Modefied Method For Synthesis and Charecterization of Aza- Ascorbic Acid

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Abstract
A process was carried out to synthesize Aza-ascorbic acid using ascorbic acid as a starting material. It is based on closing sites (5, 6) then acetylation of sites (2, 3) to protect the gropes on these positions from side reactions. The produced compound, after acetylation, was treated with (ammonia- methanol) solution to give the substituted amide of ascorbic acid.
The hydroxyl group on position (4) was converted to benzene sulphonate which is characterized as a good leaving group.
Using a good neucleophilic agent will give a chance for amino group in amide derivative of ascorbic acid to attack the sulphonate group on position 4 to produce the aza-ascorbic acid.
This method can be considered as alternative to the classical methods which are based on oxidation of the hydroxyl group at position (4) converting it to ketone group followed by cyclization of the amide derivative which is finally reduced by sodium borohydride. The synthesized product was identified by UV and IR spectra.

Introduction
On the light of biological, industrial and biochemical importance of Aza-compounds especially in drug industries as active materials in treatment of diabetes, cancer, aids and diseases caused by viral infections [1], also having the ability and activity as drugs for treatment of many cases of carbohydrate mediated diseases [2]. Many methods are proposed in the literatures describing the pathway of converting ascorbic acid molecule to aza-ascorbic acid, starting with closing sites (5, 6) and then ammination. One of the proposed methods for closing (5, 6) sites, is the treatment of ascorbic acid with dimethoxy ethane in a water bath in presence of dioxane and trifloroacetic acid [3].
On the other proposal is to treat ascorbic acid with benzaldehyde and triethyl orthoformate in presence of dimethylformamide and trifloroacetic acid [4].
The described methods in literature, (to insert the amine group), was by ammination process in which ammonia or dimethylamine were used with out catalyst [5].
In the above mentioned methods, methyl sulphonyl chloride in presence of pyridine, as a solvent, was added, utilizing the specific charecter for methyl sulphonyl group as a good leaving group [6]. After that, dimethylamine in presence of dimethyl formamide was added to yield the molecule bearing the amine group on one side and the non protected cyclic ring on the other side [7].
The aim of the present research is to find a new method to synthesize aza-ascorbic acid using ascorbic acid as a starting material, characterized with simplicity and by using moderate conditions to synthesize the product.
This method is based on closing the sites (5, 6), by using acetone and hydrochloric acid, to obtain (5,6-O-isopropylidene-L-ascorbic acid), which will treated with acetic anhydride, in presence of pyridine to give (5,5-*O*-isopropyllidene-2,3-diacetyl-L-ascorbic acid), in which the sites (2,3) are protected by acetyl groups.
The lactonic ring was opened and amino group was inserted, using a solution of ammonia in methanol. Additionally the same results were obtained when ammonia as a gas and as liquid used. The obtained compound (5, 6 – *O* – isopropylidene - 2, diacetylgluconamide) was treated with benzene sulphonyl chloride in presence of pyridine to close the ring and produce the azo-ascorbic acid.

Experimental
All chemicals used in this research were with high purity (Analar) and supplied by well known companies.
IR spectra were carried out by IR (philips) PU 9512 infra red spectroscopy.
UV spectra were carried out by UV (philips) PU 8720 UV/VIS scanning spectroscopy.
Melting point was measured by (microscope hot stage method) described by F.G. Mann and B.C. Saunders [8].

1. Preparation of 5,6-di-*O*-isopropylidene-L-ascorbic acid(1). [9]
Through a saturated solution of L-ascorbic acid (0.05 mole of L-ascorbic acid dissolved in 100ml. of freshly distilled acetone) a stream of hydrochloric acid gas (prepared by reaction of sodium chloride with sulfuric acid [10]) was passed with a continuous stirring under 28ºC for 30 minute. (80) ml. of n-hexane was added to the obtained mixture with stirring. The product was washed four times with a mixture of (acetone: hexane) in a ratio of (4: 6), dried under vacuum. (11.37 gm.) white crystals, of melting point (222-219ºC). The product was identified with UV and IR spectra as shown in tables(1,2).

2. Preparation of 5,6-*O*-isopropylidene-2,3-di-O-acetyl-L-ascorbic acid(2)
To a solution of (0.05 mole) of compound (1) dissolved in (50 ml.) pyridine, (50 ml.) of acetic anhydride was added drop wise, using a separating funnel, in a period of (2 hr.) with continuous stirring. The reaction mixture was cooled to 20º C and left for (24 hr.) at lab. temperature then dried under vacuum.

Oily orange product was obtained (11.20 gm.) with a percentage yield of 85%. It was identified with IR and UV spectra as shown in tables (1, 2).

3. Preparation of 5,6-isopropylidin- 2,3-di-*O*-acetyl gluconamide(3)[11]

To (0.04 mole) of compound (2), and by using separating funnel, (100 ml.) of ammonia in methanol (ammonia gas was prepared using the method described in [10]) was
added drop wise (30 drop/min.) with continuous stirring for (2 hr.) and by controlling the reaction mixture at (0-25ºC) and pH of (9.5-10).
The reaction vessel was closed tightly and left for (24 hr.) at lab. temperature. (11.020 gm.). Oily red product obtained with 82% percent yield, it was identified by UV and IR spectra as shown in tables (1, 2).

4. Synthesis of Aza-ascorbic acid [12]

To a solution of (0.03 mole) of product (3) dissolved in (50 ml.) pyridine, (50 ml.) of benzene sulphonyl chloride was added drop wise with continuous stirring and controlling the reaction temp. at (0-25ºC) using ice bath. The reaction mixture was kept for (24 hr.) at lab. temperature after the addition of (10 gm. sodium ethoxide). The contents of the vessel poured in ice-cold water with stirring for (3 hr.).
The organic layer was passed through chromatographic column with dimensions of 17 cm. length and 1.5 cm. diameter containing 10 cm. silica gel (230-400 mesh), followed by 5 cm. magnesium sulphate layer, the later was followed by 20 cm. charcoal decolorizing powder[13].
The rate of addition through the column was 25 drops/ min. Oily product was obtained, it was purified by preparative TLC thickness of (2mm.). Its melting point was (215 – 217ºC) and its molecular weight was 299. It was identified by UV and IR spectra. The results are listed in tables (1, 2).

5. Technique of UV spectroscopy

The specters of obtained compounds (1, 2, 3, and 4) were measured as highly diluted solutions (1mg. of compound diluted with 100 ml.), then one portion was used in a cell of 1cm. thickness. The absorbance (A) was plotted on Y- axis while the wave length was on the X-axis. The absorbance was converted to the molecular absorbance factor (E) [14].

Technique of Infra red spectroscopy

The infra red specters of the obtained compounds were measured in liquid phase using liquid films, while for solid state (KBr) disk was used [15].

Results and Discussion:

1. Ultra violet specters:

In fig. (1) the ascorbic acid gives one maximum peak at wave length of (243 nm.) and its E value was (556), while product (1), which was obtained after closing sites (5, 6) gives a maximum peak at (249), and E value of (508), as it is show in fig. (2).
Additionally, product (2), obtained after addition of acetic anhydride to product (1), gives two absorbance bands at (253, 258 nm.) and its E value was (7.3), as it is shown in fig. (3), while three absorbance bands were appeared concerning product (3) at (253, 258, 264 nm.) and E value was equal to (11) as it is clear from fig. (4).
Fig (5) shows the ultra violet specter of Aza-ascorbic acid [16], in which one absorbance band is appeared at (272 nm.) with a value of E equal to (22). Table (1) gives the values of wave lengths and E for the prepared compounds.

2.Infrared specters:

Table (2) gives the infrared spectral data for the prepared compounds (1,2,3,4). The absorption band regions and types of groups appeared in compound (4) are clearly comparable with those obtained in compounds (1, 2, 3). Figure (6) shows the IR specter of the synthesized aza-ascorbic acid, in which the appearance of C-N group stretching at the region of (1400 cm^{-1}) and C-N group bending at (1130 cm^{-1}) followed by absence of C-O-C group, is an evidence for the formation of the synthesized product [16

The reaction process:

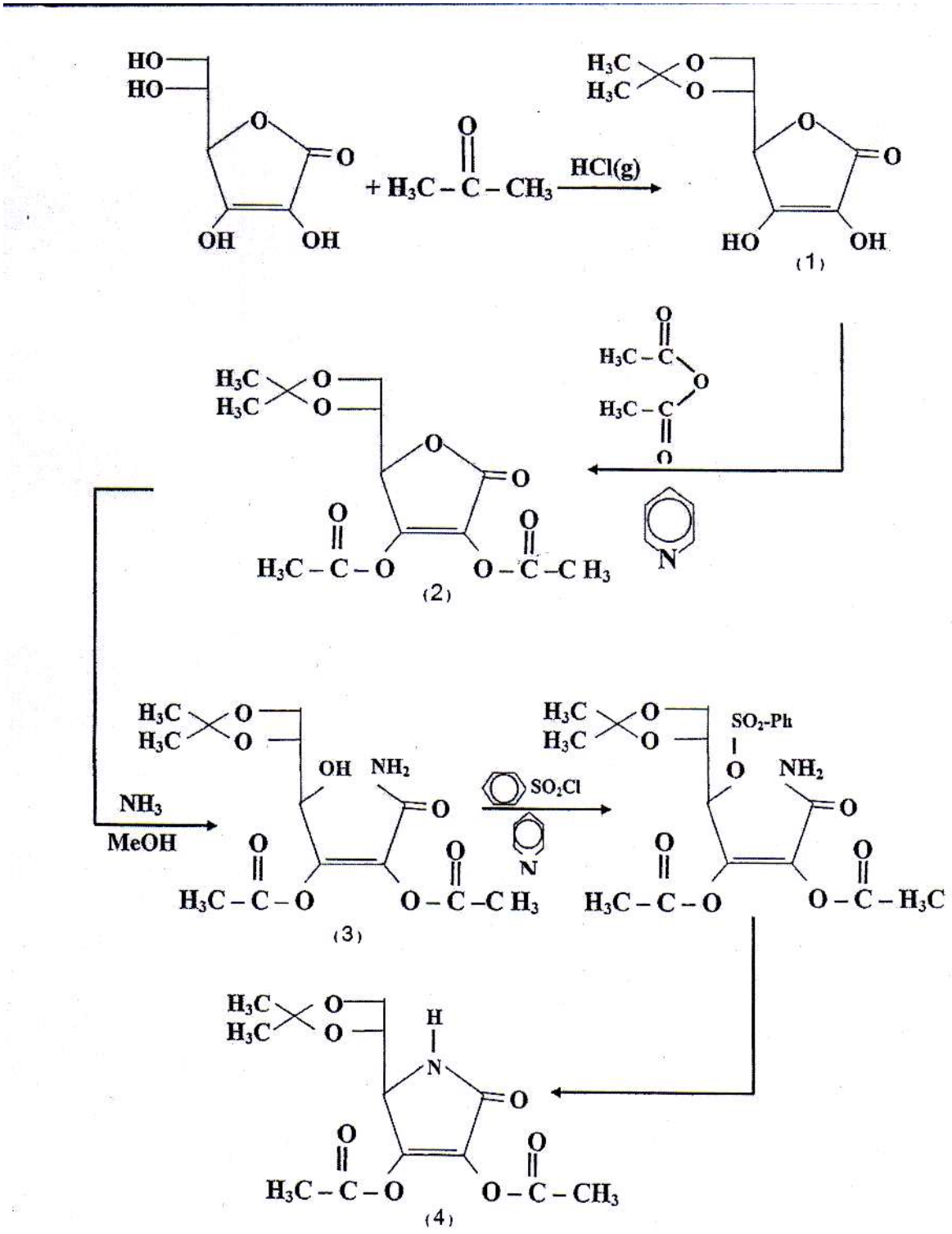


Table (1): Values of wave lengths and E for the prepared compounds

Compound	Wave length	E - Value
Ascorbic acid	2.43	556
Compound(1)	249	508
Compound (2)	253,258	7.3
Compound (3)	253,258,264	11
Compound (4)	272	22

Table (2) The spectral data of prepared compounds (1,2,3,4)

Compound	Group	*V(cm^{-1})*	Description
Ascorbic acid	0H	3100-3300	Stretching
	-C=O	1653	Strong band stretching
	C-H	1400-1450	Asymmetric deformation
Compound (1)	O-H	3350	Symmetric stretching
	C-H	2800	Aliphatic
	C=O	1750	Lactones stretching strong sharp band for molecule
	O=C-O-C	1000-1150	Stretching (saturated)
	C=C	1600	Stretching
Compound (2)	C=O	1700-1800	Stretching 2 bands, anhydride
	-C-O-C	1250	Stretching ring anhydride
	CH_3	1400	Asymmetric deformation
	C-O-C	1050-1130	Stretching Acyclic ring anhydride
Compound (3)	N-H	3350	Stretching
	O-H	3200-3400	Stretching
	CH_3	1400-1450	Asymmetric
Compound (4)	N-H	3600	Stretching
	N-H	1680	Bending
	C=C	1600	Stretching
	C=O	1700	Stretching
	C-N	1400	Stretching
	C-N	1130	Bending
	$(CH_2)_n$	700-800	Aliphatic

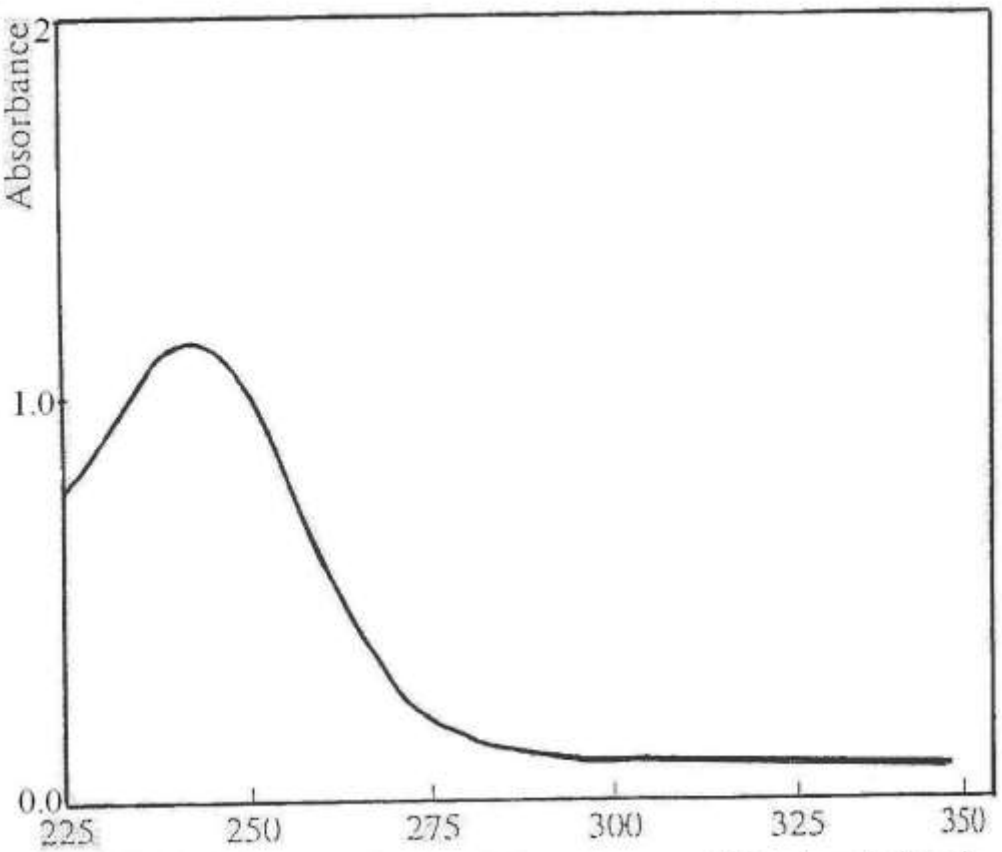


Fig.(1): UV Specter for Ascorbic acid

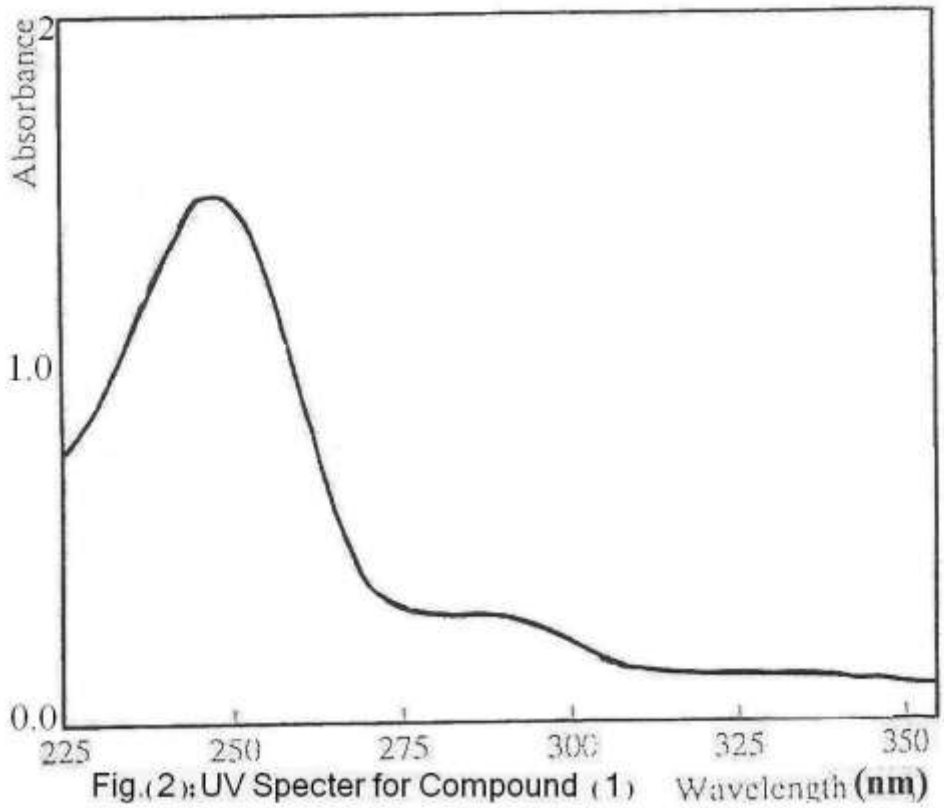


Fig.(2): UV Specter for Compound (1)

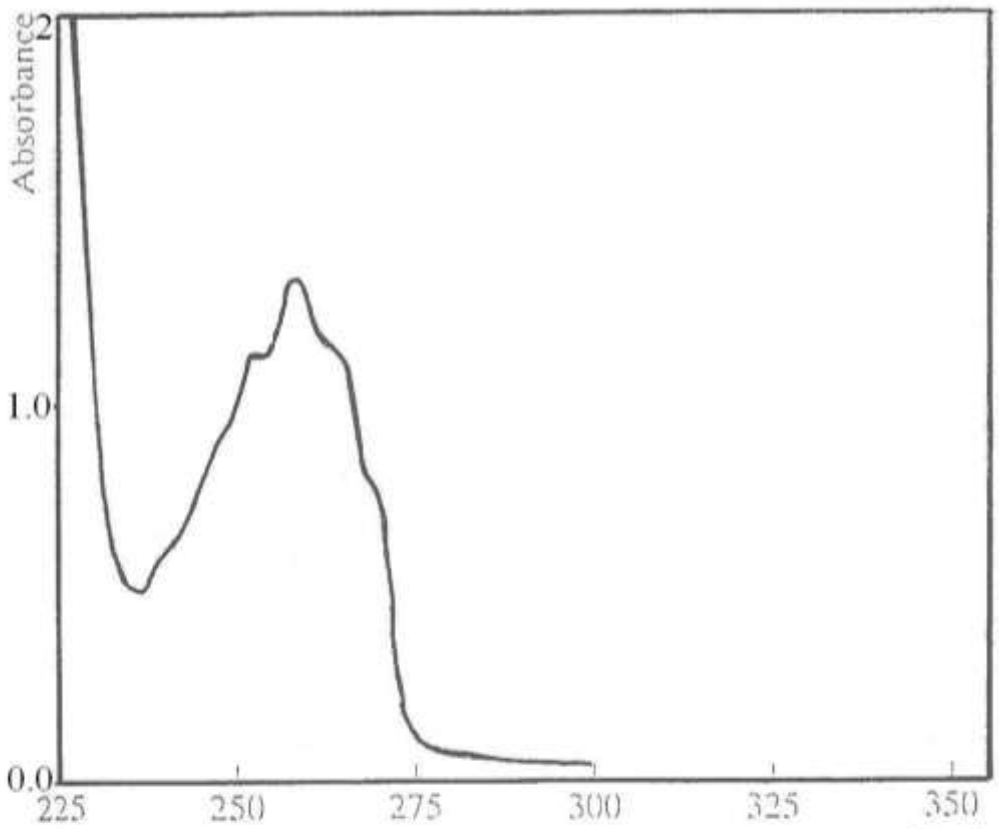


Fig.(3): UV Specter for Compound (2)

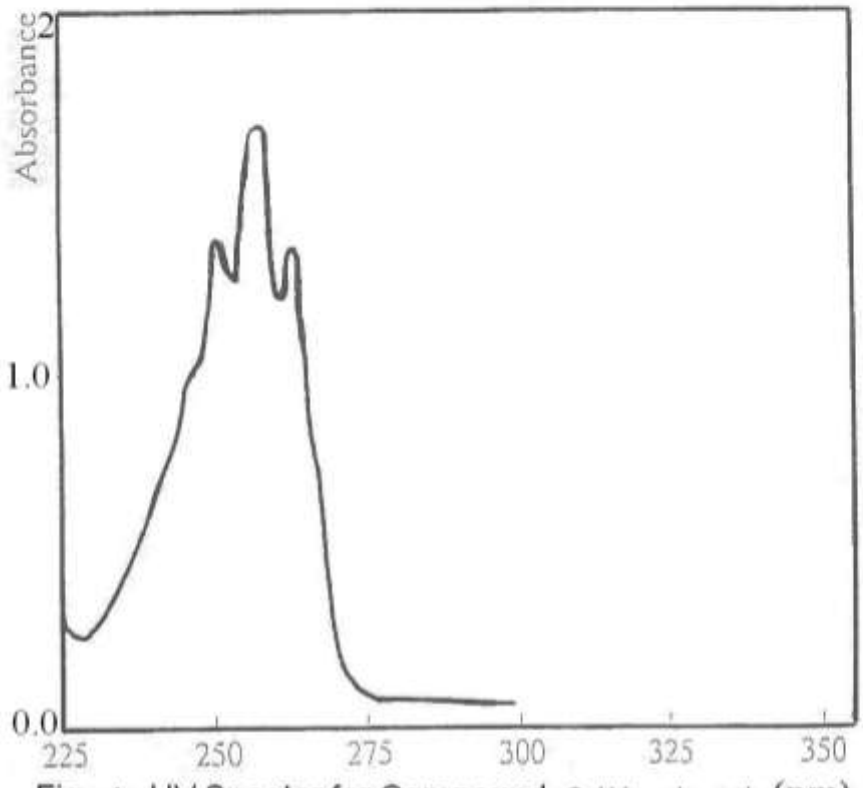


Fig.(4): UV Specter for Compound (3)

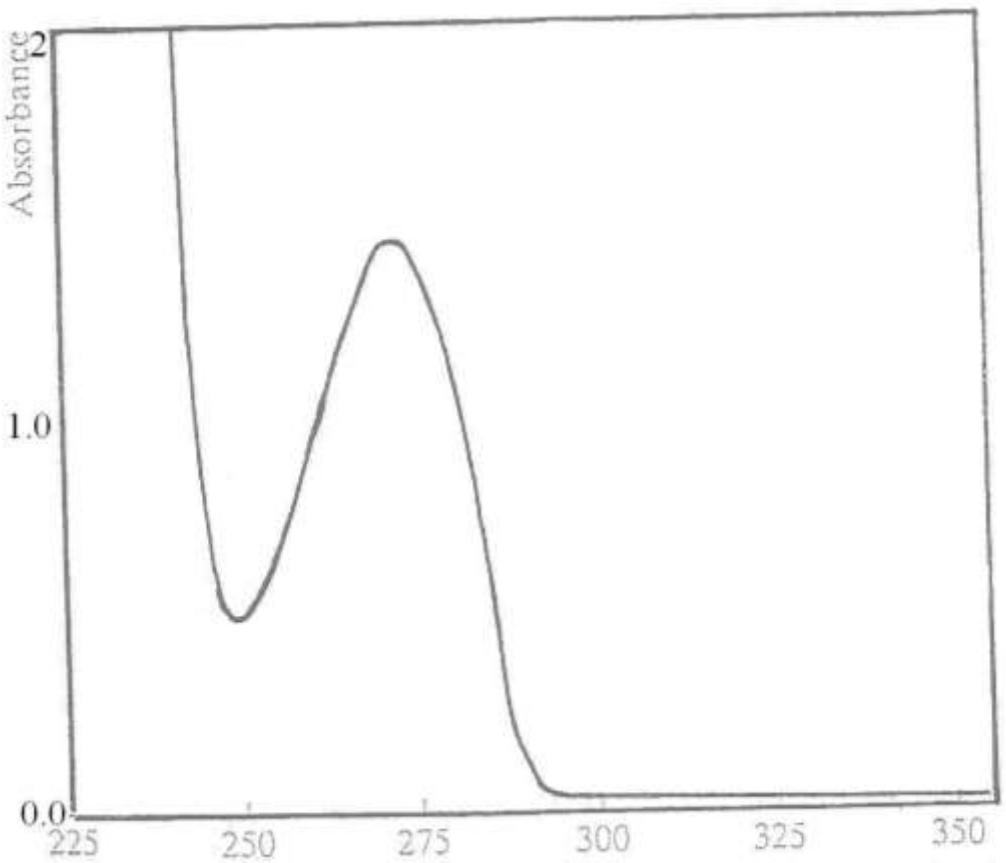
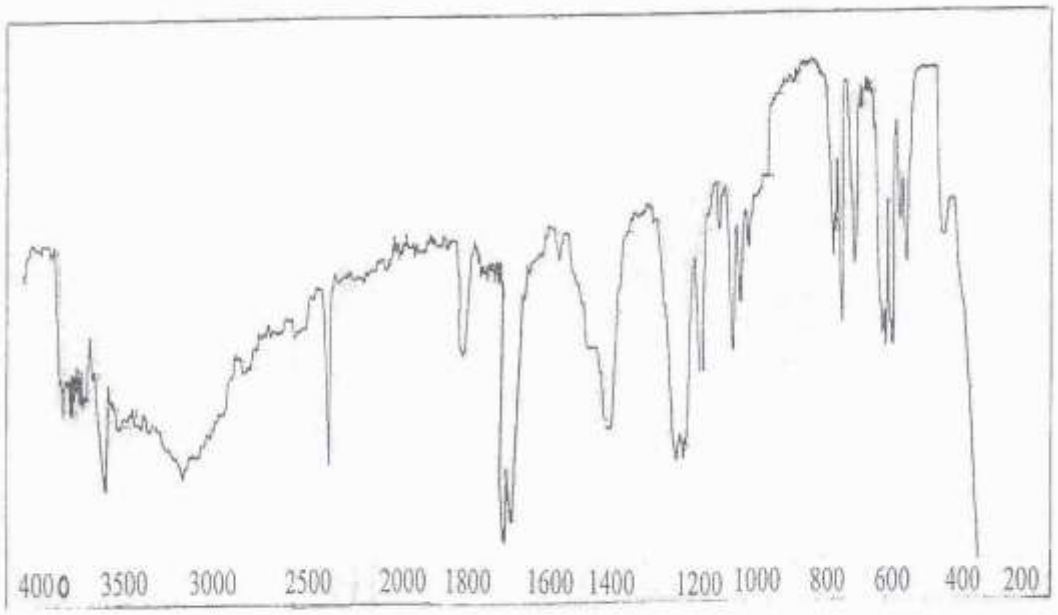


Fig.(5): UV Specter for Compound (4)



Fig(6): IR Specter for compound(4)

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طريقة محدثة في تخليق وتوصيف حامض الازا–اسكوربيك

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الملخص

يتضمن البحث تحضيرحامض الازا– اسكوربك من فيتامين سي وبمراحل متعدده . حيث تتضمن الطريقه غلق المواقع(٥و٦) بواسطة الاستايل الحلقي ثم تمت مفعلة الاخير مع حامض الخليك اللامئي بوجود البيريدين حيث تمت استلة المواقع(٢و٣) .

ان غلق المواقع(٥ و٦) سببه عدم السماح لهذه المجاميع من الدخول في تفاعلات جانبيه. كما تمت مفاعلة هذا المشتق مع(الامونيا – الكحول المثيلي) ليعطي المشتق الاميدي لحامض الاسكوربيك المعوض. ولغرض اجراء عملية الغلق تم تحويل مجموعة الهيدروكسيل رقم (٤) الى مجموعة بنزين سلفونيت كونها مجموعه مغادره جيده.

عند اجراء التفاعل بوجود نيوكليوفيل متعادل او مشحون كان قد اعطى الفرصه لمجموعة الامين في المشتق الأميدي لحامض الأسكوربيك بالهجوم على مجموعة البنزين سلفونيل في ذرة الكاربون(٤) لتعطيى حامض الازا– الاسكوربيك .

ان هذه الطريقه تعتبر بديلا عن الطريقه الكلاسيكيه التي تعتمد على اكسدة مجموعة الهيدروكسيل لذرة الكاربون(٤) وتحويلها الى مجموعة كيتون ومن ثم تحضير مشتق الأميد الحلقي وأختزاله بواسطة بوروهدريد الصوديوم. تم تشخيص المركبات الناتجه بتقنية طيف الأشعه تحت الحمراء وفوق البنفسجيه.