



Synthesis and Kinetic Study for the Interconversion Process of Some 2'-Hydroxychalcones to their Corresponding Flavanones



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Abstract

In this work, five substituted 2'-Hydroxychalcones were prepared using Claisen - Schmidt condensation and used as a synthone for substituted flavanones via base catalyzed isomerization process. The latter process has been studied kinetically using HPLC technique in (8:2) (CH₃CN:CH₃OH) medium at different temperatures (298 K - 318 K). The obtained results were inconsonance with a four-step mechanism which considered the existence of phenoxide ion as the key intermediate. The reaction was achieved as a pseudo first order reaction in which the rate of the studied compounds followed the sequence 1>2>3>4>5, and the activation energy had the same sequence for these compounds. The reaction rate was affected by the electronic behavior of the different substituents at ring B since they played an important role in the stability of the intermediate that led to the final product.

Keywords: 2-Hydroxy chalcones, flavanones, phenoxide ion, isomerization

Introduction

"Chalcone" is the name given by Kostanecki and Tambor [1] for compounds that possess 1,3-diaryl-2-propene-1-ones framework [2,3]. Chalcone is an open-chain flavonoid in which two aromatic rings (A & B) joined by three carbon atoms i.e. α,β -unsaturated carbonyl system [4]. Compounds with such system will have great chance to undergo electron transfer reactions. These compounds have been used as precursors of all other flavonoids. Flavanone, as a part of flavonoids family that consist of a 2,3-dihydro-2-phenylchromene-4-one framework in which the three-carbon bridge between the phenyl groups (figure 1), is commonly cyclized with oxygen (ring C) [5]. These substances are widespread in plants and with an array of biological activities [6]. Natural or synthetic chalcones and flavanones have been reported to possess various biological activities that involve the antioxidant [7,8], antiangiogenic [9-11], anticancer [12,13], antimicrobial [14-16], anti-inflammatory [17-19], cardiovascular [20,21], antimalarial [22,23], and many other activities. These

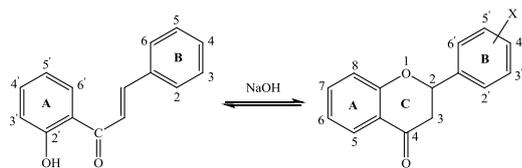
and other observations encouraged us to synthesize a number of substituted 2'-hydroxychalcones via Claisen-Schmidt condensation of substituted aromatic aldehydes and 2-hydroxyacetophenone in basic medium, and then study their transformation into their corresponding flavanones. Since the knowledge of the acid-base properties plays an important role in the development of pharmaceutical formations and studies in medicaments stability [24,25], the isomerization of 2'-hydroxychalcone - flavanone was extensively studied by means of HPLC technique in order to get further information about acid base behavior of flavanone of chemical and biological interest [26]. The rate constants for these reactions were determined, and they were clearly affected by substituents at ring B. Furthermore, the chalcone - flavanone ratio have been determined during (0.5-1) and after 48 hours. Study was carried out in a special type of cell, that was placed in the HPLC apparatus in order to estimate retention time (t_R) for compounds under study.

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X= 1=4-Cl, 2= 4-Br, 3= H, 4= 4-CH₃, 5=4-N(CH₃)₂
Figure (1): General equation for 2'-hydroxychalcone-flavanone isomerization.

Experimental

Instrumentation:

Melting points were determined on a Stuart SMP30 Advanced Digital Melting Point Apparatus (UK), and they were uncorrected.

Ultra - Violet spectra were recorded on a Shimadzu UV - 1650 pc, UV - Visible spectrophotometer (Japan), using chloroform as a solvent.

Infrared spectra were recorded on a Bruker Alpha, FT - IR Spectrophotometer (Germany).

Proton NMR spectra were recorded on a Bruker Biospin 400 MHz, Germany, using TMS as internal reference, and d⁶- DMSO as a solvent.

Reaction temperature controlled by means of water bath (Haake NK22) ± 0.15 °C, Germany.

HPLC experiments were performed on a Shimadzu LC2010 AHT liquid chromatograph. Acetonitrile was used as solvent while the mobile phase was a combination of acetonitrile and methanol (8:2) (v/v) with a flow rate of 1 mL/min at 12 Mpa as pressure into the column to make sure that all components were separated. Separation was achieved using a column (type C8 block heating Shim-pack 4.6 mm interior and 150 mm long) with particle size of 5 µm. The analyte was identified by means of UV-visible detector which was thermally controlled by an internal heater. Kinetic

General procedure for preparation of 2'-hydroxychalcone derivatives [27,28]

An equimolar amount (25 mmole) of an appropriate substituted benzaldehyde and 2-hydroxyacetophenone was placed in a (100 mL.) round bottomed flask containing (40 mL.) of ethanol. The mixture was stirred and then slowly treated with aqueous solution of sodium hydroxide (2 g. in 5 mL. of water). After (3 - 4) hours, stirring was stopped, since a thick mixture was formed, in which a deep yellow solid mass of the chalcone sodium salt has been formed, and therefore, stirring was no longer effective. The mixture was kept at room temperature

overnight. The precipitate was then filtered off and washed with a little amount of ether. The solid was treated with (100 mL.) of ice - cold water and acidified by (10 %) of hydrochloric acid to give the chalcone which was collected by filtration, washed thoroughly with cold water, and recrystallized from ethanol to give the final compounds (1-5). The physical properties are listed in table (1).

Table 1 : Physical properties for compounds (1 - 5)

No.	R	M. Formula	rec. m.p. °C	m.p. °C lit*[29]	Yield %	Color
1	4-Cl	C ₁₅ H ₁₁ ClO ₂	205-207*	145-146	20	yellow
2	4-Br	C ₁₅ H ₁₁ BrO ₂	259-260*	138-139	25	yellow
3	H	C ₁₅ H ₁₂ O ₂	89-90*	89-90	75	yellow
4	4-CH ₃	C ₁₆ H ₁₄ O ₂	123-126*	117-118	35	yellow
5	4-N(CH ₃) ₂	C ₁₇ H ₁₇ NO ₂	179-181*	170-171	40	red

General procedure for flavanone synthesis

2'- hydroxychalcone (8.93 mmole) was placed in a (100 mL.) round bottomed flask and dissolved in (10 mL.) of absolute ethanol by heating the mixture on a steam bath. The mixture was removed from the steam bath when all the solid was dissolved, then treated by (30 mL. of 1.5 %) of dilute aqueous sodium hydroxide solution with gentle shaking. The mixture turned red and became cloudy. The mixture was kept overnight at room temperature. The resultant solid was then filtered off and washed thoroughly with cold aqueous ethanol (80:20, water:ethanol), and recrystallized from ethanol to give white crystals of flavanone (6), m.p. 77 - 78°C in (60 %) yield.

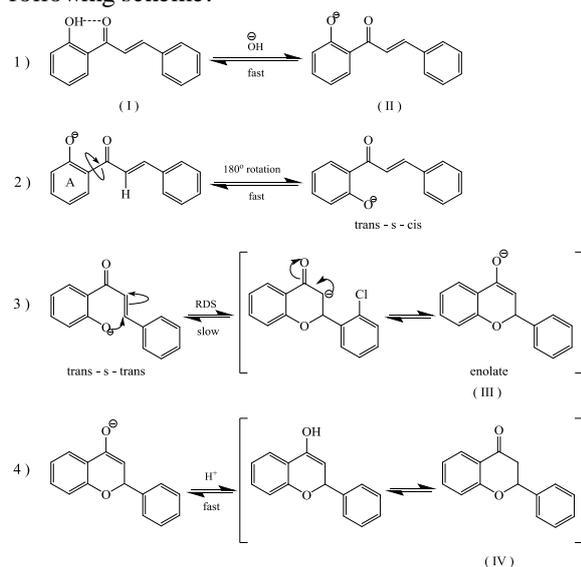
Kinetic experiments

The rate of reactions was preliminary determined by HPLC at appropriate λ_{max} that was estimated by scanning the components using UV-Visible spectrophotometer. These experiments were achieved by mixing 1 mL (1x10⁻³M) of 2- hydroxy chalcone with 1 mL of NaOH (1x10⁻² M) in the reaction cell which was kept thermostated. Afterwards, 20 µL of the above mixture was introduced into HPLC apparatus in order to start the measurements of studying the isomerization process. These measurements were recorded at different temperatures (between 25-45°C) for each reaction, and the follow up was continued until the reaction was 75-85% completed.

Results and Discussion

Flavanone skeleton is present in a wide range of synthetic and naturally occurring products exhibiting various interesting pharmacological activities as mentioned previously. These compounds can be synthesized traditionally through a two-step reaction. The first one involves the formation of substituted 2-hydroxy chalcones (1-5) via Claisen - Schmidt condensation of 2-hydroxy acetophenone and appropriate aromatic aldehydes in basic medium. The structures of the synthesized compounds were established by means of physical and spectroscopic methods (as shown in tables 1 and 2).

The second step involves the formation of flavanone which was achieved through base catalyzed intramolecular conjugate addition of the phenoxide ion to the β -carbon of chalcone as shown in the following scheme:



Scheme 1: General scheme for the isomerization process of (2'-hydroxychalcone-flavanone)

According to the scheme, the molecular structure of 2'-hydroxychalcone derivatives exist in the more

Table (2) : some spectral data for compounds (1 – 6)

No.	IR (KBr) ν (cm ⁻¹)				UV(CHCl ₃) λ max (nm)	¹ H-NMR (d ₆ -DMSO) δ ppm
	O-H	C=O	C=C	C-O-C		
1	3267	1640	1598	----	338	-----
2	3446	1667	1571	----	256	-----
3	3444	1639	1572	----	316	-----
4	3287	1640	1558	----	345	12.6 (s, 1H, OH) , 8.27 (dd, 1H, H-6') , 8.0 (d, 1H, H β) , 7.75-7.85 (m, 3H, H α , H-2, H-6) , 7.57 (ddd, 1H, H-4') , 7.47 (ddd, 1H, H-5') , 7.38 (d, 2H, H-3, H-5) , 7.3 (d, 1H, H-3') , 2.38 (s, 3H, CH ₃) .
5	3446	1643	1576	----	450	-----
6	----	1690	----	1228 , 1148	325	-----

stable formula (I), since an intramolecular hydrogen bonding (IHB) can be formed between the phenolic hydroxyl group and the carbonyl oxygen atom [30,31].

A four-step mechanism has been suggested, in which the first step involves the generation of phenoxide ion (II) that has an equilibrium state with the starting chalcone.

The latter ion involves a repulsion force between the negative charge and the carbonyl oxygen, and this will lead to two important internal rotations: the first force ring A to rotate in 180° about (C₈-C_{aromatic}) single bond at step two (figure 2), while the second will be about (C₇-C₈) single bond that changes the trans-s-cis into the trans-s-trans conformer which means that this ion will be in the right position for ring closure.

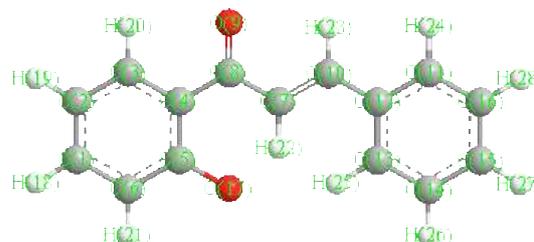


Figure (2): 3-D structure of 2-hydroxy chalcone
Formation of ring C takes place in the third step in which O₁₇ is joined with C₁₀ through an intramolecular 1,4-addition of the phenoxide ion to β -carbon of the conjugated system affording anion (III). The structure of the latter anion is a resonance hybrid between the keto form (the negative charge on the α carbon atom) and predominantly the enol in which the charge on the oxygen atom of the carbonyl group [32,33]. This step is thought to be the rate-determining step (RDS) which led to 1st order process and the substituents at ring B played an important role on rate, since the electron withdrawing

groups (EWG) enhance the reaction rate, thus compounds 1 and 2 react faster than others. This in

This step is considered as the rate determining step
 A_M : Maximum absorbance of intermediate.

Table 4: Correlation coefficient & Standard deviation

Temp. K	1		2		3		4		Com. 5	
	R	σ/min	R	σ/min	R	σ/min	R	σ/min	R	σ/min
298	0.989	0.175	0.962	0.457	0.989	0.175	0.990	0.152	0.965	0.189
303	0.991	0.525	0.989	0.184	0.991	0.525	0.977	0.267	0.942	0.144
308	0.991	0.165	0.982	0.071	0.968	0.119	0.973	0.306	0.985	0.177
313	0.984	0.309	0.978	0.147	0.996	0.189	0.988	0.114	0.982	0.196
318	0.996	0.119	0.991	0.165	0.975	0.466	0.309	0.157	0.983	0.323

contrasts with the effect of the electron donating groups (EDGs) which decrease the reaction rate, since they strengthen the hydrogen bonding (IHB) and decrease the electrophilic character for β - carbon (figure 3), as shown in table (3).

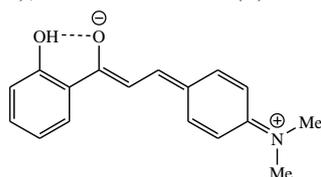


Figure (3) : Effect of EDGs of compound (5) on the strength IHB and the reaction center

The enolate ion abstract a proton from the reaction medium in the final step to give the enol form that tautomerize to the more stable keto form, i.e. flavanone (IV)

Kinetic measurements

The interconversion process of substituted 2'-hydroxychalcone to their corresponding flavanones in basic media at pH=11.5 followed a four steps pathway that involved the formation and disappearance of the phenoxide ion (II), thus $r = k_{\text{obs}}$ [2-hydroxy chalcone], and the observed rate constant (k_{obs}) corresponded to k [flavanone], was calculated. In this case, the disappearance of one mole of the reactant corresponded to the formation of one mole of intermediate and if the transformation was complete then it would be converted to the final product (i.e. flavanone), and the maximum absorbance of the intermediate A_M will be either or at least proportional to the initial absorbance A_0 of chalcone if the reaction was complete.

The rate constants were evaluated by fitting the absorbance time data to appropriate pseudo first order rate equation using linear regression programmer.

$$\ln \frac{[2\text{-hydroxychalcone}]_0}{[2\text{-hydroxychalcone}]_t} = \ln \frac{[\text{intermediate}]_0}{[\text{intermediate}]_t} = \ln \frac{[\text{flavanone}]_0}{[\text{flavanone}]_t} \quad \text{-----(1)}$$

$$\ln \left(\frac{A_M - A_0}{A_M - A_{t_1}} \right) = k_1 t \quad \text{-----(2)} \quad \text{(For intermediate formation process rate. enolate formation)}$$

A_t : Absorbance for formation of intermediate at any time.

A_0 : Absorbance of intermediate at zero time (zero time base line).

k : Rate constant for formation of intermediate.

In this case, the disappearance of the 2'-Hydroxychalcone will be equivalent to the formation of the intermediate ($A_M - A_t$) at any time

$$\ln \left(\frac{A_M - A_{\infty}}{A_{t_2} - A_{\infty}} \right) = k_2 t \quad \text{-----(3)} \quad \text{For}$$

intermediate disappearance and its conversion to the corresponding flavanone

A_t : Absorbance for disappearance of intermediate at any time.

A_{∞} : Absorbance at infinite time for disappearance of intermediate.

$A_M - A_{\infty}$: Equivalent to initial concentration of intermediate.

$A_t - A_{\infty}$: Equivalent to intermediate concentration at any time.

k : Rate constant for disappearance of intermediate.

The obtained results came in a good agreement with the proposed mechanism; these results were influenced by the nature of the substituents, and they have been summarized in table (3).

Table 3: Rate constant for compound (1 - 5)

Temp. K	Com. 1	Com. 2	Com. 3	Com. 4	Com. 5
	10^4 k/min	10^4 k/min	10^4 k/min	10^4 k/min	10^4 k/min
298	6.461	2.437	1.294	0.944	0.625
303	8.283	4.834	3.742	2.321	1.613
308	9.629	6.324	4.233	2.756	2.375
313	23.132	13.343	5.017	3.664	2.698
318	26.731	14.562	8.237	7.524	4.371

The reaction was obviously reversible, since an equilibrium state was established between chalcone and flavanone during kinetic runs.

3.2. Activation energy and Entropies of activation effects

Activation energy values are of great importance to discern the mechanism under all circumstances studied here.

In principle, a unimolecular reaction is of the simplest kind of elementary reaction since it involves the isomerization (including intramolecular ring closure) or decomposition of a single isolated

reactant molecule. It is well known that this type of reactions is the first order process in the high concentration (or pressure) region of the reactant [34].

For the isomerization reaction with different substituents, it can be noticed that all of their E_a vary in linear manner.

Low E_a means fast reaction and vice versa, this observation can be explained by the formation of enolate which results in decreasing the repulsive forces during the cyclization leading to a higher chance for the formation of flavanone. Unlike (EDG), the (EWG) stabilizes the intermediate (III), thus activation energy for compounds under study were found to vary in the following order ($1 > 2 > 3 > 4 > 5$) as shown in table (5).

The reaction rate was controlled by other factors like the frequency factor (the A-factor) and its corresponding entropy of activation ΔS^\ddagger . These factors estimate the rigidity of the cyclic transition state that was produced through the ring closure process.

The formation of more rigid cyclic transition state was indicated from low values of A-factor or negative ΔS^\ddagger leading to a faster process.

These factors are related to each other by the following equation [35]:

$$A = \frac{e k T}{h} e^{\Delta S^\ddagger / R k} \quad \text{Boltzman constant, } h = \text{Planck constant, } T = \text{mean temperature}$$

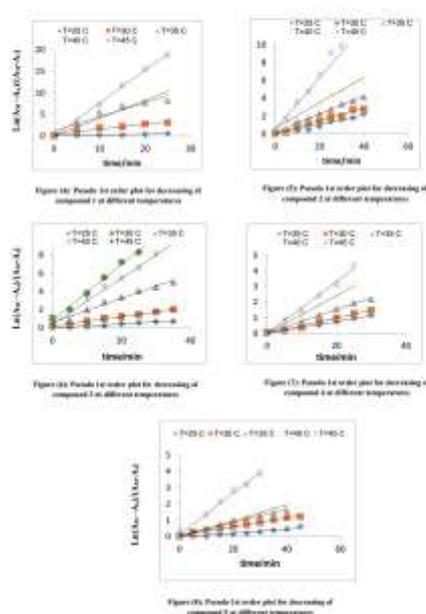
Table 5: Arrhenius parameters and entropies of activation

Comp. No.	$E/kJ.mol^{-1}$	A-factor/ min^{-1}	$\Delta S^\ddagger / JK^{-1} mol^{-1}$ at 308 k	$\Delta G^\ddagger / kJ.mol^{-1}$
1	71.35	1.080×10^9	-77.39	22.3607
2	92.75	1.06×10^{11}	-58.96	20.9890
3	114.56	3.93×10^{11}	-48.70	19.8901
4	118.01	4.96×10^{11}	-20.96	18.8621
5	127.75	5.02×10^{11}	-34.50	18.1693

From the above equation, a value of $A = 10^{13.5} s^{-1}$ corresponds to $\Delta S^\ddagger = 0$, the reduction in this value will lead to a negative entropy of activation.

Negative value of ΔS^\ddagger for the cyclization process indicates the formation of restricted enolate which suffers from lack of certain degree of freedom compared to neutral chalcone, thus the reduction in both of A-factor and ΔS^\ddagger values will affect the rate

constant values and consequently the reaction rate. These values were affected by the substituents at ring B since electron withdrawing groups reduce the ΔS^\ddagger values resulting in a more rigid and aligned (more stable) T.S and thus, a faster cyclization process, while electron donating groups cause a relative increase in ΔS^\ddagger values, so the values of ΔS^\ddagger will follow the order ($1 > 2 > 3 > 4 > 5$).



From the data listed in table (6), we can note that there is an equilibrium state between flavanone and chalcone after 1 hour of the reaction, but after 48 hours the equilibrium tends to proceed toward flavanone formation, as shown in figures (9 and 10).

Table 6: The ratio between flavanone and chalcone after time

No.	R. time (flavanon) min	R.time (chalcone) min	Ratio (flavanone:chalcone)%	
			After 1 hour	After 48 hours
1	2.775	3.204	(63.12:36.88)	(82.56:17.74)
2	1.244	1.504	(57.78:42.22)	(71.28:27.72)
3	2.775	3.204	(46.53:53.47)	(64.53:35.47)
4	2.664	2.963	(41.65:58.35)	(61.03:38.97)
5	2.637	2.673	(39.78:60.22)	(58.85:41.15)

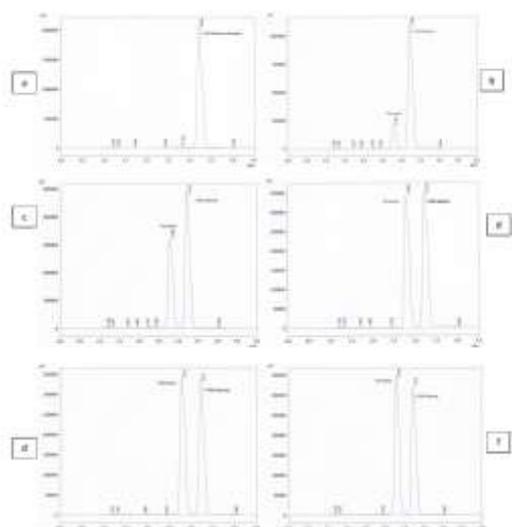


Figure (9): Chalcone-Flavanone isomerization HPLC-Chromatograms at different times compound(1)

a- Chalcone only b- after 5 min. c- after 10 min. d- after 15 min e- after 20 min. f- after 30 min.

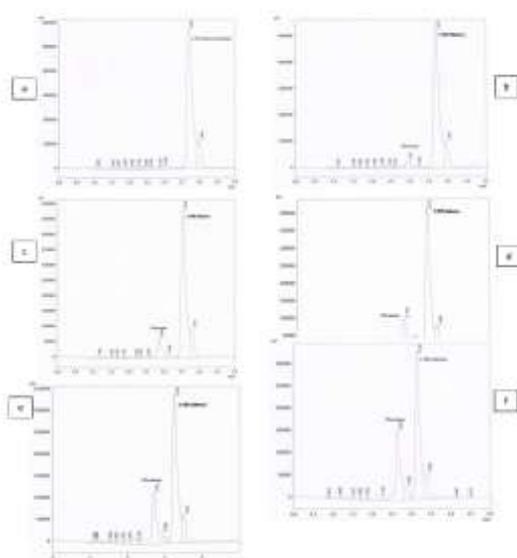


Figure (10): Chalcone-Flavanone isomerization HPLC-Chromatograms at different times compound(5)

a- Chalcone only b- after 5 min. c- after 10 min. d- after 15 min e- after 20 min. f- after 30 min.

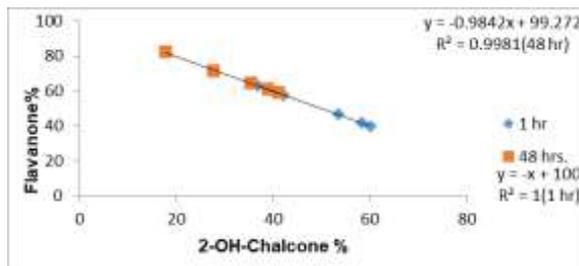


Figure (11): The relationship between chalcone-flavanone isomerization

Finally, from figure (11) clearly we can notice that all compounds under study followed the same mechanism.

CONCLUSION

According to the data obtained from our work which involved the synthesis of 2-hydroxychalcones and their conversion to the corresponding flavanones, we noted that the isomerization process could be achieved through a four-step mechanism in which the RDS was the ring closure process of the phenoxide ion which resulted in the formation of the enolate ion (i.e. 3rd step). The reaction was affected by the type of substituents at ring B of the synthesized chalcones, since electron withdrawing groups enhanced the reaction rate by increasing the electron deficiency at β – carbon and stabilizing the intermediate. This is in contrast with the electron donating groups which reduce the reaction rate due to destabilization of the intermediate (enolate ion). Furthermore, we could note that the equilibrium state was shifted toward the more stable flavanone after 48 hours

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