Journal of Kerbala University , Vol. 13 No.2 Scientific . 2015

Synthesis and the solubility study of new 2-substituted tetracycline derivatives using phthalic anhydride

تحضير معوضات جديدة للتتراسايكلين في الموقع 2 باستخدام حامض الفثالك اللامائي ودراسة الذوبانية لها

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Abstract:

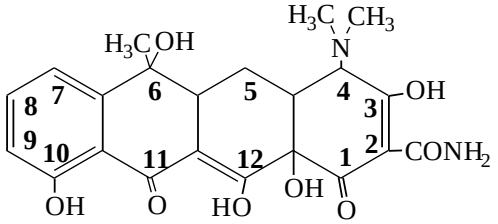
Two derivatives of tetracycline at position 2 have been synthesized via different ways. The first derivative involved reaction of phthalic anhydride with tetracycline at cooled condition to producing N-tetracycline phthalamic acid (II). The second derivative involved reaction of phthalic anhydride with tetracycline at hot condition to producing N-tetracycline phthalimide (III). Structures of the prepared compounds were elucidated on the basis of UV, FTIR and ^{1}HNMR spectral data which agreed with the proposed structures. The solubility of the new derivative increase in polar solvent corresponding to tetracycline. The biological activity of the compounds suppose to be more than tetracycline and it was still to be doun .

الخلاصة

تم تحضير مشتقين لمركب التتراسايكلين في الموقع 2 وبطريقتين مختلفتين ، المشتق الاول التفاعل تضمن مفاعلة التتراسايكلين مع الفثالك اللامائي بأستخدام التبريد للحصول على حامض N– تتراسايكلين فثال اميك (II) والمشتق الثاني يتضمن تفاعل التتراسايكلين مع الفثالك اللامائي باستخدام الحرارة للحصول على N – تتراسايكلين فثال ايمايد (III). تم تشخيص المركبات المحضرة باستخدام كروموتوغرافيا الطبقة الرقيقة ، مطيافية الاشعة الفوق البنفسجية ، الاشعة تحت الحمراء و الرنين النووي المغناطيسي البروتوني وكانت جميع النتائج مطابقة لما هو متوقع. دراسة الذوبانية اثبتت زيادة في الذوبانية للمشتقات الجديدة في المذيبات القطبية مقارنة بالتتراسايكلين. الفعالية البايلوجية للمركبات المحضرة يعتقد ان تكون اكبر من التتراسايكلين نفسة وهي قيد الانجاز.

Introduction:

Tetracyclines (I) belong to a family of structurally related compounds that are widely used as antibiotics.[1-3] Since their discovery in 1943, this class of antibiotics has found use in the clinical treatment of a wide variety of infections microorganisms[4].



(I)

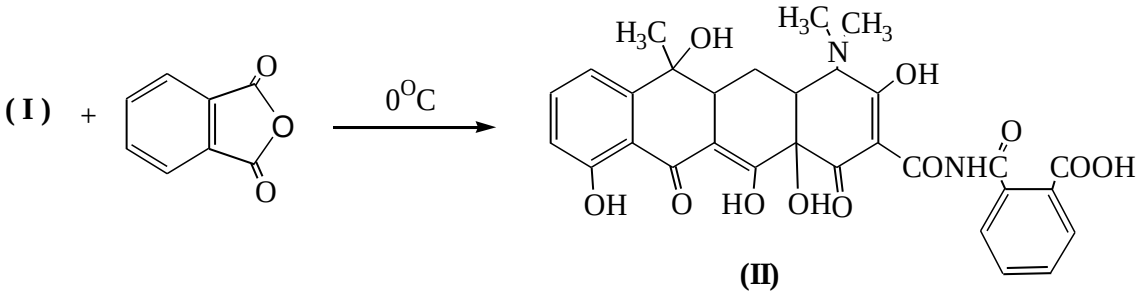
Since 1944 tetracycline's have been important for the treatment of bacterial infections when it was introduced into clinical use. The advantages of these compounds were that they inhibited a much wider spectrum of gram-positive and gram-negative organisms,[5,6] but there are some resistance against gram-negative bacteria. [7] In subsequent years, lengthy programs directed at the chemical modification of tetracycline at 8,9 and 4 position to yielded few medically useful derivatives[8-10]. When a drug is orally administered, solubilization of the drug is essential for bioavailability because only the dissolved drug can be absorbed.[11,12] Therefore, the solubility of a drug directly affects its clinical application. [13]As a result numerous chemical modification of tetracycline have been reported at an amide group in 2-position 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.[14] 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.[15] Phthalimide and phthalic acid are an interesting class of compounds with a large range of applications [16]. Phthalimides have served as starting materials and intermediates for the synthesis of many types of alkaloids and pharmacophores. [17] Recently, phthalimide and some of its derivatives have proved to have important biological effects similar or even higher than known pharmacological molecules nd so their biological activity is being a subject of biomedical research[18-21]. This study deal with the synthesis of a new tetracycline derivatives (scheme 1 and 2) by replacement of one hydrogen of 2-carboxamide nitrogen to produced derivative (II) also replacement of two hydrogen of 2-carboxamide nitrogen to produced derivative (III). The solubility study of the new tetracycline derivatives containing phthalimide and phthalamic acid moiety predicate increase water solubility than tetracycline.

Experimental:-

For anhydrous reaction, glassware was dried overnight in an oven at 120^0C and colled in a desiccator over anhydrous $CaSO_4$ or silica gel. Reagents were purchased from fluca (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}HNMR spectra carried out with Bucker WM-400 (400 MHZ FTNMR) spectrophotometer using TMS (Tetramethyl Silane) as internal reference (chemical shift in ppm). Purity of the prepared compounds was checked by TLC (Thin Layer Chromatography) on silica gel plates and spot were visualized by exposure to iodine vapours. The physical data of the prepared compounds and the spectroscopic data are presented in Table (1).

Synthesis of N-tetracycline phthalamic acid derivative (II)

Tetracycline (0.67gm, 0.001mol) was dissolved in (2 ml) DMF then added TEA (0.8 ml) with stirring for 1 hr. in ice bath. The solution of phthalic anhydride (0.17 gm,0.001mol) in (0.5 ml) DMF was added to the first solution as drop at 0-10 ^{o}C. The mixture was stirred at room temperature for 15 hr, then (11 ml) of dry ether was added . The oily layer was separated from reaction mixture and by using long cooling the oily yield converted to crystal solid of phthalamic acid derivative of tetracycline (II).

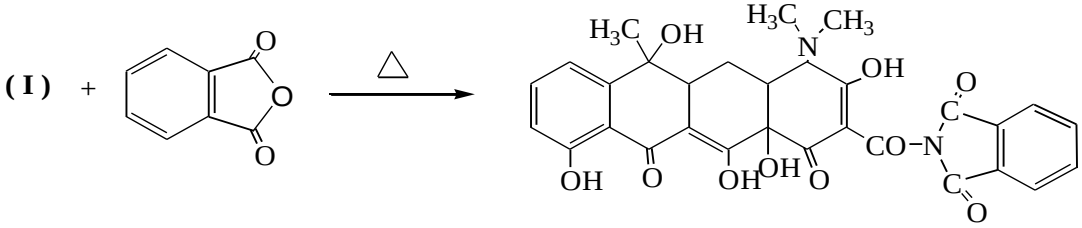


(II)

Scheme: 1- Syntheses of tetracycline derivative (II)

Synthesis of N-tetracycline phthalimid derivative (III)

Equimolar amount of tetracycline (0.1 mol) and phthalic anhydride (0.1mol) were fused together in Pyrex round bottom flask. The fusion was performed in an oil bath per-heating to 145-150 ^{o}C for 30 min., during the first 10 min, the mixture was stirred occasionally to allow proper mixing of the reaction. The sublimed phthalic anhydride which deposited on the walls of the flask was pushed down in to the reaction mixture by means of a glass rod. The mixture was left undisturbed during the next 20 min. The reaction mixture was carefully cooled until the liquid mass solidified. The solid mass was dissolved in ether and filtered, to yield yellow syrup of compound (III).

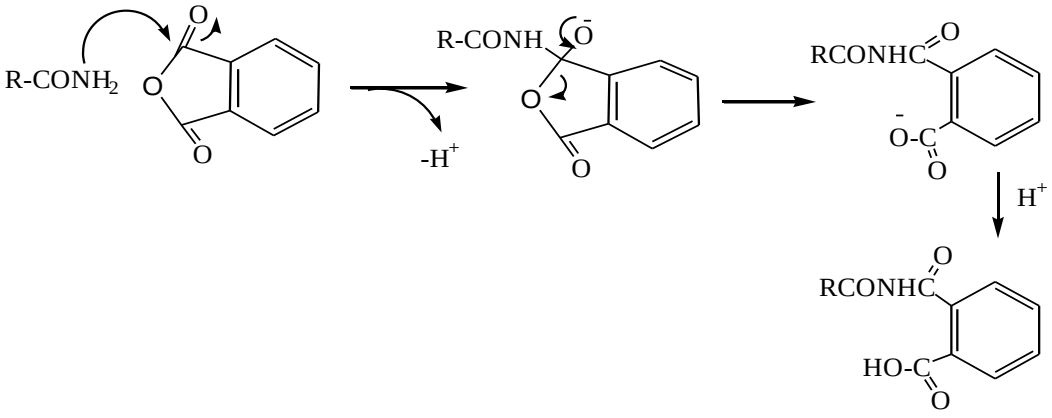


(III)

Scheme: 2- Syntheses of tetracycline derivative (III)

Results and discussion

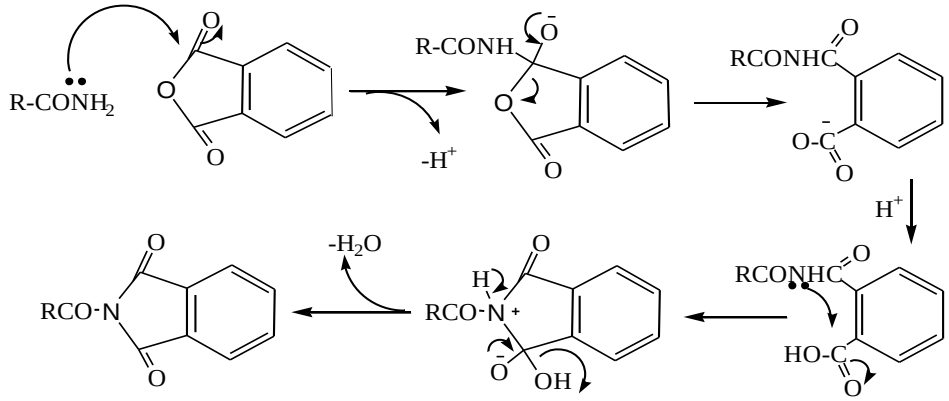
N-tetracycline phthalamic acid was prepared by reaction of tetracycline with phthalic anhydride in DMF as a solvent at 0^{0}C to produced derivative (II) that recrystallizes from ethanol to produced 80% yield as yellow crystal, R_f (0.8), m.p 176^{0}C . The goal of this reaction to furnish another carboxyl group since reaction performed at low temperature was found to be stopped at the step of amide bond formation by the following mechanism (scheme 3)



Scheme: 3- mechanism for Synthesis of tetracycline derivative (II)

The reaction of phthalic anhydride with the amide group of tetracycline at elevated temperature is performed by either fusion at a temperature near 180^{0} C, or by allowing it to be heated under reflux in dioxane or toluene suspension[22] to remove two hydrogen atoms of 2-carboxamide nitrogen then produced derivative (III) in 50% yield. The fusion method applied in this work was found to be a simple one, less time consuming and gave products with low yield. The suspension method was found, because of prolong heating, to caused partial decomposition of

tetracycline. To avoid high temperature in our procedure, the fusion was performed at relatively lower temperature (145-150^{o} C) with increasing the time course to 30 min. In this method, phthalic anhydride was condensed with the amino function of carboxamide according to the general equation illustrated in scheme -2-. The proposed mechanism for this reaction
had been involves nucleophilic attack of the amino group on one of the carbonyl carbon atoms of phthalic anhydride (scheme 4).



Scheme: 4 mechanism for Syntheses of tetracycline derivative (III)

FTIR studies.- In the FTIR spectra of N-tetracycline phthalamic acid (II) showed disappearance of ν(N-H) absorption bands and showed strong absorption bands at 3485 cm^{-1} and at 3275 cm^{-1} due to ν(O-H) carboxylic and ν(N-H) amide. Other absorption bands appeared at 1685 and at 1506 cm^{-1} belong toν(C=O) carboxylic, ν(C=O) amide and ν (C=C)aromatic respectively as seen in fig.(1). The FTIR spectra of N-tetracycline phthalimid (III) also showed disappearance of ν(N-H) absorption bands proving success of dehydration reaction and appearance of two bands at 1788 cm^{-1}and 1712 cm^{-1} due to asym., and sym., bands of ν(C=O) imide, a few strong characteristic bands for ν(C-H)aromatic were observed in 3018cm^{-1} as seen in fig.(2).

UV–Vis studies.- UV–Vis spectra of the compounds were measured in DMF using10^{-2} as well as 10^{-4} M solutions, for N-tetracycline phthalamic acid (II) showed two very strong bands at 244 nm, 267 nm and for N-tetracycline phthalimid (III) showed exhibited two very strong bands at 259 nm and 280 nm, and weak band at 440 nm, 246 nm and 463 nm all these absorption due to $\pi\rightarrow\pi^*$ and $n\rightarrow\pi^*$ transition as seen in table (1)

H-NMR studies.- ^{1}H-NMR spectra of compound (II) showed signals at δ=(7.3-8.3) ppm belong to aromatic and protons at δ=(5.4) ppm for NH proton. a clear signal appeared at δ = 10.53 ppm belong to (OH) carboxylic proton as seen in fig.(3). ^{1}HNMR spectrum of compound (III) showed disappearance of (OH) carboxyl proton and (NH) amide proton signal and appearance of two signals at δ = (7.14-7.56) and (7.89 - 8.47) ppm belong to protons of two aromatic rings as seen in fig.(4).

solubility studies.- the solubility study of the tetracycline derivatives using deferent types of solvent improve increase solubility of these derivatives than free tetracycline.

biological activity studies.- biological activity of the compounds suppose to be more than tetracycline and it was still to be doun .

Acknowledgements

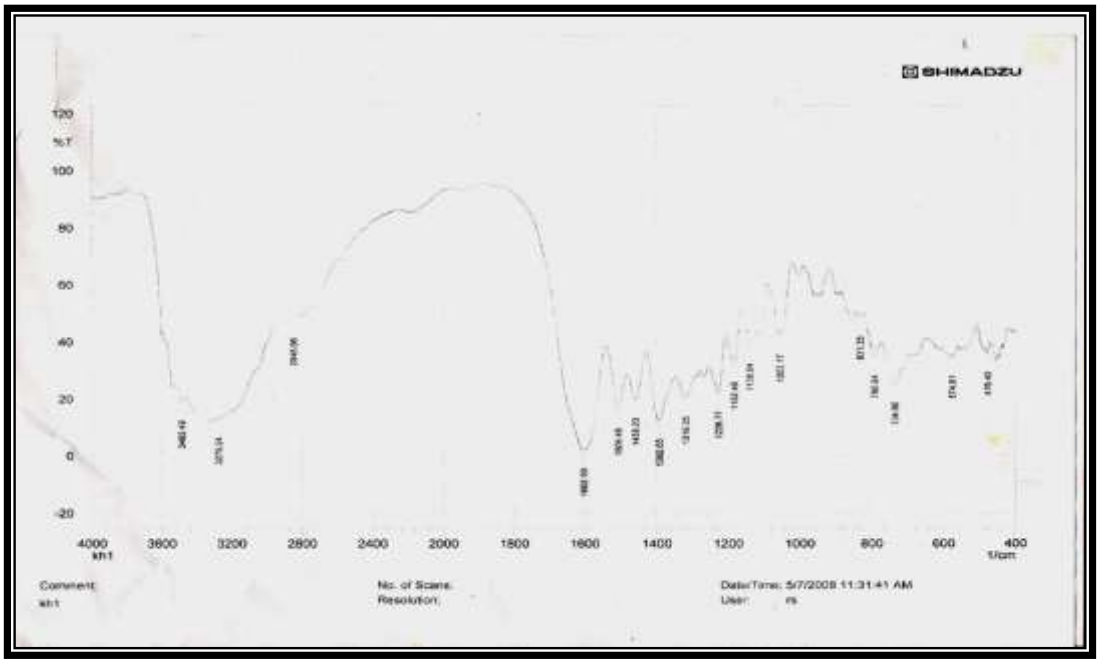
For the financial support, thanks give to chemistry department college of Education of pure science/ University of Karbala for offering requirement to facilities this work, also compound thanks for Samarra drug industries for supplying me the tetracycline drug.

Table (1): spectroscopic data and physical property of compounds [II and III]

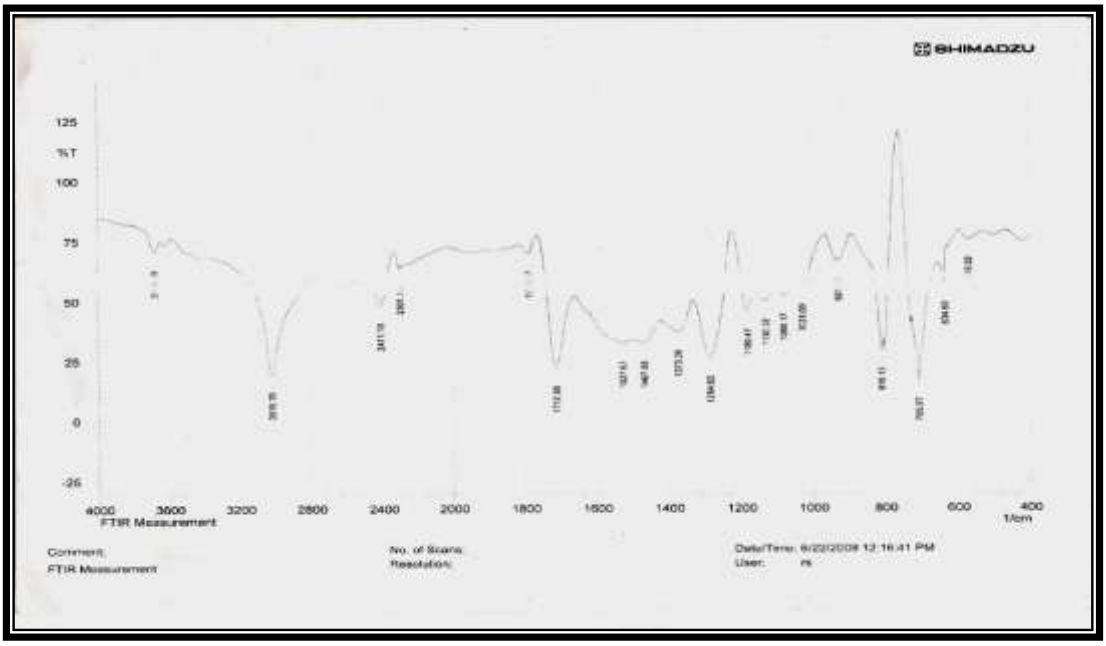
Comp. No.	FTIR spectral data cm^{-1}			^{1}H-NMR spectral data ppm		
	ν(O-H) Carboxylic	ν(N-H) Amide	ν(C-H) Aromatic	δ(H) Carboxylic	δ(H) Amide	δ(H) Aromatic
II	3485	3275	3062	10.5	5.3	7.3-8.3
	Molecular formula	Color	Melting Points °C	Yield %	Solvent of Recrystall.	λ_{max} (nm)
	$C_{30}H_{28}O_{11}N_2$	Yellow	176	80	Ethanol	244, 267
III	ν(C-H) Aromatic	ν(C=O) Imide	ν(C-N) Imide	δ(H) Aromatic	δ(H) Aliphatic	
	3018	1712	1284	7.3-8.4	1-2.5	
	Molecular formula	Color	Melting points°C	Yield %	Solvent of Recrystall.	λ_{max} (nm)
	$C_{30}H_{26}O_{10}N_2$	Yellow	Syrup	50	-	259 , 280

Table(2):The solubility study of compounds [II and III]

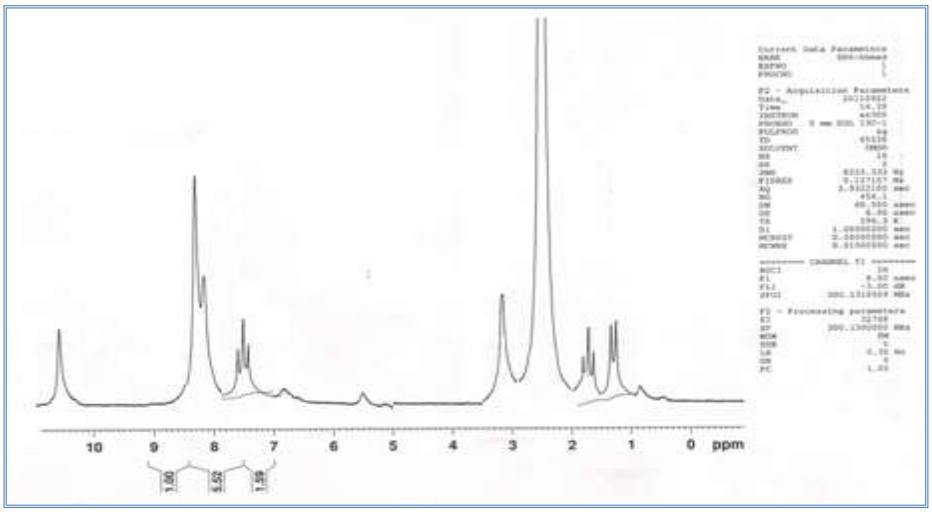
Comp. No.	water	Acetone	Methanol	10%HCl	10%NaOH	DMF	DMSO
Tetracy.	_	_	_	+	++	+	+
II	+++	+	+	+	+	+	+
III	+	_	+	+	+	+	+



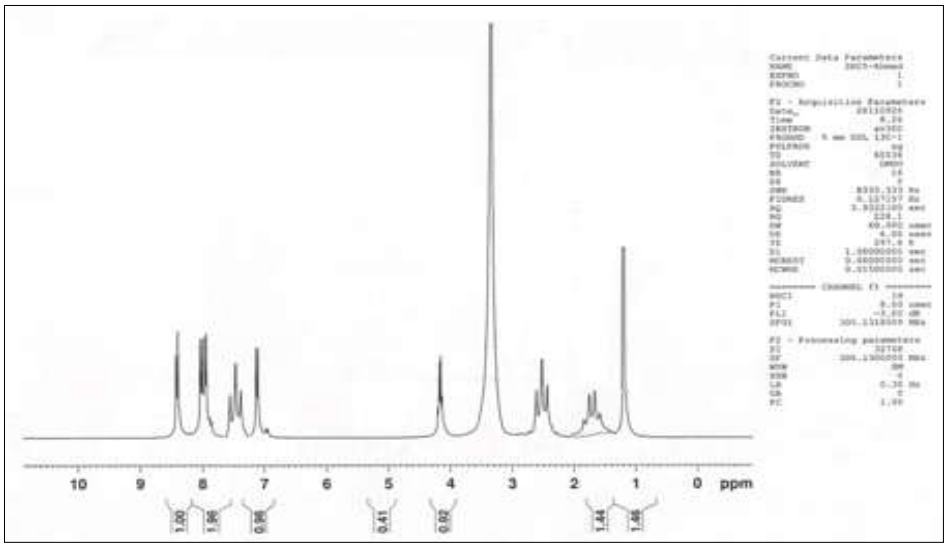
Fig(1): FT-IR spectrum of compound (II)



Fig(2): FT-IR spectrum of compound (III)



Fig(3): H-NMR spectrum of compound (II)



Fig(4): H-NMR spectrum of compound (III)

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