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Synthesis and characterization of new 2-Substituted Tetracycline derivative using β-D-fructopyranose

تحضير وتشخيص مشتق جديد للتتراسايكلين في الموقع 2 باستعمال الفركتوبايرانوس

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Abstract:

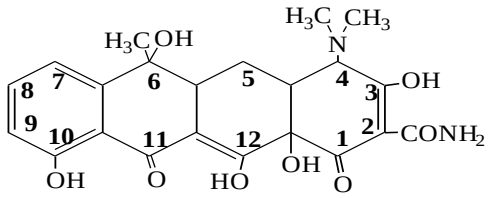
derivative of tetracycline at position 2 has been synthesized via way involved treatment of 1-chloro diacetone fructose with tetracycline to give derivative (IV), then the protected groups were removed using diluted acid. The Structures of the prepared compounds were elucidated on the basis of UV, FTIR and ^{1}H-NMR spectral data which agreed with the proposed structures. The solubility studies proved that the new derivative of tetracycline be more soluble in polar solvent compared than free drug.

الخلاصة

تم في هذا البحث تحضير مشتق جديد للتتراسايكلين في الموقع 2 عن طريق مفاعله التتراسايكلين مع 1-كلورو ثنائي اسيتون فركتوز للحصول على المشتق *N* –تتراسايكلين ثنائي اسيتون فركتوز (IV)، ثم اجراء رفع حماية لمجاميع الهيدروكسيل للحصول على المشتق (V) باستعمال حامض مخفف . تم تشخيص المركبات المحضرة باستخدام كروموتوغرافيا الطبقة الرقيقة ، مطيافية الأشعة الفوق البنفسجية ، الأشعة تحت الحمراء و الرنين النووي المغناطيسي وكانت جميع النتائج مطابقة لما هو متوقع. دراسة الذوبانية للمشتق (V) بينت زيادة الذوبانية لة مقارنة بالتتراسايكلين . الفعالية البايلوجية للمشتق المحضر يعتقد ان يكون اكبر مقارن بالتتراسايكلين والنتائج قيد الانجاز.

Introduction:

Tetracycline (I) were first discovered in 1948 by Benjamin Duggar[1] as natural product produced by species of Streptomycin, and have since proven to be an economically valuable drug class over the past 6 decades.



(I)

The highly modification chemical scaffold of the tetracycline (fig. 1) afford them versatility and allows them to interact with a variety of biological targets.[2] Tetracycline antibiotics are effective against a broad spectrum of microorganisms including Gram-positive bacteria, Gram-negative bacteria as well as eukaryotic protozoan parasites.[3-5]

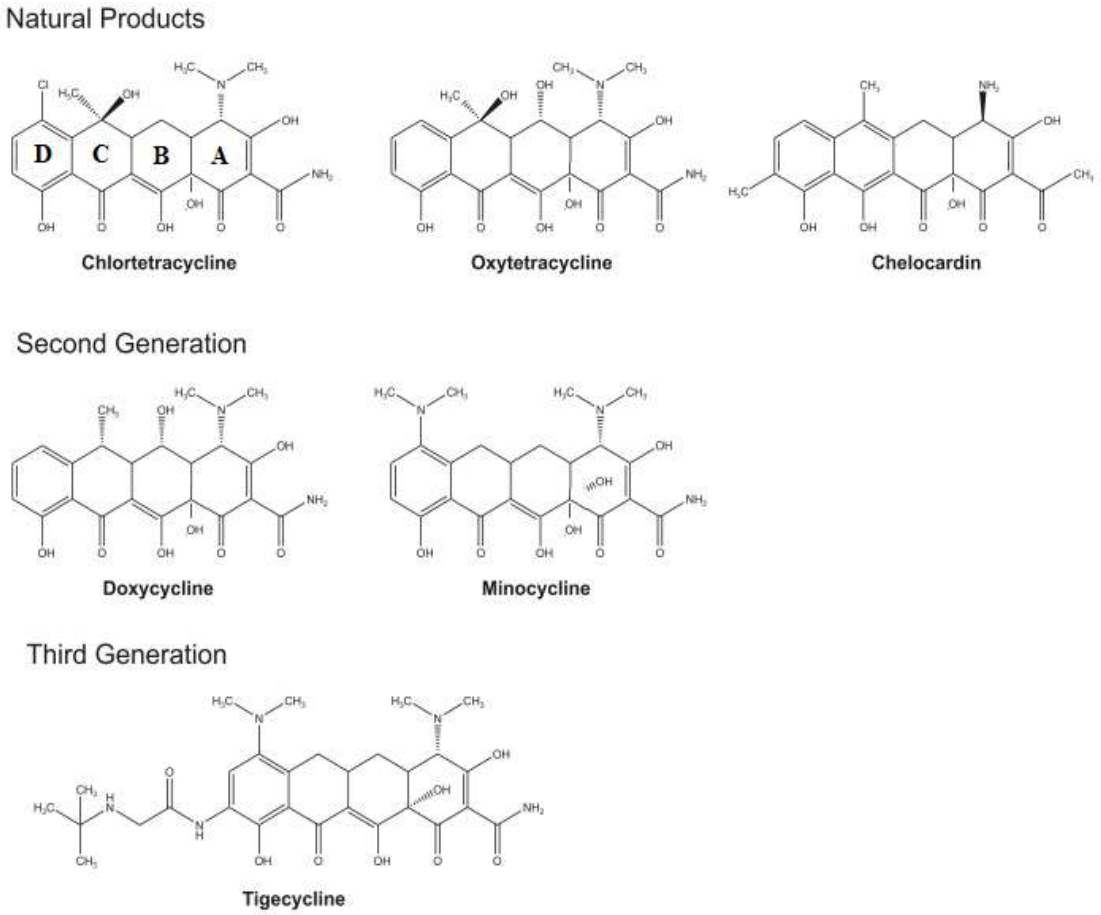


Fig. 1. Selected members of the tetracycline family of antibiotics.

The tetracycline polyketide [6] carbon skeleton is comprised by four linearly fused carbocyclic rings (A–D) adorned with up to six contiguous stereocenters and a congested array of acid- and base-sensitive functionality. [4] The western D-ring is phenolic and electron-rich. The A-ring exhibits polar functionality including a dimethylamino group and a key pharmacophoric [7] □-keto carboxamide (vinylogous carbamic acid).

In subsequent years, lengthy programs directed at the chemical modification of tetracycline at position 9,8 yielded few medically useful derivatives[8-14]. As a result, numerous chemical modification of tetracycline have been reported at an amide group in position- 2 to prevent the bacterial resistance. Many research found that the interning of (-CH_2-NR_2) group (N-amino methelation) through the amino group, will give tetracycline derivative which has good medical property also found that substitution of bulky groups for one of the hydrogen's on amid nitrogen not cause any appreciable loss in the activity.[15] In fact substitution of a pyrolidino methyl group increases the water solubility of tetracycline about 2500 times without change in activity by condensing of tetracycline with a pyrolidine and formaldehyde in the presence of t-butyl alcohol, this derivative is very soluble in water and provides a mean of injection the antibiotic in a small volume of solution.[16] In the last few years, carbohydrate mimetic have become an emerging area in drug design. Several application of carbohydrate mimetic are currently on clinical trial to increase the activity, bioavailability and selectivity of drugs[17,18]. This study deal with the synthesis of a new tetracycline derivatives (scheme 1) by replacement of one hydrogen of 2-carboxamide nitrogen. The new tetracycline derivatives containing fructosyl moiety which were predicated to have useful biological activities and more water solubility than tetracycline.

Experimental

For anhydrous reaction, glassware was dried overnight in an oven at 120^0C and cooled in a desiccators over anhydrous $CaSO_4$ or silica gel. Reagents were purchased from Fluka (Switzerland) or sigma (Louis. USA). Melting points were obtained with Buch, 510 melting point apparatus. Infrared (FTIR) spectra were recorded on Beckman I.R 8 spectrophotometer . Tetracycline anhydrous was supplied from Samarra drug industries. The purity of this compound is checked according to m.p and Meric index. U.V spectra were carried out using an Hp 8452A diod array spectrophotometer. ^{1}H-NMR spectra carried out with Bucker WM-400 (400 MHZ FTNMR) spectrophotometer using TMS as internal reference (chemical shift in ppm). Purity of the prepared compounds was checked by TLC on silica gel plates and spot were visualized by exposure to iodine vapors. The physical data of the prepared compounds are presented in Table (1) and the spectroscopic data are presented in Table (2).

General methods for the compounds synthesis

***Syntheses of 2,3:4,5-di-O-isopropylidine-β-D-fructopyranos* (II).** Compound (II) was prepared according to previously published procedure.[19]

***Syntheses of* 1-*chloro-2,3:4,5-di-O-isopropylidine-β-D-fructopyranose* (III)**

Compound (II) was refluxed with anhydrous carbon tetrachloride (CCl_4) in the presence of triphnyl phosphen at 70^0C for 90 h., triphnyl phosphen oxide was separate from the mixture after all 10 hr. the cooled solution was filtered through kieselguhr evaporation under reduce pressure and purification of the residue by use of column chromatography (EtOAc). FTIR (KBr cm^{-1}): (2983), (C-H str), (1250-1050 C-O-C str),(650 C-Cl):H-NMR (δ 1.55ppm,H aliph.),UV:(λ_{max}) 249 .The ^{1}H-NMR spectra is shown in fig.1

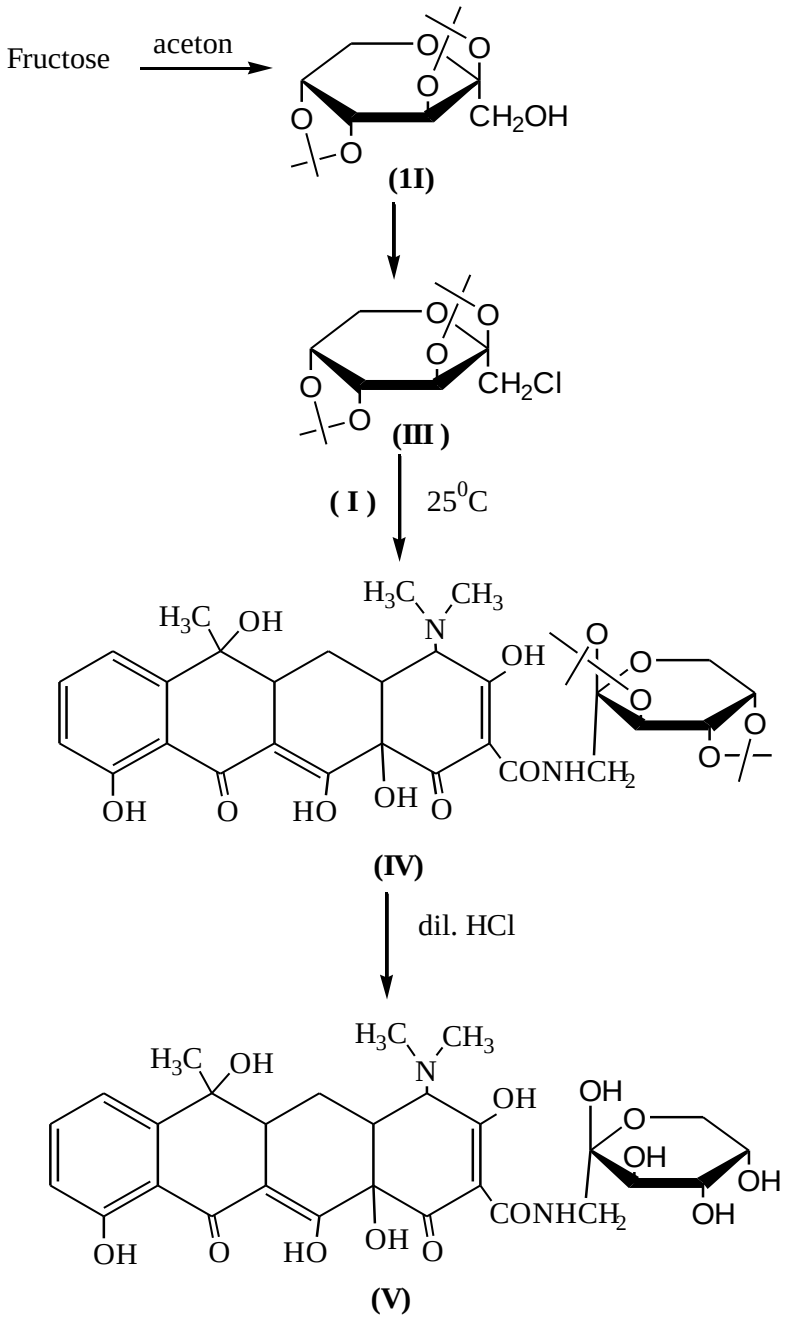
Synthesis of *N-(*diacetone fructose) tetracycline (IV) To (2.5 mmol, 1.1 gm) of tetracycline in (50 ml) of CH_3CN and (2.5 ml) of Et_3N was added (2.5 mmol 0.7 gm) of 1-chloro 2,3:4,5 di-O-isopropylidine-β-D-fructopyranose, the reaction mixture was stirred at 25^0C for 10 hr., the solution was concentrated under reduced pressure, purification of the residue by using column chromatography (EtOAC/ CH_3OH 8.5:1.5) afford (IV) as a yellow sime solid in 66% yield, R_f(0.6).

Synthesis of *N-(* fructose) tetracycline (V)

Compound (IV)(0.001 mol) was dissolved in (10 ml) chloroform, then added 0.5 N of HCl, with stirring for 10 hr. at 25^0C , 50% of $KHCO_3$ was added, the reaction mixcture was poured on the colled water (50 ml) and extructed with chloroform, washed with water dried over $MgSO_4$.

Results and discussion

In this work new convenient method and spectroscopic studies of N-tetracycline derivative via β-D-fructose was prepared. In the first step, carboxamide of tetracycline was reacted with 1- chloro-2,3: 4,5- di- *O* -isopropylidine -β-D-fructopyranose (III) to form Synthesis of *N-(*diacetone fructose) tetracycline (IV) then stirring with 0.5 N of HCl to removed the protected groups to give compound (V) (Scheme 1).



Scheme: 1 Syntheses of tetracycline derivative (V)

FTIR studies.- the FTIR spectra of compound (III) showed disappearance of ν(O-H) absorption bands and showed absorption bands at 650 cm^{-1} , also compound (IV) showed disappearance of ν(N-H) absorption bands of carboxamide in tetracycline (I) and appearance bands at 3320cm^{-1} and 1250cm^{-1}-1050cm^{-1} of ν(N-H) amide and (C-O-C) of acetyl, also compound (V) showed absorption a proud bands 3340 cm^{-1} of ν(O-H) of three carboxyl groups, this is good indicated for complete the reaction as seen in fig.(2-4).

UV–Vis studies.- UV–Vis spectra of the compounds N-tetracycline derivative (IV) showed absorption bands at 244 nm and 354 nm, all these absorption due to π→π* and n→π* transition as seen in table (2)

H-NMR studies.- ^{1}H-NMR spectra of compound (IV) showed the appearance of signals at δ=(3.1) for (CH) aliphatic protons of tow acetyl group and signals at δ=(7.1- 8.2) ppm to protons of one aromatic ring.

solubility studies.- the solubility study of the tetracycline derivative (V) was determined following Gude et al.[20] using deferent types of solvent improve increase solubility than free tetracycline. biological activity studies.- *biological activity* of the compounds expected to be more than tetracycline and it was still to be don.

Table (1): FTIR spectral data of compounds [III-V]

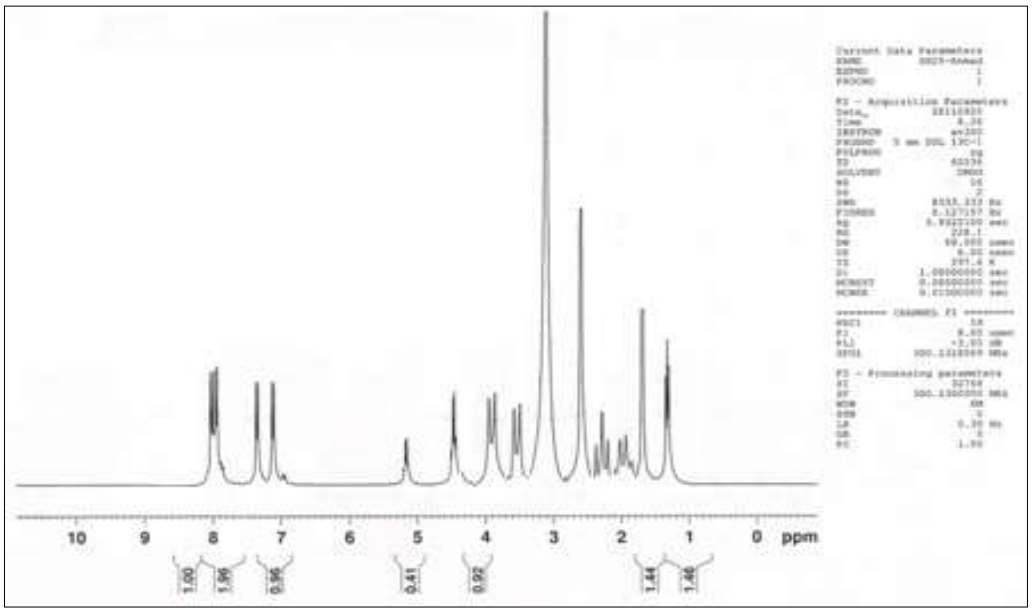
Comp. No.	FTIR spectral data cm^{-1}			^{1}H-NMR spectral data ppm		
	ν(C-Cl)	ν(C-O) Acetal	ν(C-H)	δ(H) Alip.	δ(H) Halide	δ(H) Arom.
III	650	1050-1250	2983	-	1.55	-
IV	ν(C-H) Aromatic	ν(C-O) Acetal	ν(N-H) Amide	δ(H) Aromatic	δ(H) Aliphatic	δ(H) Acetal
	3011	1050-1250	3264	7-8.1	1-2.3	3.1
V	ν(C-H) Aromatic	ν(O-H) alcoho.	ν(C=O) Amide			
	3055	3373	1778	-	-	-

Table (2): Physical properties of prepared compounds [II-IV]

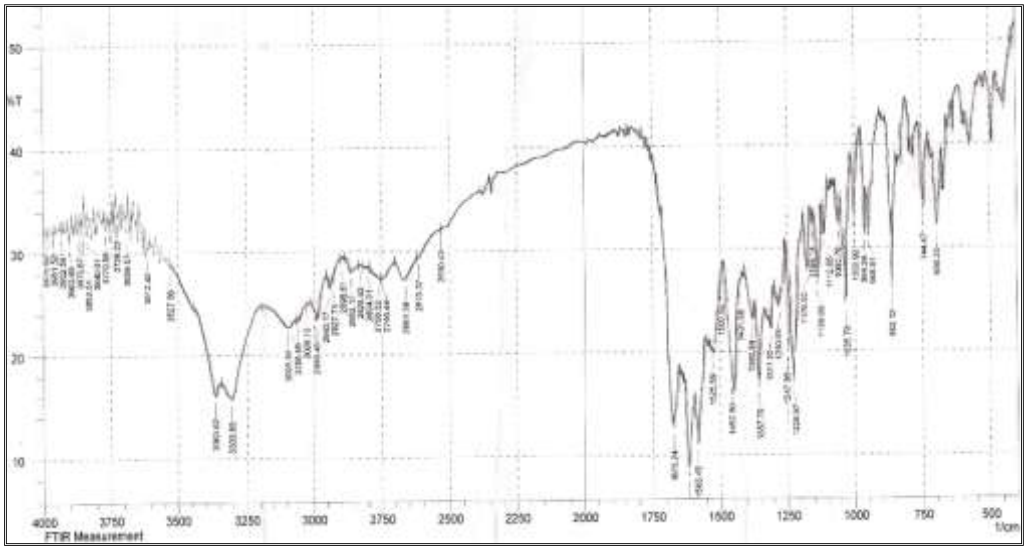
Com. No.	Molecular formula	Color	m. p °C	Yield %	Solvent of Recrystall.	λ_{max} Nm
III	$C_{12}H_{19}O_5Cl$	Yellow	140	82	-	249
IV	$C_{38}H_{42}O_{13}N_2$	Brown	sime solid	66	ethanol	244, 354
V	$C_{28}H_{34}O_{13}N_2$	Yellow	syrup	50	ethanol	234, 342

Table(3):The solubility study of derivatives compound

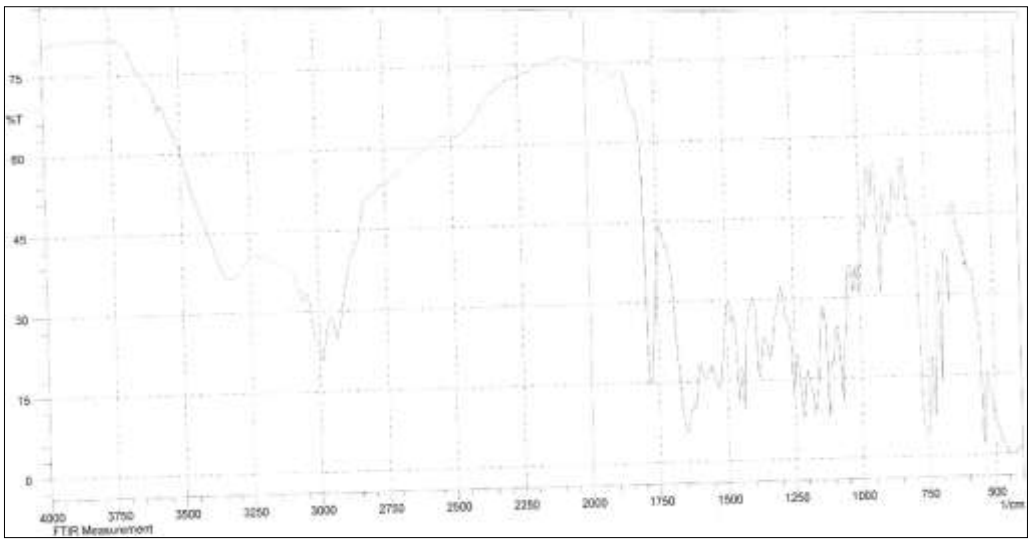
Com. No.	water	Ethanol	Methanol	CCl_4	hexane	DMF	DMSO
Tetra.	-	-	-	-	-	+	+
IV	+	+	+	-	-	+	+
V	++	+++	++	-	-	+	+



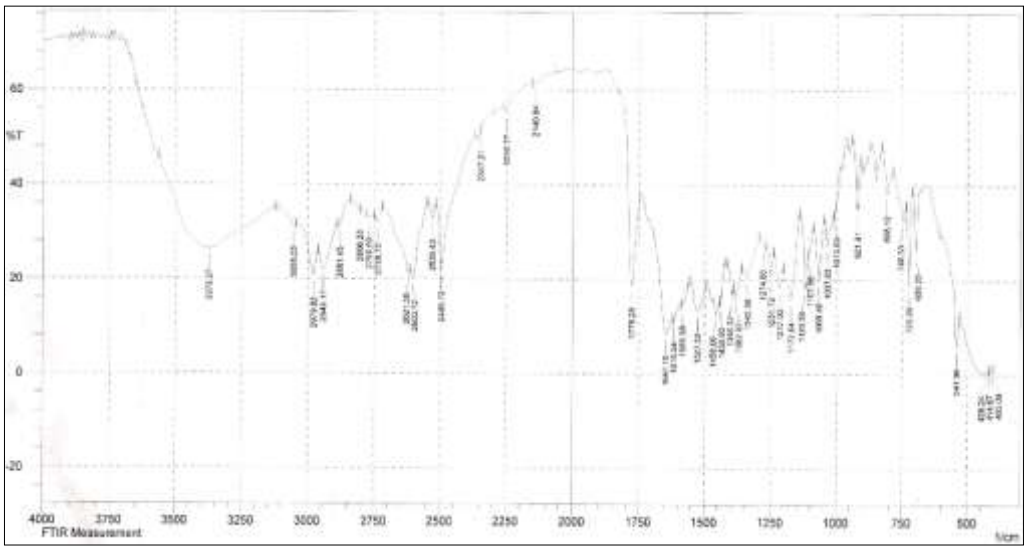
Fig(1): ^{1}H-NMR spectrum of compound (IV)



Fig(2): FTIR spectrum of compound tetracycline



Fig(3): FTIR spectrum of compound tetracycline(IV)



Fig(4): FTIR spectrum of tetracycline derivative (V)

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