركب (3) باستخدام حامض الخليك (65%) حيث اعطى المركب (4). بعدها تمت اكسدة المركب (4) ببرايودات الصوديوم لينتج الالديهايد (5) الذي يتفاعل مع البولي فنيل الكحولي ليعطي مشتقات الاسيتال للبوليمر(6).

شخصت المركبات المحضرة بواسطة كروماتوغرافيا الطبقة الرقيقة (TLC) واطياف الاشعة تحت الحمراء(FTIR) وبعض من هذه المركبات تم تشخيصه بواسطة اطياف الاشعة فوق البنفسجية والمرئية (U.V-Vis) واطياف الرنين النووي المغناطيسي (^{1}HNMR) واطياف كاربون الرنين النووي المغناطيسي (^{13}CNMR).

تم تقييم فعالية البوليمر المحضر ومعقدات البوليمر مع بعض الايونات ضد البكتريا وقد اعطت نتائج جيدة.

Introduction:

Polymer based drug delivery system have attracted much attention due to their ability to perform multiple critical functions. These functions include delivering therapeutic or other bioactive agents to a specific site via a targeting mechanism, increasing the circulation times of drugs in the body, protecting drugs from degradation, or increasing the bioavailability of poorly soluble drugs [1].

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 [2].

It's known that cyclic acetal can be synthesized by the reaction of vicinal diol with carbonyl group.

Polyvinyl alcohol was left to react with erythro ascorbic acid through its carbonyl group to get the cyclic acetal which was obtained in acid media. The cyclic acetal derivative was separated, characterized and the biological activity was measured against four types of bacteria and it showed a biological activity.

Experimental:

Melting points were determined by electro thermal Stuart melting point apparatus and are uncorrected. IR spectra (in KBr) were recorded on Shimadzu FT infrared spectrophotometer. ^{1}H and ^{13}CNMR spectra were recorded on Ultra Shield (300 MHz) spectrophotometer with tetramethyl silane as internal standard. Electronic spectra were obtained using a (U.V-Vis) spectrophotometer type Shimadzu, (160A).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.

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 powdered L-ascorbic acid (57mmol, 10g) (1) in (100ml) of dry acetone.

After addition of n-hexane (80ml), stirring and cooling in an ice-water, the supernatant was decanted. The precipitate was washed four times with of acetone-hexane mixture (4:7) (v/v) (154ml), cooling in an ice-water and removal of supernatant after each addition. The last precipitate was dried under reduced pressure to give (2) (11.7g, 95.35%) as a white crystalline residue, m.p (206-208˚C). R_f (0.68) (benzene: methanol, 5:5) (v/v). FTIR (KBr, cm^{-1}): 3240, 3062 (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) (Out Of Plane).

Synthesis of 2,3-*O*-dibenzoyl-5,6-*O*-isopropylidene-L-ascorbic acid (3):

Benzoyl chloride (129mmole, 15ml) in dry pyridine (50ml) was added dropwise to a cold solution of compound (2) (46mmole, 10g) .The reaction mixture was left stirring for 24hrs ,and then kept in dark place at room temperature for 22hrs.

The mixture was poured into ice-water and stirred for 20 minutes, the supernatant was decanted. Extraction with chloroform (150 ml). The organic layer was washed with water, dilute hydrochloric acid (5%) (2 × 100ml.), water, saturated aqueous sodium hydrogen carbonate (100ml) and water. Dried over anhydrous magnesium sulfate. The solvent was evaporated to give a brown syrup, which was precipitated with amixture of chloroform and petroleum ether (60-80˚C) (1:5) (v/v) to give (3) (15g, 76.5%) as a pale brown solid, m.p (83-85˚C). R_f (0.73) (benzene: methanol, 5:5) (v/v). FTIR (KBr, cm^{-1}): 3062 (C-$H_{ar.}$), 2985 (C-$H_{ali.}$), 2931 (C-$H_{ace.}$), 1751 (C=$O_{lac.}$), 1662 (C=$O_{est.}$), 1627 (C=$C_{ali.}$), 1600 (C=$C_{ar.}$), 1261-1118 (C-O), 900-600 δ(C-H) (Out Of Plane).

Synthesis of 2,3-*O*-dibenzoyl-L-ascorbic acid (4):

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

Benzene (40ml) was added to the solution and evaporated the organic solvent (repeat this process four times).The solid residue was recrystallized from chloroform and diethyl ether to yield (4) (7g, 77.7%) as a white crystals, m.p (115-116˚C), R_f (0.35). FTIR (KBr,

cm^{-1}): 3406 (O-H), 3074 (C-$H_{ar.}$), 2939 (C-$H_{ali.}$), 1716 (C=$O_{est.}$), 1600 (C=$C_{ar.}$), 1273-1118 (C-O), 900-600 δ(C-$H_{ar.}$) (Out Of Plane).

Synthesis of pentulosono-γ-lactone-2,3-enedibenzoate (Erythro ascorbic acid aldehyde) (5):

Asolution of (4) (26mmol, 10g) in absolute ethanol (60ml) was added drop wise to the stirred solution of sodium periodate (5.6g) in distilled water (60ml) at (0˚C). After 15 minutes, ethylene glycol (0.5ml) was added as drop wise, the stirring was continued for 1 hour at room temperature.

The mixture was filtered and water (40ml) was added to the filtrate. Extracted with ethyl acetate (3×50ml), the extracts dried by anhydrous magnesium sulfate. The residue was evaporated and recrystallized from benzene to yield a pure product (5) (4g, 44.4%) as a white crystals, m.p (110-112˚C). R_f (0.63) (benzene: methanol, 6:4) (v/v). FTIR (KBr, cm^{-1}): 3080 (C-$H_{ar.}$), 2839, 2677 (C-$H_{ald.}$), 1689 (C=$O_{ald.}$), 900-600 δ(C-$H_{ar.}$) (O.O.P.). ^{1}HNMR ($CDCl_3$): δ(4.97) ppm (s, 1H, H_4), δ (7.28-8.17) ppm (m, 10H, aromatic), δ(11.4) ppm (br, 1H, CHO). ^{13}CNMR ($CDCl_3$): δ(172.44) ppm (C=O), δ(133.83) ppm (C-3), δ(133.47) ppm (C-2), δ(130.23-128.03) ppm ($C_{ar.}$), δ(77.46) ppm (C-4). The signal of aldehydic carbonyl was disappeared due to it showed out of the scale [3].

Synthesis of polyvinyl acetal (6):

Compound (5) was dissolved in a mixture of benzene (8 ml) and ethanol (2ml) with two drops of HCl. PVA (Mw = 14000, 0.5 gm) was added to the mixture with vigorous stirring at (40 – 50)°C for 24 hr. 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=$O_{benzoate}$), 1279-1068(-C-O-$C_{ac.}$), 923-680(C-$H_{ar.}$).

Results and Discussion:

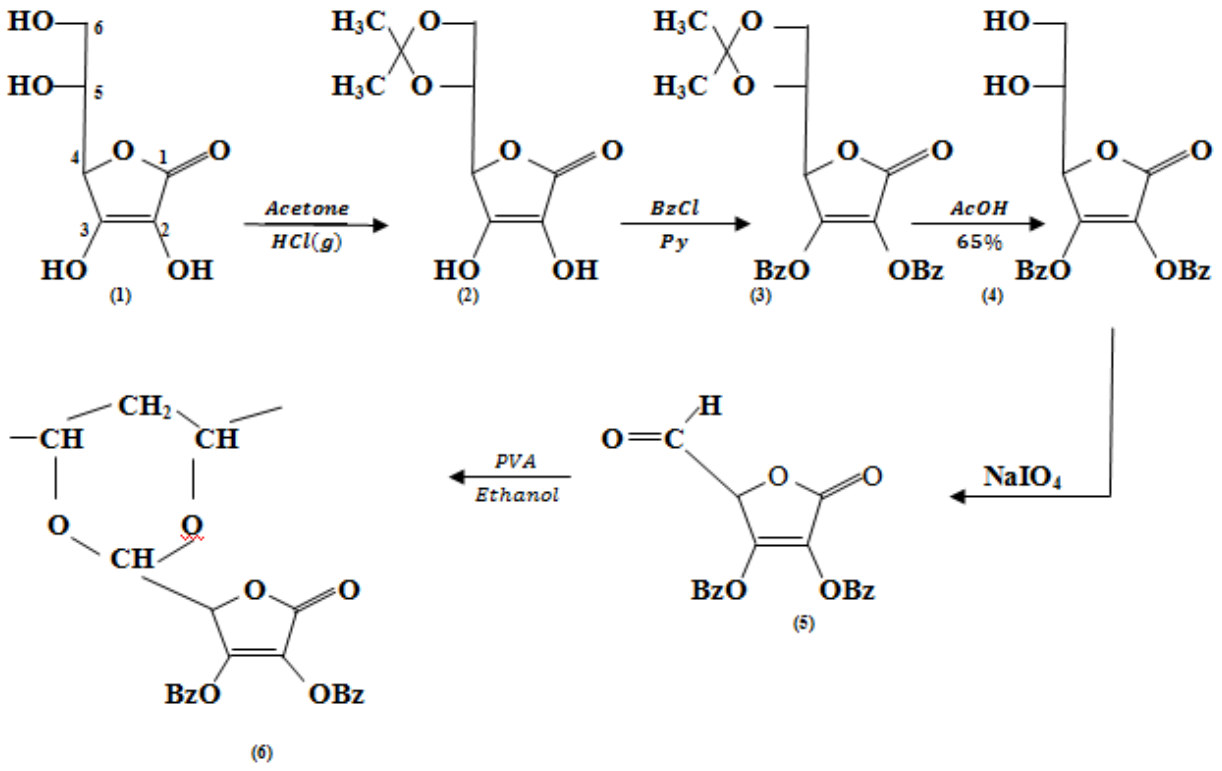
L-ascorbic acid (1) is one of the natural anti oxidant present in biological system because of its activity to attack the free radicals and other reactive oxygen species, as the literatures points to the great role which ascorbic acid plays to prevent a number of diseases and it importance in food industry.[4, 5]

One strategy allows the synthesis compound (6), in (5) steps starting from L-ascorbic acid, 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[6] method. This is followed by esterification of the hydroxyl groups at C-2 and C-3 positions with excess of benzoyl chloride in dry pyridine.

The FTIR spectra for compound (2) and (3) were confirmed the formation of compound (3) by disappearance of the bands for (O-H) of compound (2) and exhibited the band at (1662) 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 [7] (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.[8],[9] 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 (1689) cm^{-1} for (C=O) in compound (5) spectrum. The structure of (5) was confirmed by ^{1}HNMR which exhibited a signal at δ(11.4) ppm for (CHO) and was characterised by ^{13}CNMR and (U.V-Vis) spectrum which showed one peak at (295) nm (33898 cm^{-1}) (ε_{max} = 156 $molar^{-1}cm^{-1}$) assigned to ($n \longrightarrow \pi^*$) transition.The FTIR spectra for compound (6)confirm the formation of the polyacetal by disappearance of the band (1689)cm^{-1} for ($C=O_{ald.}$) and the appearance of the band (1279-1068)cm^{-1} for ($-C-O-C_{ac.}$).



Scheme (1) the scheme of prepared compounds.

Evaluation & susceptibility testing of antibacterial 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.

The broth dilution method:

For quantitative estimates of antibiotic activity, dilutions of the antibiotic may be incorporated into broth or agar medium, which is then inoculated with the test organism. The lowest concentration that prevents growth after overnight incubation is known as the minimum inhibitory concentration (MIC) of the agent [10].

Graded amount of antimicrobial substances are incorporated into liquid bacteriologic media. The media are subsequently inoculated with test bacteria and incubated. The end point is taken as at amount of antimicrobial substance required to inhibit the growth or to kill the test bacteria.

Table(1): Effect of PVA acetal (6) on the growth of selected isolated bacteria.

Isolates	Gram's stain	Concentration gm / ml				
		0.099	0.066	0.049	0.033	0.0166
Escherichia coli	-ve	-	-	-	+	+
Klebsilla Pneumonia	-ve	-	-	-	-	+
Pseudomonas Aeruginosa	-ve	-	-	-	+	+
Streptococcus Pyogenes	-ve	-	-	-	+	+

Effect of polymeric complexes as antimicrobial agents:

The polyvinyl acetal has been treated with elements like "silver, cupper, Cobalt, Nickel and Manganese" to give polymer-metal complexes and study as antibiotic agents. Polyvinyl acetal metal complexes were found to posses' good antibacterial activity.

Table (2): Effect of PVA acetal complex with silver salt on the growth of selected isolated bacteria.

Isolates	Gram's stain	Concentration gm / ml				
		0.099	0.066	0.049	0.033	0.0166
Escherichia coli	-ve	-	-	-	-	-
Klebsilla Pneumonia	-ve	-	-	-	+	+
Pseudomonas Aeruginosa	-ve	-	-	-	-	+
Streptococcus Pyogenes	-ve	-	-	-	-	+

Table (3): Effect of PVA acetal complex with cupper salt on the growth of selected isolated bacteria.

Isolates	Gram's stain	Concentration gm / ml				
		0.099	0.066	0.049	0.033	0.016
Escherichia coli	-ve	-	-	-	-	-
Klebsilla Pneumonia	-ve	-	-	-	-	+
Pseudomonas Aeruginosa	-ve	-	-	-	-	-
Streptococcus Pyogenes	-ve	-	-	-	-	-

Table (4): Effect of PVA acetal complex with manganese salt on the growth of selected isolated bacteria.

Isolates	Gram's stain	Concentration gm / ml				
		0.099	0.066	0.049	0.033	0.0166
Escherichia coli	-ve	-	-	+	+	+
Klebsilla Pneumonia	-ve	-	-	-	-	+
Pseudomonas Aeruginosa	-ve	-	-	-	+	+
Streptococcus Pyogenes	-ve	-	-	+	+	+

Table (5): Effect of PVA acetal complex with nickel salt on the growth of selected isolated bacteria.

Isolates	Gram's stain	Concentration gm / ml				
		0.099	0.066	0.049	0.033	0.0166
Escherichia coli	-ve	-	-	-	-	-
Klebsilla Pneumonia	-ve	-	+	+	+	+
Pseudomonas Aeruginosa	-ve	-	-	+	+	+
Streptococcus Pyogenes	-ve	-	-	-	-	+

Table (6): Effect of PVA acetal complex with cobalt salt on the growth of selected isolated bacteria.

Isolates	Gram's stain	Concentration gm / ml				
		0.099	0.066	0.049	0.033	0.0166
Escherichia coli	-ve	-	+	+	+	+
Klebsilla Pneumonia	-ve	+	+	+	+	+
Pseudomonas Aeruginosa	-ve	+	+	+	+	+
Streptococcus Pyogenes	-ve	-	+	+	+	+

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