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Biochemical study of Ascorbic Acid derivatives as a pro_drug

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Abstract

In this research new derivatives of L-ascorbic acid (vitamin C) have been synthesized. These derivatives have been obtained by the esterification.
Selective esterification of C-3 and C-2 hydroxyl group required protecting the two hydroxyl groups at 5-6-position by conversion of vitamin C to 5-6-O-isopropylidene derivative (I).
3-(acetyl salicyloyl)-5-6-O- isopropylidene-L-ascorbic acid (III) was synthesized by treatment of (I) with acetyl salicyloyl chloride (II).
The purity of the compounds were characterized by thin layer chromatography (TLC) and infrared spectroscopy (IR).
The Kinetic study for compounds (1,3) was carried out using different buffer at pH (4,8) over 30 hours at different intervals of periods using UV spectroscopy which showed that these compounds were hydrophobic. The released aspirin was increased as the pH is increased and extended time.

Introduction

In 1958 Albert initially coined the term "pro_drug" and used it to refer to pharmacologically inactive compound that is transformed into an active substance by either chemical or metabolic means(I).
Design & synthesis of pro-drug have been used to solve various problems associated with the use of many drugs. There are several advantages that may be gained by generating a pro-drug, such as increase absorption, alteration of pain at the site of injection if the agent is given parentally, elimination of an unpleasant taste associated with the drug, decrease toxicity, decrease metabolic inactivation, increase chemical stability,
and a prolong or shorten action, which ever is desired in particular agent(2).
Some pro-drug relay upon to its full of life form , for example, hetacillin is a pro-drug form of ampicillin in which the amide nitrogen α-amino functionalities have been allowed to eract with acetone to given an imidazolidinone ring system (4,5).
This has the effect of decreasing the basicity of the amino group and reducing protonation in the small intestine, so that the agent would become more lipophilic. Vitamin C is well known as L-ascorbic acid because of its acidity character and its effectiveness in the treatment and prevention of scurvy. Ascorbic acid is derived from glucose via the glucuronic acid pathway.
The enzyme L-gluconolacton oxidase which is responsible for the conversion of gluconolacton to ascorbic acid which is absent in primates, making ascorbic acid required in the diet(6).

Experimental

Synthesis of 5,6-*O*-isopropylidene-L-ascorbic acid(I).

A Saturated solution of L-ascorbic acid (10 g)in 100 ml of freshly distilled acetone, (HCl) gas was bubbled at room temp. for 20 minutes, to this solution n-hexane (80 ml) was added , stirred, and decanted. The residue was washed with acetone-hexane (4:7) for times, then the solvent was removed under reduced pressure to give compound (I).

Synthesis of *O*-acetyl salicyloyl chloride(II) [7]

To a dry power acetyl salicylic acid (19.7mmole) in claisen flask was added redistilled thionyl chloride (1.74 ml) & the mixture was refluxed for 6 hours or until evolution of hydrogen chloride ceases. The reaction mixture was left to cool, the Condenser was removed & the flask was heated at 60 $C^0$for 3 minutes with occasional shaking.

Excess thionyl chloride was removed under reduced pressure to give compound (II).

Synthesis of 3-(acytyl salicyloyl)-5,6-*O*-isopropylidene L-ascorbic acid(III)

To an ice-cooled solution (-12C°) of 5-6-O-isopropelidene L-ascorbic acid(I) (4.12 g)in pyridine, was added, drop wise, acetyl salicyloyl chloride(II)(4.37g). The resulting reaction mixture was kept at room temp. for 24 hours, and then the cold distilled water (300 ml) & chloroform (400 ml) were added. The organic layer was separated, dried over anhydrous ($MgSO_4$), filtered& the solvent was removed under reduced pressure (trace of pyridine was removed under reduced pressure by co evaporation with toluene).The remaining syrup was purified on a silica gel column to give compound(III).

Identification of new derivatives by Thin layer chromatography (TLC)

The identify compounds(I,II,&III)were determined by TLC using aluminum plates coated with (0.25 mm)layer of silica gel F_{254} as stationary phase, while the mobile phase is (benzene: ether)(8:2), compounds were detected by iodine vapor.

All synthesized compound were purified on silica-gel column.

Drug released study

The drug released study of aspirin released from the compounds, 150 mg for each compound was comprised at high pressure to form disc which is placed with stirring in 25 ml of buffer solution and the concentration of aspirin released from the hydrolysis was followed in the supernatant (3 ml) by recorder the UV absorbance at 277 nm for period 0-30 hrs.

Solutions: Buffer solution:

A-The buffer solution (phosphate buffer),(pH=4&8) was prepared by dissolving(1.58 g& 2.37 g) from (Na_2HPO_4) in 100 ml distilled water (some drop of H_3PO_4 were added to fixed the pH).

B-The buffer solution (pH=8&10) by was prepared by dissolved (5.04 g&6.3g) from Na_2CO_3 & (2.69 g &3.36 g) of ($NaHCO_3$) in (1L) distilled water (8).

Results and Discussion

The IR spectrum of compound (I) show stretching band at 3240 cm^{-1} for (O-H) , 2910 cm^{-1} for (C-H aliphatic) (acetyl linkage), 1755 $cm^{-1}$for (C=O) lactone.

The IR spectrum for acetyl salicyloyl chloride (II) showed the stretching bands at 1780-1800 cm^{-1} for (C=O) carboxylic acid chloride and 883 cm^{-1} for (C-Cl), and disappearance of the stretching bands for (O-H) carboxylic acid.

Finally, The IR spectrum for compound (III) showed stretching bands at 2950 (C-H) aliphatic, 1753 cm^{-1} (C=O) ester, 758(C-H) aromatic (9).

The qualitative determination for compound 1,2,and3 was carried out using silica gel as stationary phase forTLC, while the mobile phase consist of a mixture of hydrocarbon carriers(Toluene, chloroform dioxane or benzene) &polar organic modifiers(acetone, butanol, ethanol or acetic acid).

The advantages in screening the sample by TLC prior to HPLC are determination the contaminants that may absorb to the stationary phase in HPLC column & determination of solvent conditions necessary for a successful separation of compounds(1,2,and 3).

Table (1): The physical properties for derivatives compound.

Compound number	Formula	Physical state	Rf.
1	$C_9H_8O_6$	White crystals	0.02
2	$C_9H_7O_3cl$	White crystals	0.122
3	*$C_{18}H_{18}O_9$*	*Brown crystals*	*0.056*

Pro-drug agent that contain carboxylic acid or alcohol functionalities can often be prepared by conversion to an ester.

This is the most commonly seen type of pro-drug, due to the ease with which the ester can be hydrolyzed to give the active drug.

Decreasing the water solubility of a drug by the formation of a prodrug may have additional benefits beyond simply increasing absorption.This result when the drug begins to dissolve in the mouth and then is capable of interacting with taste receptors (10).

In this research the concentration of aspirin at different intervals of time and various pH values was followed at fixed wave length at 277 nm, it was found result indicated the released concentration correlated positive with the increasing of pH value. The gastric irritation will be decrease by the condensation of aspirin with vitamin C because the ester linkage will expected to be cleaved in the colon librating aspirin vitamin C (11).

Table (2): Concentration of aspirin released by compound II in pH= 4.

Time (hr.)	Concentration of aspirin released (M)	% released
0	0	0
0	0	0
0.083	0	0
0.167	1.69×10^{-5}	0.152
0.25	2.53×10^{-5}	0.22
0.34	3.38×10^{-5}	0.304
0.5	4.22×10^{-5}	0.38
1	7.62×10^{-5}	0.68
1.25	1.59×10^{-5}	1.43
1.5	2.22×10^{-4}	2
2	3.56×10^{-4}	3.2
2.75	3.64×10^{-4}	3.27
3	4.43×10^{-4}	3.99
4	5.56×10^{-4}	4.17
6	3.42×10^{-3}	5.91
8	3.67×10^{-3}	2.08
24	3.98×10^{-3}	33.09
26	4.09×10^{-3}	35.91
28	4.16×10^{-3}	36.88
29	4.53×10^{-3}	37.5
30	4.98×10^{-3}	38.32

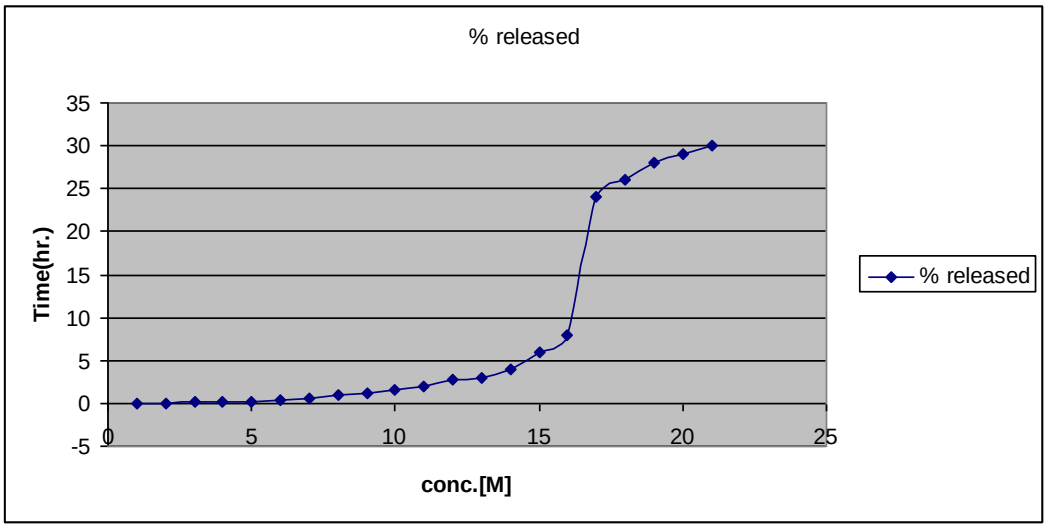

Figure (1): The percent of aspirin released by compound II in pH= 4.

Table (3): Concentration of aspirin released by compound II in pH= 8.

Time (hr.)	Concentration of aspirin released (M)	% released
0	0	0
0.083	3.38×10^{-5}	0.304
0.167	8.47×10^{-5}	0.76
0.25	9.32×10^{-5}	0.86
0.34	1.01×10^{-4}	0.91
0.5	1.1×10^{-4}	0.99
1	1.99×10^{-4}	1.79
1.25	4.38×10^{-4}	3.94
1.5	4.63×10^{-4}	4.17
2	2.15×10^{-3}	19.43
2.75	2.36×10^{-3}	21.3
3	2.66×10^{-3}	24
4	3.29×10^{-3}	29.6
6	3.42×10^{-3}	30.8
8	3.93×10^{-3}	35.4
24	5.19×10^{-3}	46.8
26	5.25×10^{-3}	47.3
28	5.32×10^{-3}	48
29	5.48×10^{-3}	49.4
30	5.56×10^{-3}	50.1

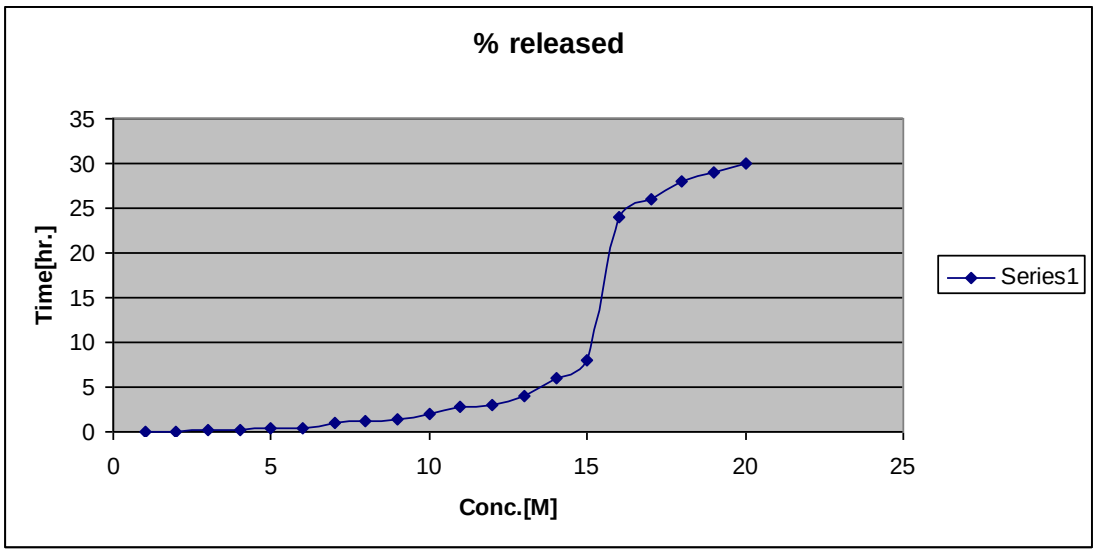

Figure (2): The percent of aspirin released by compound II in pH= 8.

Table (4): Concentration of aspirin released by compound III in pH= 4.

Time (hr.)	Concentration of aspirin released (M)	% released
0	0	0
0.083	0	0
0.167	3.38×10^{-6}	0.053
0.25	1.69×10^{-5}.	0.1
0.34	2.53×10^{-5}	0.16
0.5	3.38×10^{-5}	0.213
1	7.12×10^{-5}	0.45
1.25	8.47×10^{-5}	0.53
1.5	1.59×10^{-4}	1
2	2.11×10^{-4}	1.3
2.75	2.42×10^{-4}	1.52
3	3.8×10^{-4}	2.06
4	2.2×10^{-3}	2.4
6	2.61×10^{-3}	13.9
8	2.81×10^{-3}	16.4
24	2.88×10^{-3}	17.7
26	3.03×10^{-3}	18.2
28	4.32×10^{-3}	19.1
29	4.47×10^{-3}	20.4
30	5.21×10^{-3}	22.14

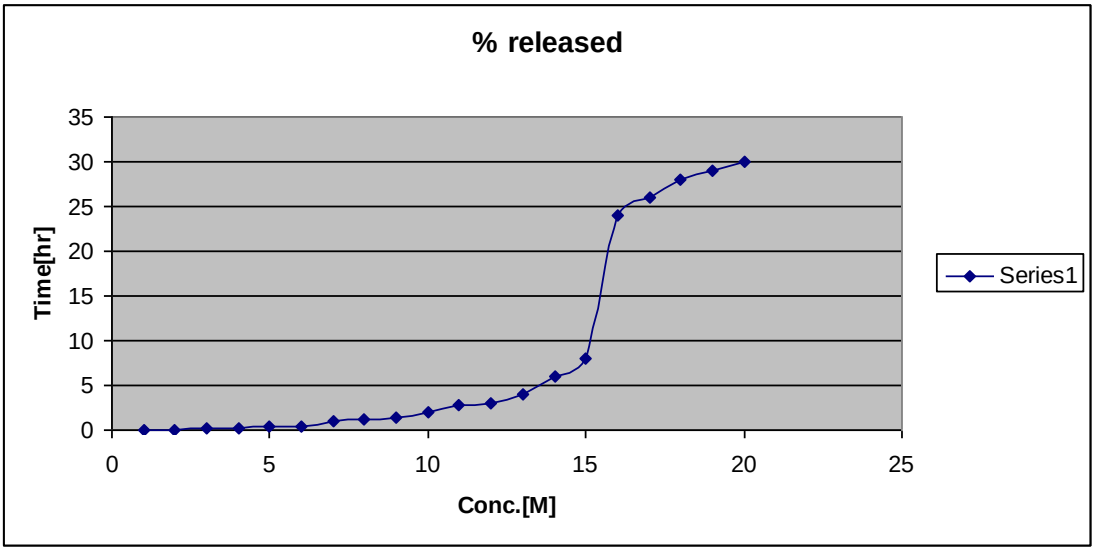

Figure (3): The percent of aspirin released by compound III in pH= 4.

Table (5): Concentration of aspirin released by compound III in pH= 8.

Time (hr.)	Concentration of aspirin released (M)	% released
0	0	0
0.083	1.69×10^{-5}	0.1
0.167	2.56×10^{-5}	0.26
0.25	3.34×10^{-5}	0.32
0.34	4.22×10^{-5}	0.42
0.5	5.07×10^{-5}	0.46
1	7.13×10^{-5}	0.58
1.25	8.77×10^{-5}	2
1.5	3.17×10^{-4}	2.4
2	3.94×10^{-4}	2.7
2.75	4.4×10^{-4}	3
3	4.8×10^{-4}	3.4
4	5.45×10^{-4}	4.51
6	7.16×10^{-4}	6.4
8	1.015×10^{-3}	12.4
24	1.98×10^{-3}	41.6
26	6.6×10^{-3}	41.8
28	6.63×10^{-3}	42.5
29	6.67×10^{-3}	45.1
30	7.24×10^{-3}	45.6

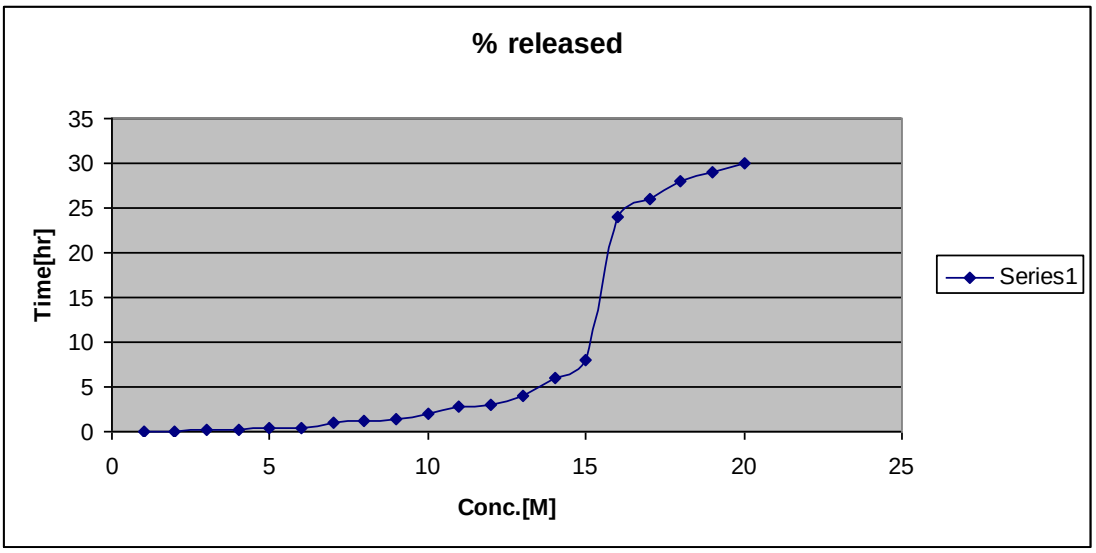

Figure (3): The percent of aspirin released by compound III in pH = 8.

Wilson & Gisvold (1998) refers to the hydrolytic release of the bioactive agent (carboxymethyl cellulose-2,2- dichloropropionates)(CMC ester) is mainly dependent on the hydrophilicity of the CMC ester. In the case of containing enzymatic cleavable the release can be accelerated by addition of esterase. The release of 2,2-dichloropropanic acid from CMC ester at 30 C^0 & pH_7 without

addition of esterase was 8% after 100 hours while the release of 2,2-dichloroproponic acid from CMC ester at $30C^0$ & pH_7 with addition of esterase was 50% after 100 hours.

Conclusion

Drug released study, hydrolysis of derivatives increase when pH increase &the gastric irritation will be decrease by the condensation of aspirin with vitamin C because the ester linkage will expected to be cleaved in the colon librating aspirin & vitamin C.

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دراسة بايو كيميائية لبعض مشتقات حامض الاسكوربيك كدواء مصاحب

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الخلاصة

تم في هذا البحث تخليق عدد من المشتقات الجديدة لحامض الاسكوربيك(فيتامين C)عن طريق تفاعل الاسترة.تطلي دخول الموقع(3)تحوير الموقعين(6و5)الى المشتق الايزوبروبلدين(I). وتم الحصول على الحامض 3-احادي اسيتايل سالسيلوليل-6,5-O-ايزوبروبلدين-L-اسكوربيك(III)من تفاعل مشتق الايزوبروبلدين(I)مع كلوريد الاسيتايل سالسيلويل(II).

تم تشخيص المركبات بكروموتكرافيا الطبقة الرقيقة(TLC)وطيف الاشعة تحت الحمراء(IR). اجريت دراسة حركية باستخدام جهاز الاشعةفوق البنفسجية(UV) وباستخدام محاليل منظمة مختلفة باس هيدروجيني مختلف(PH)(4,8)و خلال فترة30 ساعة وجد من خلال هذة الدراسةان المركبات المحضرة تكون كارهة للماء.

لوحظ ان تفكك المركب الى الاسبرين وفيتامينC يزداد بزيادة قاعدية المحلول المنظم وزيادة الوقت.