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Synthesis of the new derivative 1- Diphenylamine Enamine Ascorbic Acid, and study of its biological activity

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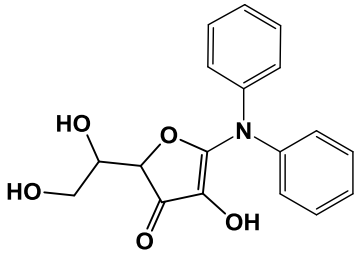
Abstract: The new enamine [1- Diphenyl enamine ascorbic acid] has been prepared according to two methods: The first method was "The reaction between ascorbic acid and diphenylamine" has been done directly, however the yield was relatively low. The second method was "the reaction between ascorbic acid and diphenylamine" which has been carried out through three steps: In the first step, the hydroxyl groups in (5, 6) position have been protected using the acetone, in the second step, the hydroxyl groups in (2, 3) position have been protected using iodide methyl. The obtained compound (5, 6-dioxolan-2, 3-metoxy ascorbic acid) have been reacted with Diphenylamine to obtain the target molecule in the third step" the structural determination has been confirmed using spectroscopy methods (FT-IR, ^{1}H-NMR, ^{13}C-NMR). The target compound gave very good biological activity against Gram-positive bacteria (Staphylococcus Aureus) and Gram-negative (Pseudomonas Aeruginosa) bacteria, with Gentamicin as a reference active compound.



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Keywords: Ascorbic acid, Diphenylamine, Enamine, Group protection, Biological activity.

اصطناع المشتق الجديد 1- ثنائي فينيل إينامين حمض الأسكوربيك ودراسة نشاطه البيولوجي

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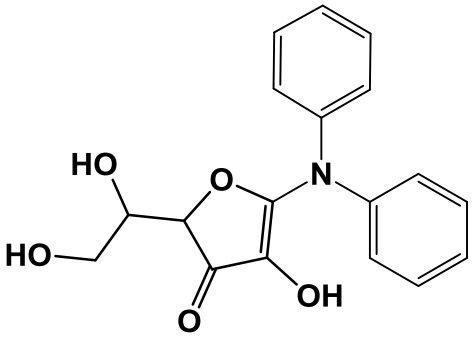
المستخلص: تم اصناع المركب الإينامينى الجديد [1- ثنائي فينيل إينامين حمض الأسكوربيك] وفقًا لطريقتين: الطريقة الأولى "تم التفاعل بشكل مباشر بين حمض الأسكوربيك وثنائي فينيل أمين، ولكن كان المردود قليلاً ". أما الطريقة الثانية: " تم تنفيذ التفاعل بين حمض الأسكوربيك وثنائي فينيل أمين من خلال ثلاث خطوات: في الخطوة الأولى، تمت حماية الزمر الهيدروكسيلية في المواقع (5 ، 6) باستخدام الأسيتون ، في الخطوة الثانية ، تمت حماية الزمر الهيدروكسيلية في المواقع (2 ، 3) باستخدام يوديد الميثيل. تم تفاعل المركب الناتج (6، 5-ديوكسولان-3، 2-ميتوكسي حمض الأسكوربيك) مع ثنائي فينيل أمين للحصول على الجزيء المستهدف في الخطوة الثالثة " ثم تم التأكد من البنية الهيكلية للمركب الناتج باستخدام طرق التحليل الطيفي (FT-IR, ^{1}H-NMR, ^{13}C-NMR). كما أعطى المركب المستهدف نشاطًا بيولوجيًا جيدًا ضد البكتيريا موجبة الجرام (Staphylococcus Aureeus) والبكتيريا سالبة الجرام (Pseudomonas Aeruginosa)، بالمقارنة مع الجنتاميسين كمركب نشط مرجعي.



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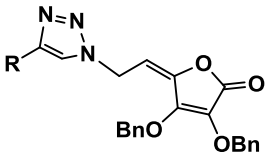
الكلمات المفتاحية: حمض الأسكوربيك، ثنائي فينيل أمين، إينامين، حماية الزمر الوظيفية، النشاط البيولوجي

1. Introduction:

Organic chemistry is one of the most important branches of chemistry, as it is linked to our daily life through the synthesis of many chemical compounds such as pharmaceuticals and others [1]. The organic synthesis through chemical reactions gave important industrial products similar properties to natural products with the same efficacy as the manufacture of perfumes, pesticides, others [2⟶4]. The enamines are among the compounds of application of importance in the industrial and pharmaceutical fields, Mannich and Davidsen are the first to develop a mechanism for forming the enamines, and many enamine derivatives have been Preparation from secondary amino compounds, ketones and aldehydes. Where many derivatives have been synthesized by new methods, and studies have proven their biological efficacy and importance in vivo and their use as medicinal preparations [5⟶7].

An important topic is the synthesis of enamines, which can be synthesized from sugary compounds such as ascorbic acid. The ascorbic acid participates in many chemical reactions to form important derivatives in some vital fields because it has biological activity on living organisms that is anti-cancer, virus, and bacteriostatic [8⟶12]. Many researchers have participated in the synthesis of organic nitrogen compounds based on ascorbic acid of great importance and effectiveness in the medical field.

The nitrogen-containing compounds are among the most important chemical compounds (organic and inorganic) that are included in many materials, whether natural or industrial, such as proteins and nucleic acids. Compound (2) is an antioxidant that impedes the growth of cancer cells, and also Compounds (3&4) are an important antiviral. [13⟶15]



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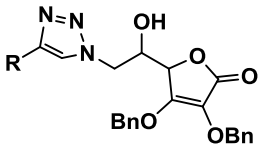

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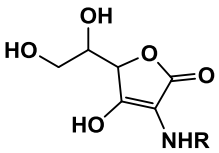


(2)

Therefore, obtaining derivatives of this acidic compound is considered to be a work of fetching pharmacological importance, especially the enamines derivatives. in this field, the synthesis of derivatives of ascorbic acid has been chosen.

In this research, the synthesis of new Enamines **(1)** has been reported, by the reaction between the carbonyl (lactone) group in ascorbic acid and the Diphenylamine as a secondary amine. The antibacterial activity of Compound **(1)** has been studied against Pseudomonas Aeruginosa and Staphylococcus aureus bacteria**.**

2. Experimental section:

2.1. Apparatus

- Proton Nuclear Magnetic Spectroscopy Model 400 MHz from Bruker, Switzerland.
- Infrared absorption spectrum model FT-IR-4100 from Japanese company SHIMADZU.
- Rotary evaporator Model 4.91 from the German company Normschiff.
- Aluminum foil chromatography sheets, silicon-coated, $60F_{254}$, measuring 20 x 20 from Merck, Germany.
- Thin-coated glass chromatography sheets, $60F_{254}$, silicon-coated, measuring 20 x 20 from Merck, Germany.

2.2. Reagents and materials

Ascorbic acid, acetone, Diphenylamine, produced by the company: SIGMA ALDRICH, CHEMLAB, Riedel-deHaen& MERCK BDH.

2.3. Experimental Procedure:

The compound **1** is obtained in two methods from the ascorbic acid:

2.3.1. First method: Direct interaction between ascorbic acid and diphenylamine

(25 ml) methanol has added to a reaction bottle that had a magnetic stirrer, a water bath, and a reversible coolant, and (1 mol; 0.176 g) ascorbic acid, the temperature of the mixture was raised to (40^0C) and (0.5 ml) of vinegar acid was added at a concentration of (80 %) As a catalyst for the reaction, after about (20) minutes (1mol; 0.169g) diphenylamine was added, and the reaction mixture was stirred at temperature (40^0 C) while monitoring the reaction progress by thin-layer chromatography (TLC) using a series of scooping (2, 1- Dichloroethane: ethanol (40%: 60%). The mixture was cooled and the unreacted acids were converted to sodium salts to obtain (PH = 7) using an aqueous solution saturated with sodium bicarbonate.

The neutral solution was filtered, and distilled water was added to the filtrate (20ml). Then the product was extracted using an appropriate amount of ethyl acetate three times. The organic phase was collected to dry using anhydrous sodium sulfate. The organic phase was re-filtered and then placed on a solvent repellant in order to get rid of the ethyl acetate and purified using Chromatography of glass plates using a dredging system consisting of (2, 1-dichloroethane: ethanol) (35%: 65%), and a white precipitate of melting point (149-148) ºC was obtained with a yield of (32%).

2.3.2. Second method: The three-steps enamine formation:

2.3.2.1. First step: synthesis (5, 6-dioxolan ascorbic acid)

In a reaction bottle equipped with a magnetic stirrer, (40 mL) of high-purity acetone and (0.5 mL) of acetyl chloride were added, the reaction mixture was stirred at room temperature for one hour, (1 mol; 0.176 g) of ascorbic acid was added and stirred Then the mixture temperature is maintained at (70 ° C) in an ice bath. The reaction was followed by thin-layer chromatography (TLC) with (25%: 75%) (dichloromethane: ethanol). At the end of the reaction, a white precipitate powder with yield (100%) is obtained. Its melting point was in the range (141-139) degrees Celsius

2.3.2.2. Second step: synthesis of 5, 6-dioxolan-2, 3-metoxy ascorbic acid

In a reaction bottle equipped with a magnetic stirrer, (10 mL) of dimethylformamide (DMF) and (1 mol, 0.138 g) of anhydrous potassium carbonate was added, the reaction mixture was stirred at room temperature for 1 hour. Then (1 mol, 0.216 g) of (5.6-dioxolane ascorbic acid) was added, followed by the TLC thin layer chromatography with (dichloromethane: ethanol) (30%: 70%). At the end of the reaction, (60 ml) of distilled water was added, the product formed was extracted by ethyl acetate three times, the organic phases were combined and evaporated, purified by preparatory TLC chromatography. A precipitate powder was obtained with yield (72%), and its melting point is (137-136) ° C.

2.3.2.3. Third step: synthesis of (1- diphenylamine enamine ascorbic acid)

In a reaction bottle equipped with a magnet, (25 mL) dichloromethane and (1 mol; 0.244 g) of (5, 6-dioxolane-2, 3-methoxi ascorbic acid) were added, and the mixture was heated to a temperature of (40° C), (0.5 mL) of acetic acid was added as a catalyst. After (20) minutes, (1 mol; 0.169 g) of diphenylamine was added and the mixture was mixed at a temperature of (40° C). The TLC reaction was followed with (dichloroethane: ethanol) (35%: 65%). The mixture was cooled, then a saturated aqueous solution of sodium bicarbonate was added to convert the excess acid into a sodium salt (pH = 7)

The solution was filtered, then 20 ml of distilled water was added, about 3 to 4 drops of halogen acid (1N) were added to remove protection from the hydroxyl groups in the protected mode. The product was extracted with ethyl acetate three times, the organic phases were collected and dried with anhydrous sodium sulfate, the organic phase evaporated, and purified with TLC chromatography. A white precipitate was obtained with a melting point (154-153) ° C and yield (67%).

2.3.2.4. Evaluation antibacterial activity of compound 1:

To study biological activity, two Petri plates were prepared using (agar medium 11), the first plate cultured with Gram-positive bacteria (Staphylococcus Aureus), and the second plate cultured with Gram-negative bacteria (Pseudomonas Aeruginosa). Three samples were prepared in each dish:

- First sample: A sample of compound 1: (APH1) at a concentration of (50µg/ml) using dimethyl sulfoxide (DMSO) as a solvent.
- Second sample: A sample of compound 1: (APH2) at a concentration of (100µg/ml) using dimethyl sulfoxide (DMSO) as a solvent.
- Third sample: a sample of the reference substance (gentamicin) (GE) using a concentration of (50µg/ml) using dimethyl sulfoxide (DMSO) as a solvent.

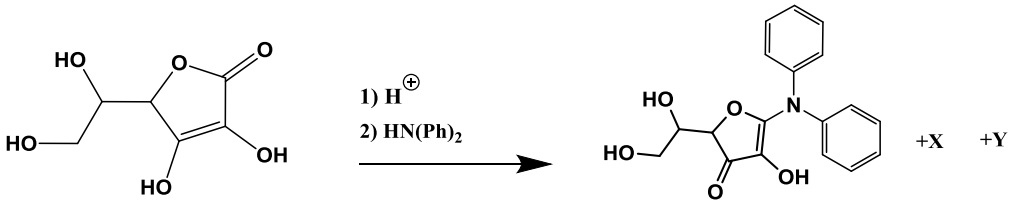
A sample was prepared from the reference material (gentamicin) (GE) using a concentration of (50µg/ml) using (DMSO). Samples were placed in a pre-prepared Petri plate with stainless steel cylinders, areas of inhibition were compared with the reference sample.

3. Results and Discussion:

The compound **1** is obtained in two methods from the ascorbic acid:

3.1. First method: Direct interaction between ascorbic acid and diphenylamine

This method involves a direct conjugation reaction between ascorbic acid and diphenylamine in the presence of an acid catalyst, which gave the required derivative and by-products according to the following reaction:



(4) (5)

The reaction time was changed to obtain a good yield, but no significant difference was observed in the yield by changing the time, as shown in Table (1):

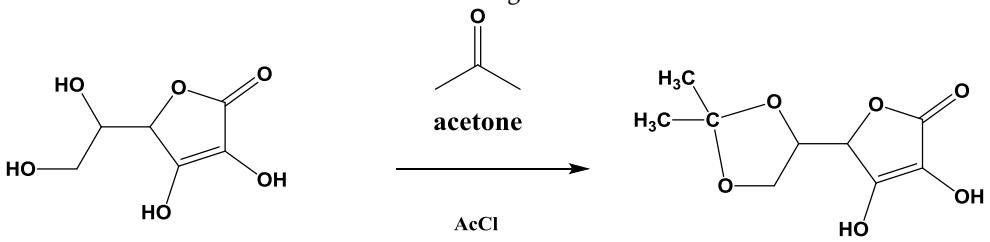
Table (1): The relationship of time to the yield

Time (h)	5	6	7
(%)Yield	30	30	31

3.2. second method: The formation of the enamine according to three steps:

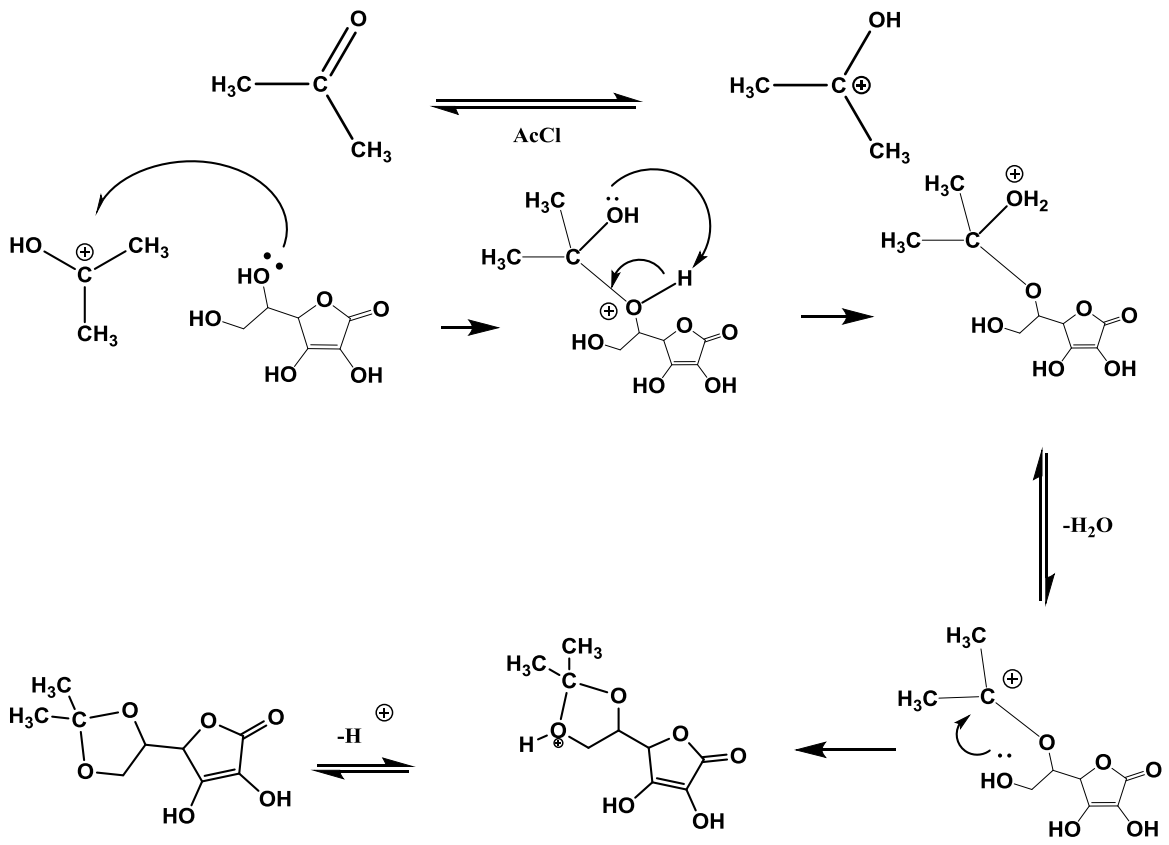
3.2.1. The First step: synthesis (5, 6-dioxolan ascorbic acid) 6:

This step includes protecting ascorbic acid's hydroxyl groups at (5, 6) positions. Where acetone reacts with ascorbic acid in acidic solution as following:



(4) **(6)**

The proposed mechanism of this reaction is presented as the following scheme:



To confirm the protection of the hydroxyl groups in position (5, 6), the infrared spectrum of the protected compound (6) (Fig. 1) was compared with the infrared spectrum of ascorbic acid (4) (Fig. 2), and the disappearance of the absorption signals within the range was observed (3100-3600) cm-1.

Figure (1) Infrared spectrum of(6.5-Dioxolan ascorbic acid) 4

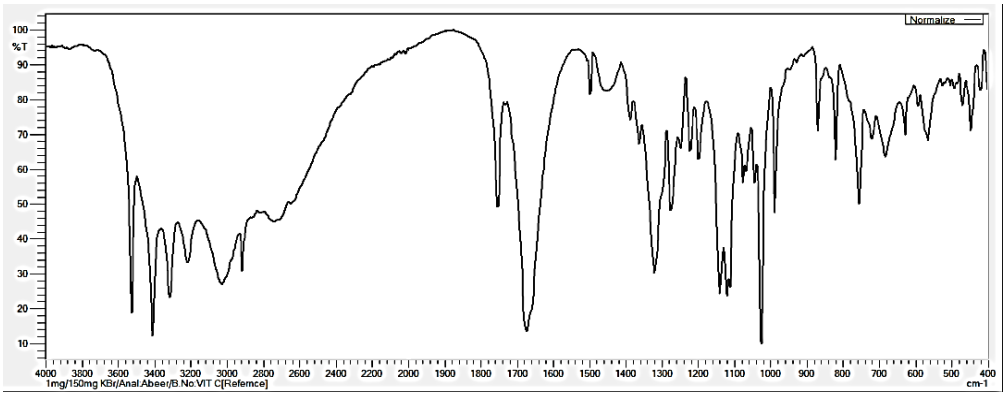
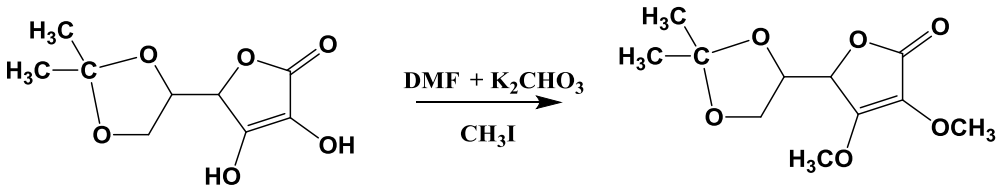


Figure (2) infrared spectrum of (ascorbic acid)

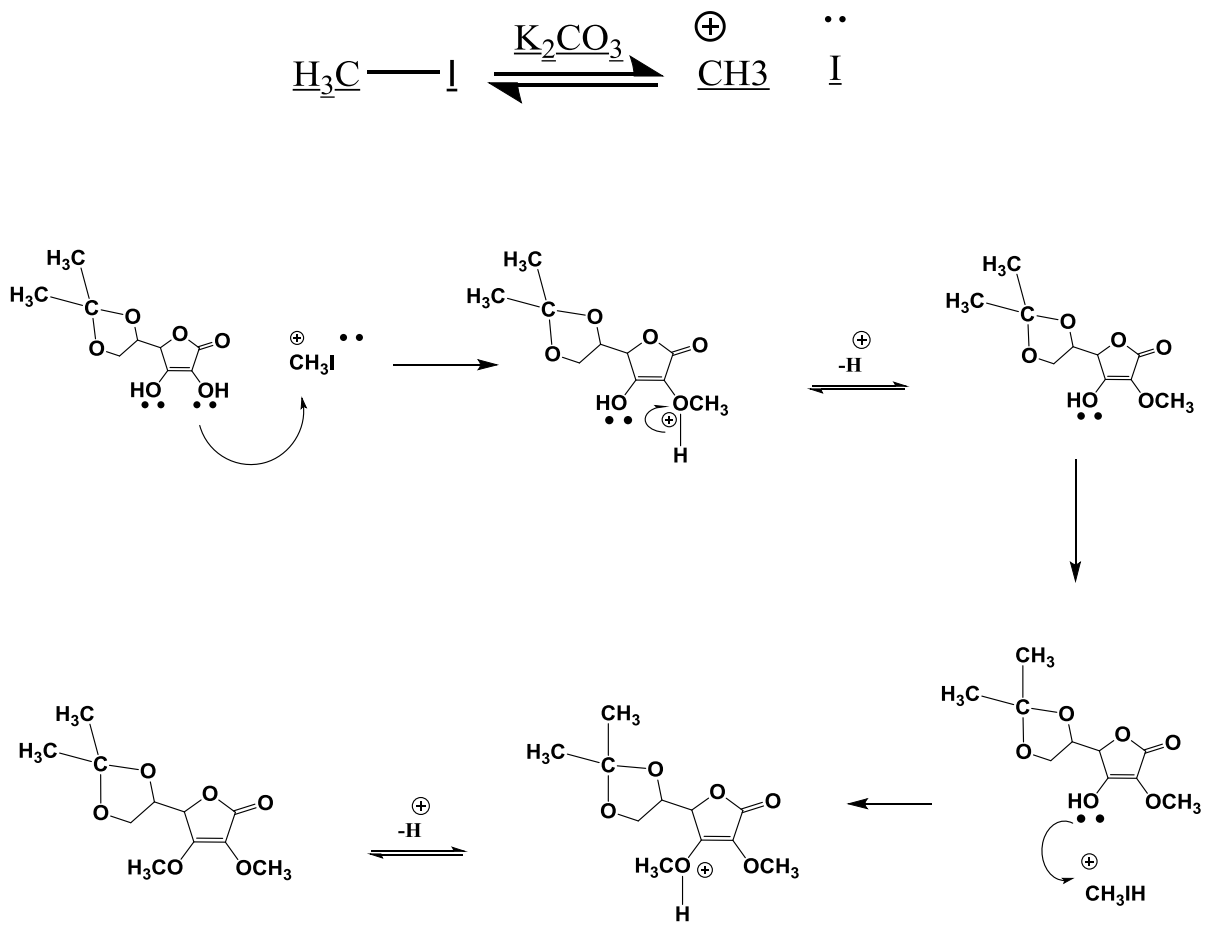
3.2.2. The second step: synthesis of 5, 6-dioxolan-2, 3-metoxy ascorbic acid 2

This step involves protecting the hydroxyl groups of ascorbic acid at (2, 3) sites with CH3I methyl iodide in an organic solvent as follows:



(6) (7)

the mechanism of reaction for the pervious formation is proposed according to the following scheme:



To confirm the protection of the hydroxyl groups in position (2, 3), the infrared spectrum of the protected compound (7) (Fig. 3) was compared with the infrared spectrum of ascorbic acid (4) (Fig. 2), and the disappearance of the absorption signals within the range was observed (3100-3600) cm-1.

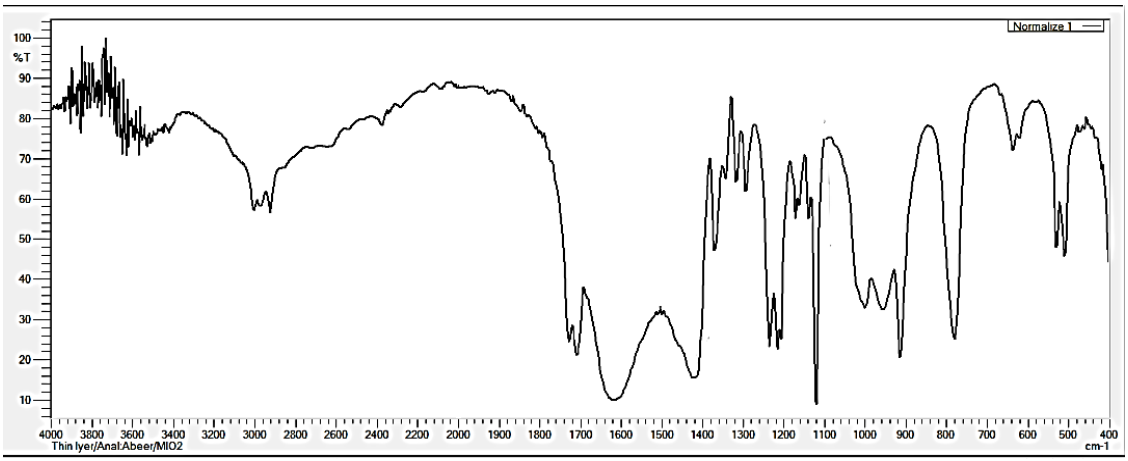
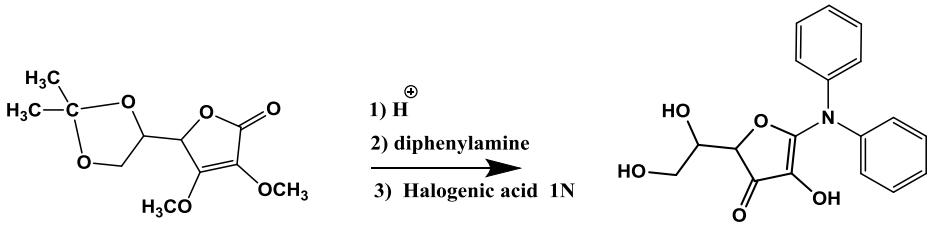


Figure (3) Infrared spectrum of (5.6- Dioxolan- 2, 3- ascorbic acid)

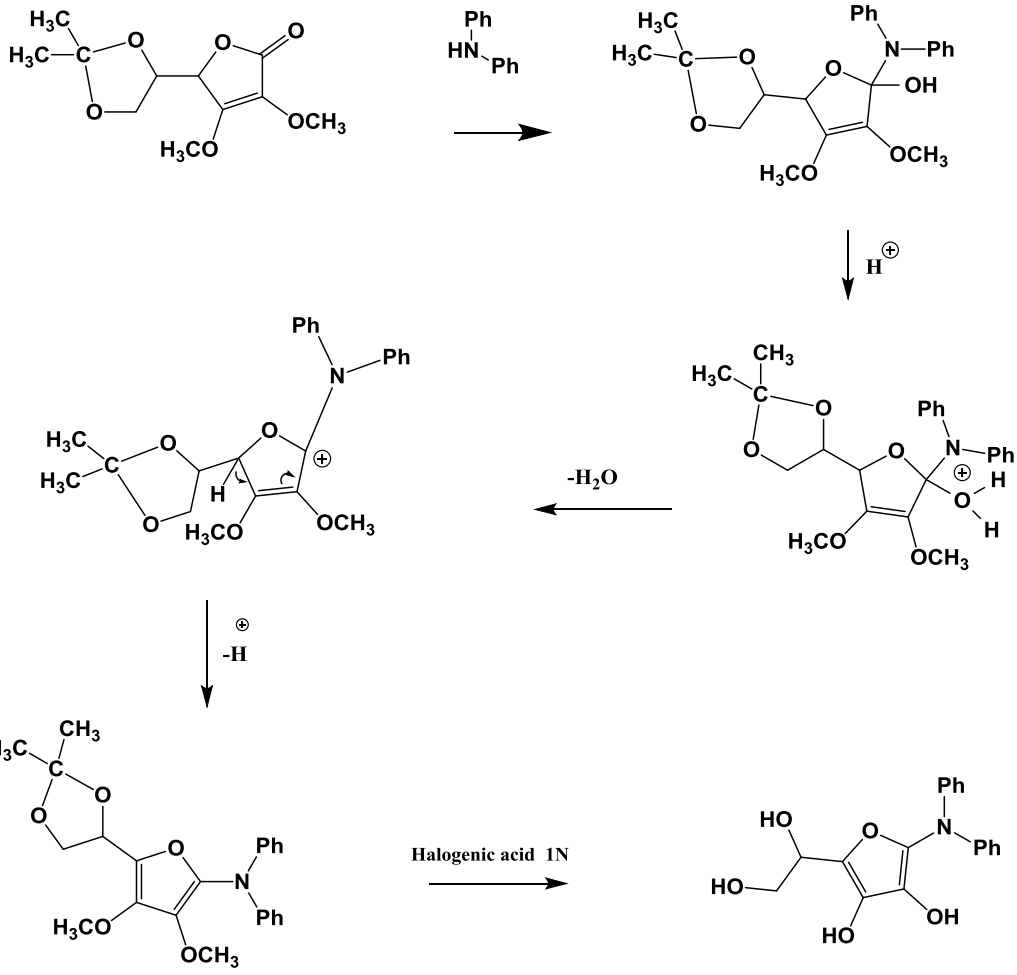
3.2.3. The third step: synthesis of (1- diphenylamine enamine ascorbic acid)1

The reaction of protected compound (2) and diphenylamine in solution in the presence of a catalyst of acetic acid gave the target molecule 1. Confirmed with TLC and extracted:

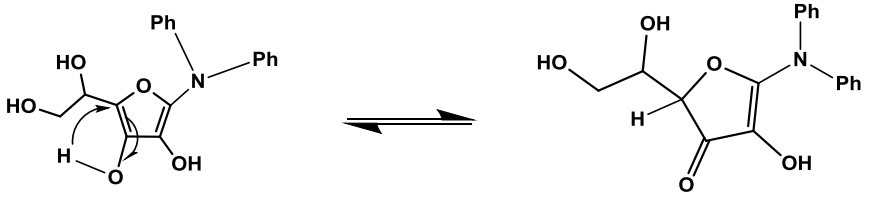


(7) **(1)**

A mechanism is proposed for the last reaction according to the following scheme:



The obtained enamine has an enol isomere, so the final form of the required compound becomes as follows:



The molecular structure of the resulting enamine (1- diphenylamine enamine ascorbic acid) was determined using spectroscopy: FT-IR, ^{1}H-NMR, ^{13}C-NMR.

Infrared spectrum of compound 1

When comparing the infrared spectrum of compound 1 Figure (4) with the spectrum of protected ascorbic acid, the disappearance of the absorption signal of one of the hydroxyl groups and a shift was observed in the absorption value of the carbonyl group indicating the formation of compound 1, In addition, a new (C-N aromatic) absorption signal is formed, and Table (2) shows some values of the functional group absorption in compound 1

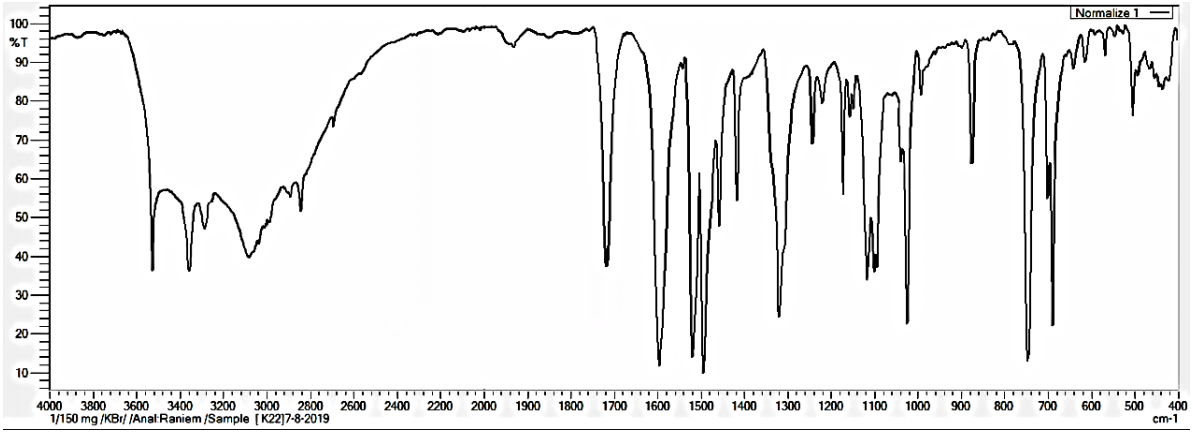


Figure (4) Infrared Spectrum of Compound 1

Table (2): IR Absorption for Compound 1

functional groups	C-O	C=O	C-N aromatic amine	C_{SP^3} – H	-OH
Stretching vibrations cm-1	1021	1715	1310	2845	3305 3381 3460

The proton Magnetic Nuclear Spectrum of compound 1

The proton Magnetic Nuclear Spectrum showed ^{1}H-NMR (Figure 5) using DMSO solvent.

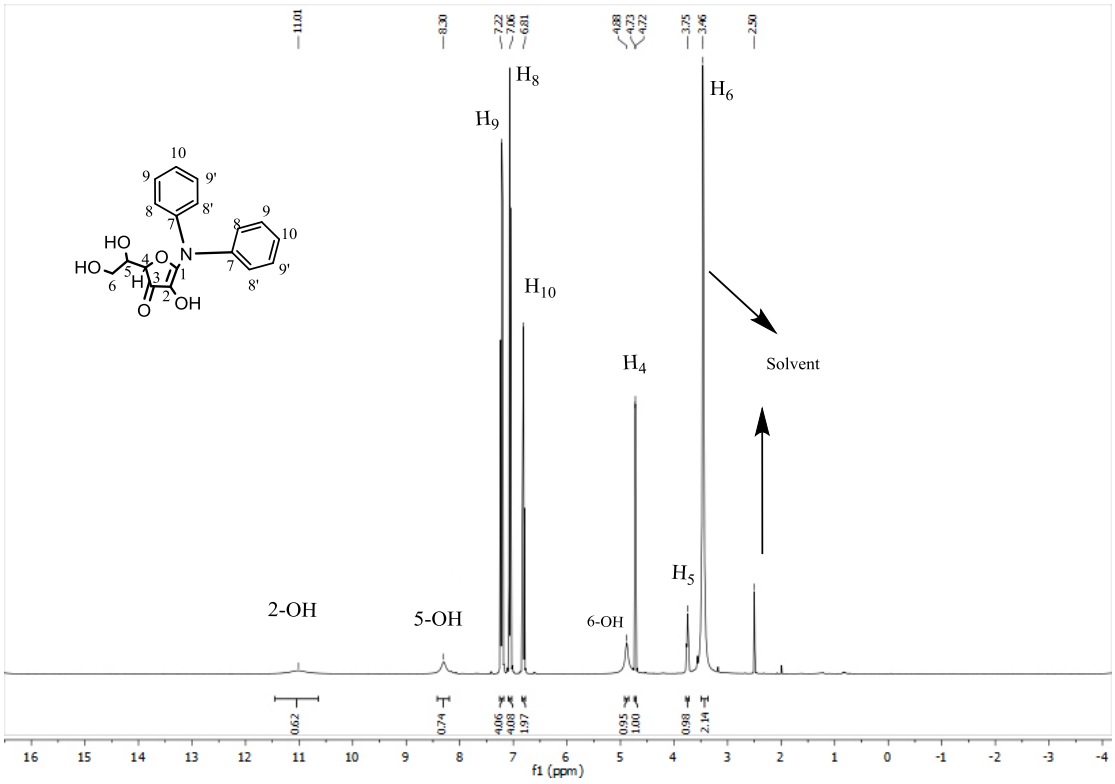
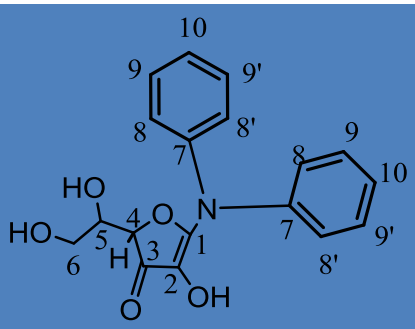


Figure (5) ^{1}H-NMR Spectrum of Compound 1

Table (2) shows the displacement values and mating constants calculated according to the device frequency of 400MHz.

Table (3):



number of the hydrogen atom	Chemical displacement δ, ppm	number of signals	Number of hydrogen atoms ((from the line of integration	Proton mating constant J(HZ)
6-OH	4.88	s	1	-
6	3.46	m	2	-
5	3.75	m	1	-
5-OH	8.3	s	1	1.43
4	4.73	d	1	1.52

2-OH	11.01	s	1	-
8, 8'	7.08	d	4	8
9, 9'	7.23	t	4	7.8
10	6.82	t	2	8.8

The carbon Magnetic Nuclear Spectrum of compound 1

The ^{13}C-NMR spectrum of the resulting compound was recorded (Fig. 6) using a solvent (DMSO), in this spectrum there are (10) signals corresponding to the carbon atoms present in this compound as in Table (4)

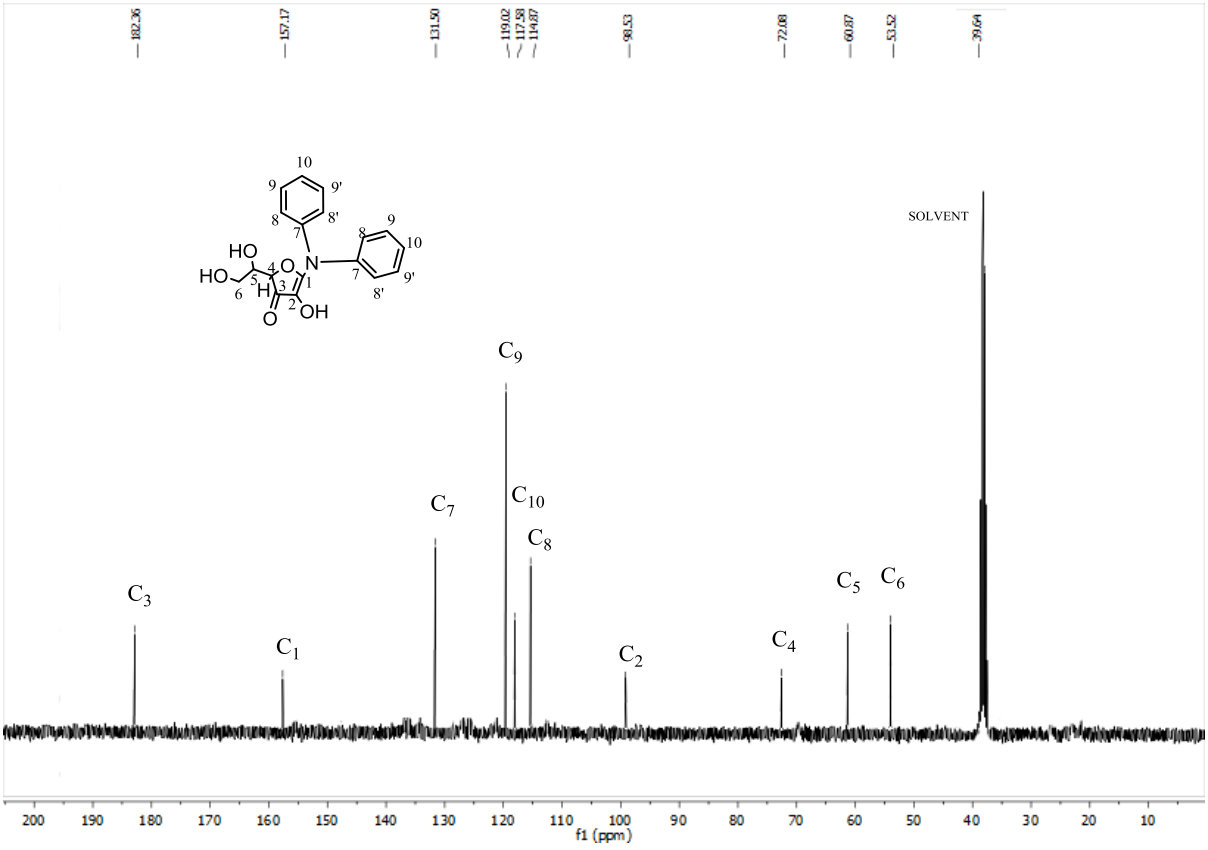


Figure (6) ^{13}C-NMR Spectrum of Compound 1

Table (4):

Carbon Atom Number	Chemical Shift δ (ppm)
1	157.17
2	98.53
3	182.36
4	72.08
5	60.87
6	53.52

Carbon Atom Number	Chemical Shift δ (ppm)
7	131.90
8	114.87
9	119.02
10	117.98

3.3. Evaluation the antibacterial activity of Compound 1:

The biological effectiveness of Compound 1 was studied on two strains of bacteria: Staphylococcus aureus and Pseudomonas Aeruginosa. Samples (APH1 and APH2) were very active on bacteria compared to the anti-inflammatory drug gentamicin. The incubation was for 30 minutes at a temperature of 37 ° C. Meanwhile, the tablets were prepared from stainless steel cylinders with an outer diameter (8 ± 0.1 mm), these tablets contain a food medium and sterile.

The following figure (7) shows the areas of inhibition, and the areas of inhibition are measured with a graduated ruler, and the results are recorded in Table (5).

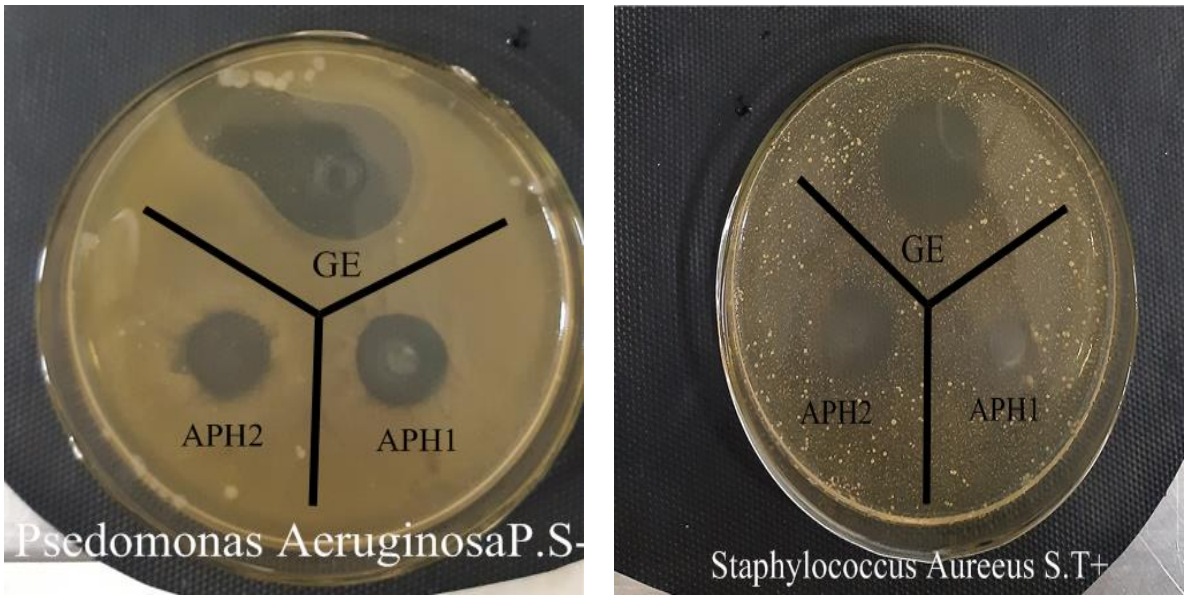


Figure (7) The zones of inhibition against to the growing of Staphylococcus aureus and Psedomonas Aeruginosa. in different concentrations

Table (5): The Activity of the resulting compound (APH), It is calculated by measuring diameter of the inhibition zone (mm).

	Compound 1		References. (mm)	
	APH 1 (50µg/ml)	APH 2 (100µg/ml)	Gentamicin (50 µg / ml)	DMSO
	Diameter of the inhibition zone (mm)			
Staphylococcus Aureus (S.T) Gram positive	13	17	19	-
Psedomonas Aeruginosa (P.S) Gram positive	17	16	20	-

Conclusion:

1- A new enamine derivative of ascorbic acid was synthesized, the optimal conditions were studied in order to obtain the highest yield.

2- We suggest synthesizing other enamine derivatives from ascorbic acid using other amine compounds.

Acknowledgments:

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