

Theoretical Study of Hydrolysis Mechanism of Khellin

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This paper describes khellin hydrolysis mechanism using semiempirical PM3 implemented in Gaussian 03 package. The calculations show that in the presence of an acidic media, an enolate molecular ion leads directly to ω -acetokhellinone while in the basic media it leads to khellinone.

Keywords: Khellin hydrolysis, PM3 calculation, Khellinone, ω Aceto khellinone

INTRODUCTION

Khellin is an active principle extracted from the seeds of Ammi visnaga Lam (Fam. Apiaceae) which is natural source of several furochromones namely, visnagin, khellin, khellol-glucoside, ammiol and coumarin as visnadin. Khellin and psoralens bind with DNA through cross-links to produce monoadducts. Thus khellin is used in photochemotherapy of dermatoses such as vitiligo and psoriasis [1-5]. It is known that hydrolysis of khellin gives very interesting derivatives namely ω aceto khellinone and khellinone (Scheme 1). These two products are important molecules for the synthesis of new khellin derivatives [6].

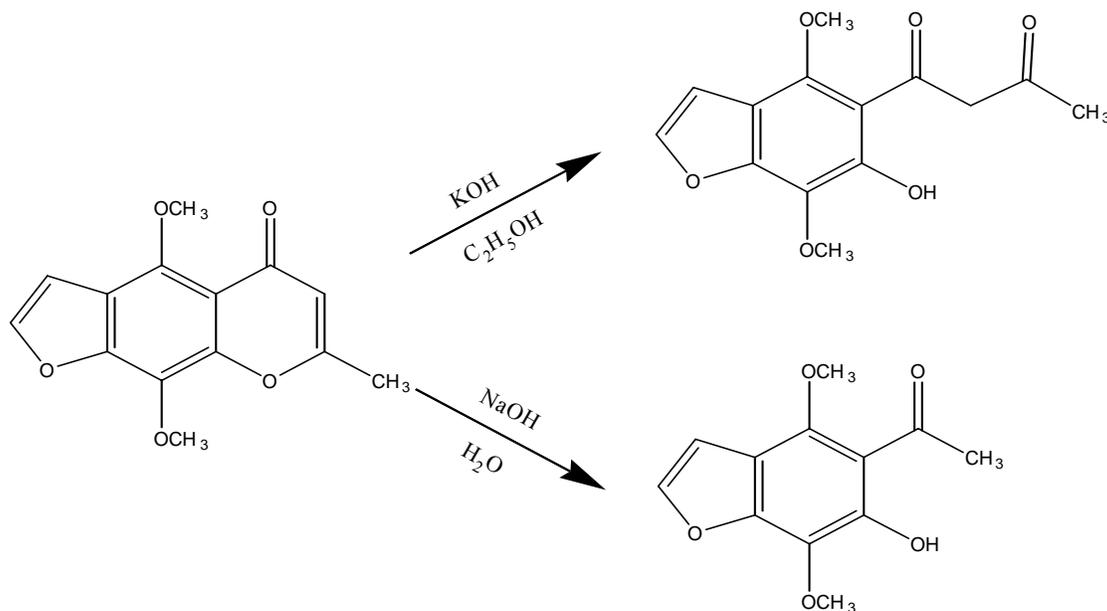
The ring-opening reaction (ROR) of chromone was investigated by *ab initio* and density functional theory methods by Kóña *et al.* [7] but calculations on the ROR mechanism of khellin have not been yet studied. The aim of

this paper is to investigate the mechanism of khellin hydrolysis using a semi empirical PM3 method implemented in the GAUSSIAN03 package to investigate the ring-opening reaction of khellin and its hydrolysis. This method is the best parametric method for this type of calculations and the most well adapted for molecules of this dimensions.

COMPUTATIONAL METHODOLOGY

All theoretical calculations were performed by GAUSSIAN 03 software package [8]. The geometries were completely optimized by the PM3 method and followed by performing the harmonic frequency analysis, at the same level of theory as the geometry optimization, to insure that the located stationary point is a true minima or a transition state. In addition, zero point corrections and thermal corrections to Gibbs free energies were also evaluated using the same method. For each step of the mechanism, the geometries were fully optimized without imposing any symmetrical

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Scheme 1. The experimental chemical pathway for khellin hydrolysis

restrictions. Furthermore, transition states are connected with reagent and products by intrinsic reaction coordinate (IRC) calculations.

RESULTS AND DISCUSSION

Synthesis of ω Acetokhellinone

The predicted mechanism of the reaction paths of the synthesis of ω acetokhellinone in the present study is described, step by step. As shown in Scheme 2, three steps are involved for the synthesis of ω -acetokhellinone reaction and below is the detailed discussion of each step.

OH⁻ addition to khellin. The addition of hydroxide ion to C2 proceeds by the formation of the ion-dipole complex 1 which is formed without a reaction barrier, leading *via* transition state TS1 to the enolate intermediate 2, which is formed by the addition of the hydroxide ion to chromone [5].

Going from khellin and OH⁻ to enolate intermediate 2 *via* TS1 involves formation of O8-C2 bond and cleavage of π bond of the C2-C3. As shown in Fig. 1, the C2-C3 bond varies from 1.357 Å in 1 to 1.367 Å in TS1 and finally 1.475 Å in 2. This indicates that the C2-C3 bond changes to a single bond in the enolate intermediate but the bond C3-C4 changes to

double bond (1.458 Å in 1, 1.447 Å in TS1 and 1.389 Å in 2). The hybridization of C2 changes to sp³ hybridization while C3 remains possessing sp² hybridized character.

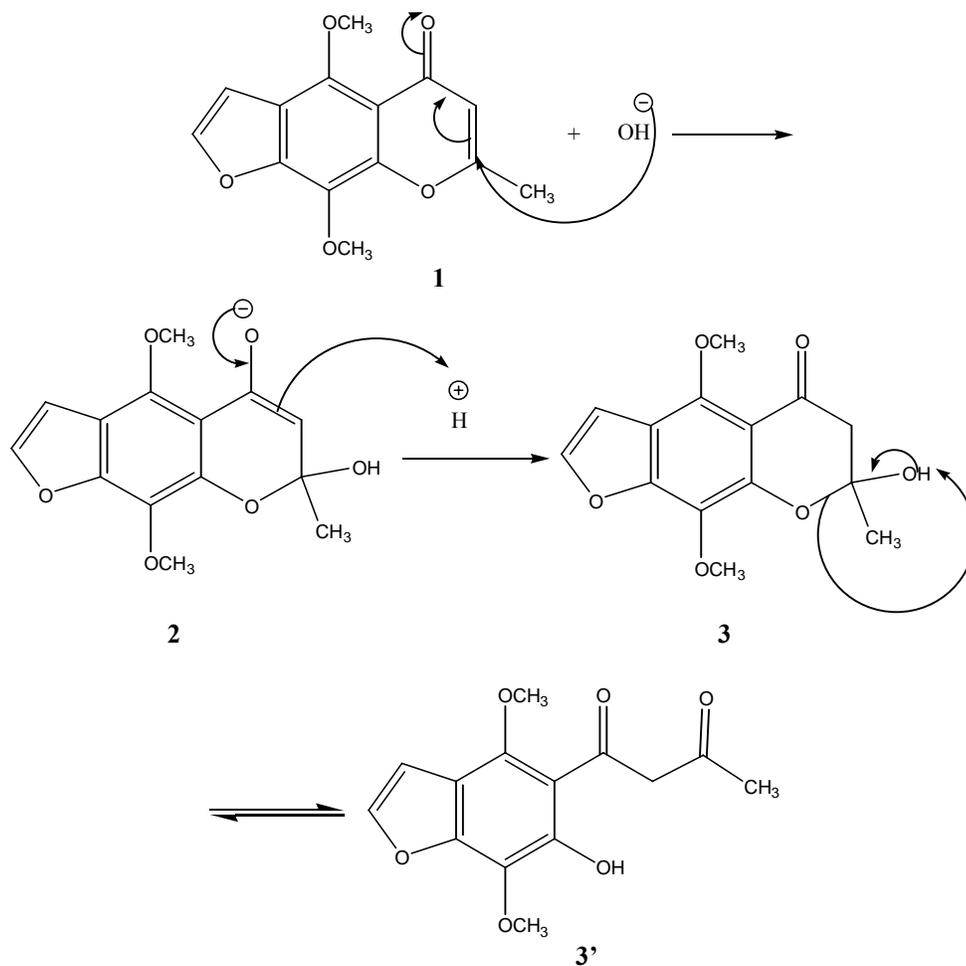
The frontier orbital of the complex 1 structure has been analyzed. The highest occupied molecular orbital (HOMO) constitutes mainly the orbitals of the atom O8 but the orbitals of C3 are implied in the lowest occupied molecular orbital (LUMO). These explain the moving of electrons from O8 to C3.

The normal mode associated with the imaginary frequency [317 cm⁻¹] describes mainly the C2-O8 bond stretching. This confirms the nature of TS1 as the site for the addition of hydroxide ion at C2. This structure has been confirmed by the IRC calculations as a correct transition state of addition of hydroxide ion to the C2 carbon of khellin.

The predicted energy barrier for this step is 9.98 kcal mol⁻¹ while the free energy of activation is 10.89 kcal mol⁻¹. The formation of enolate intermediate is exothermic -0.914 kcal mol⁻¹. Carbon C3 has the highest negative charge while C2 has the highest positive charge. These two atoms can have a major role in the reactivity of this molecule whether the media is basic or acidic.

Reactivity of enolate intermediate 2 with H⁺. The second

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Scheme 2. Mechanism of ω acetokhellinone formation from khellin

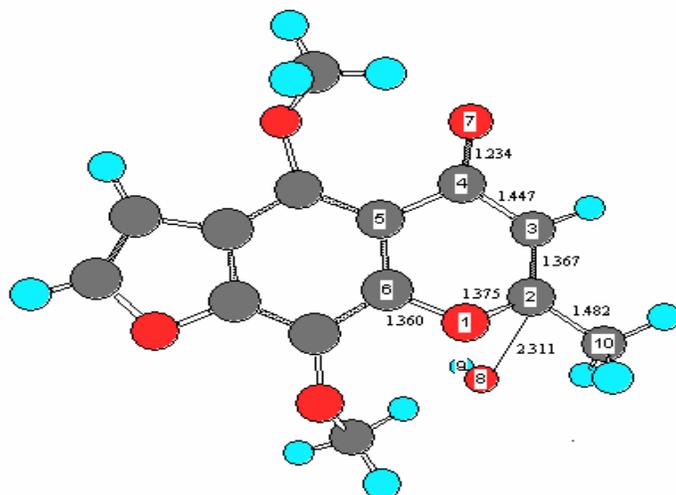


Fig. 1. PM3 calculated structure of transition state TS1. Distances are given in Å.

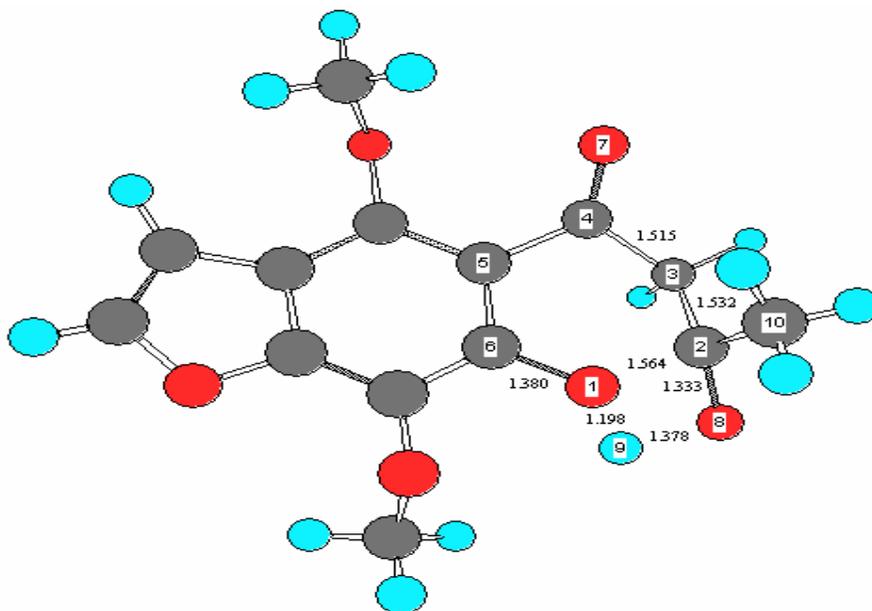


Fig. 2. PM3 calculated structure of transition state TS2. Distances are given in Å.

step of the mechanism is the fixation of the proton hydronium H^+ on carbon C3 of the enolate intermediate 2. In the presence of H^+ , molecule 2 converts into the molecule 3 spontaneously. This step has no transition state.

Hydrogen transfer. The last step of this mechanism is a rearrangement reaction. The proton of hydroxide moiety is transferred to the O1 ring oxygen.

In TS2, H9 is in bridge between the O1 and O8 at 1.198 Å and 1.378 Å, respectively. TS2 is characterized by one imaginary frequency at -2151 cm^{-1} . The analysis of this frequency indicates the stretching of H9-O1 which point to the tendency of the formation of the bond H9-O1. The energy barrier of this step is $41,953\text{ kcal mol}^{-1}$.

The hydrogen transfer process involves the opening of the pyron ring and the final product is the ω acetokhellinone (open isomer of molecule 3). ω -Acetokhellinone is more stable than molecule 3 by 3 kcal. The optimized structures and selected parameters involved in this step are depicted in Fig. 2.

Synthesis of Khellinone

The predicted reaction paths (mechanism) of the synthesis of the khellinone is shown in Scheme 3.

OH⁻ addition to the khellin. The first step is the same as

in the mechanism of formation of the ω aceto khellinone. The two products of hydrolysis proceed through the same intermediate.

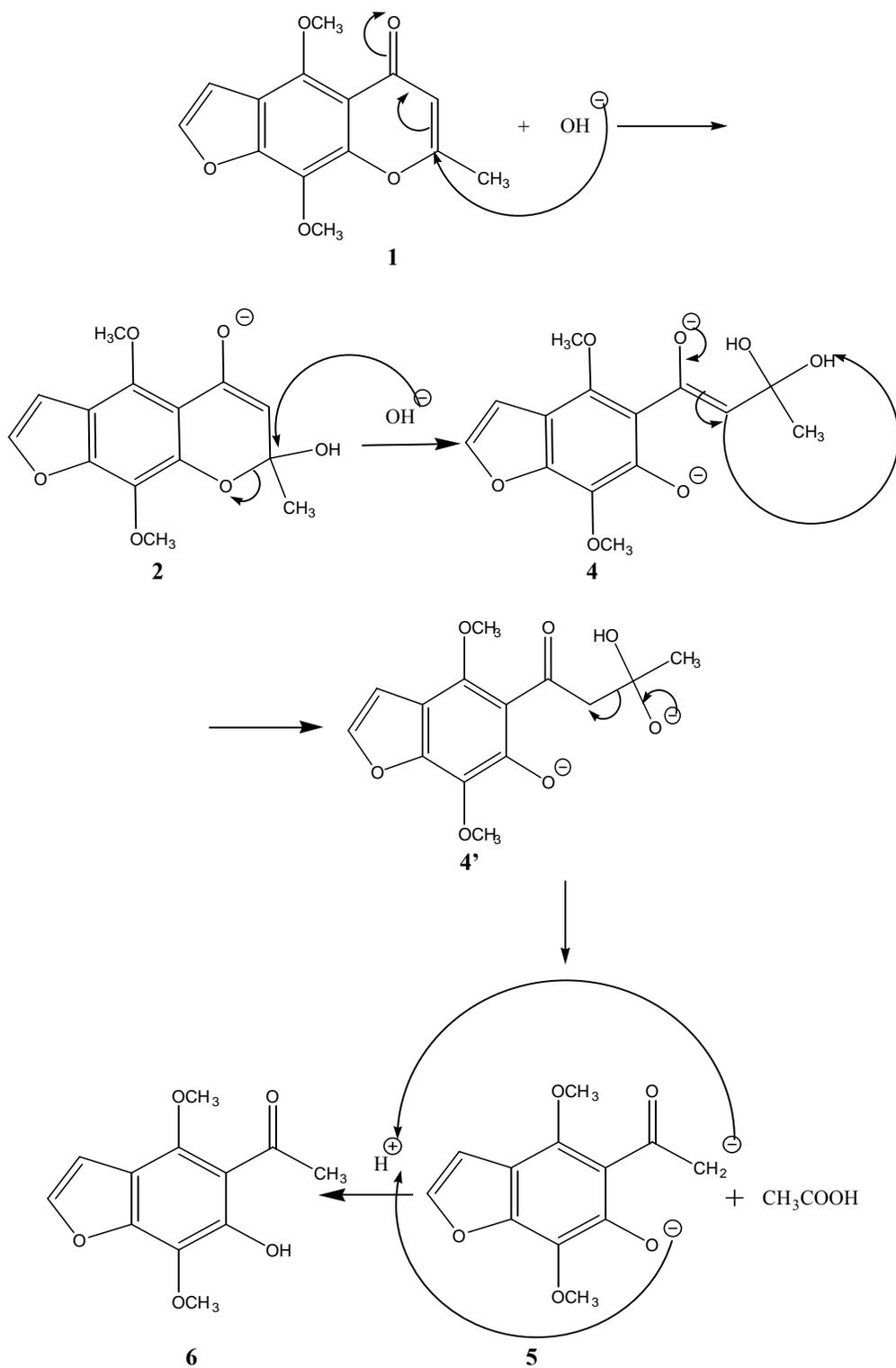
OH⁻ addition to the ion complex 2. The second step of the mechanism is the formation of the molecular ion 4 by the action of hydroxide ion with the molecular ion 2. This step involves the opening of the pyron ring. TS3 is formed when the distance between O11 and C2 is 1.933 Å.

The normal mode associated with the imaginary frequency of TS3 (754 cm^{-1}) indicates that the important stretching concern the atoms O11, C2 and O1. These stretching show the formation of the bond O11-C2 and the breaking of the bond C2-O1 as shown in the Fig. 3. The energy barrier of this step is $46.5\text{ kcal mol}^{-1}$.

Hydrogen transfer. The hydrogen transfer process involves the cleavage of the O11-H12 bond and the formation of the C3-H12 bond. On going from 4 to 4' through TS4, the bond C2-O11 becomes a carbonyl bond. The molecular ion 4 is less stable than the molecular ion 3. The calculated dipole moments (1.468 Debye for 3, 3.763 Debye for TS4 and 13.668 Debye for 4), indicating that the formation of 4 results in a markedly charge separation.

TS4 is characterized by one imaginary frequency (-2427

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Scheme 3. Mechanism of the formation of khellinone from khellin

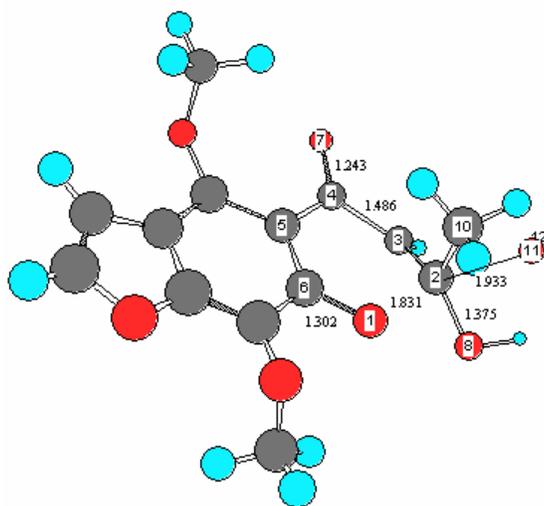


Fig. 3. PM3 calculated structure of transition state TS3. Distances are given in Å.

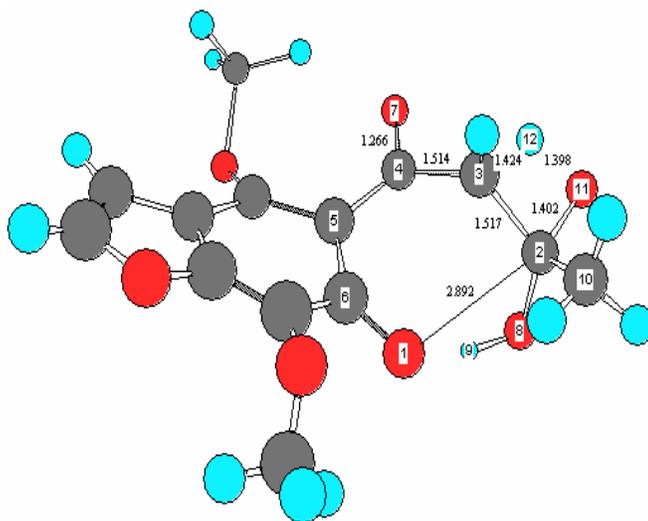


Fig. 4. PM3 calculated structure of transition state TS4. Distances are given in Å.

cm^{-1}) as shown in Fig. 4. The energy barrier of this step is 57 kcal mol^{-1} . The rearrangement of the molecular ion 4 to the molecular ion 4' gives a less stable molecule, more polar, and so more reactive.

The rearrangement of the molecular ion. The cleavages of the C2-C3 bond leads easily to molecular ion 5 and acetic acid. This step requires only 6 kcal mol^{-1} . TS5 is formed when the C2-C3 distance is 2.194 Å as shown in Fig. 5. The normal

Mode associated with the imaginary frequency (-239 cm^{-1}) describes mainly the C2-C3 bond stretching as the reason for breaking this bond. Finally, the molecular ion 5 gets hydronium ions and gives khellinone (6) without a transition state.

The total energy profile at 298.15 K and 1 atm is shown in Fig. 6 and the corresponding energies are listed in Table 1.

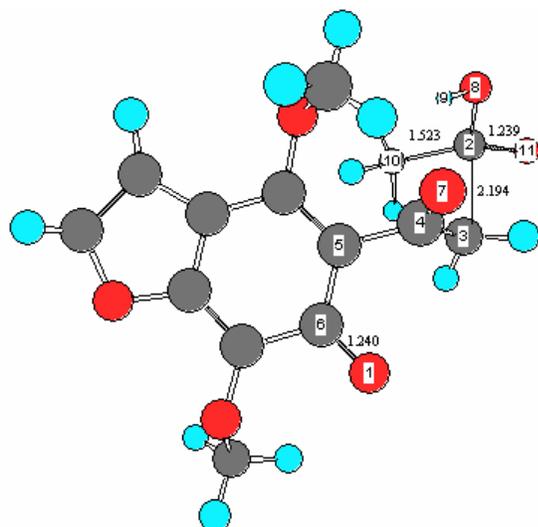


Fig. 5. PM3 calculated structure of transition state TS5. Distances are given in Å.

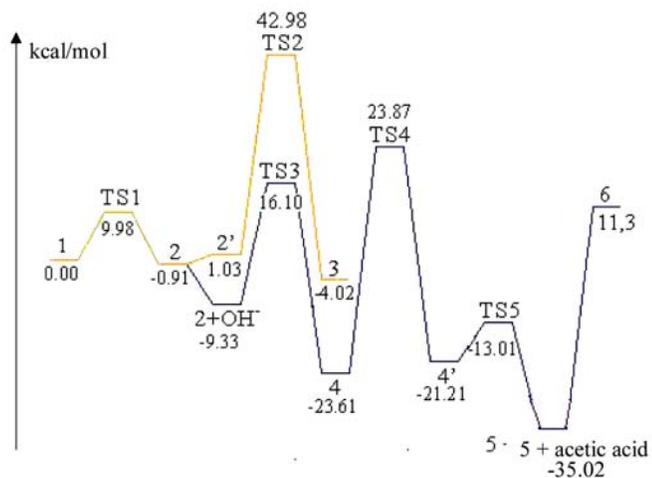


Fig. 6. Total energy of formation of ω -acetokhellinone (3) and khellinone by khellin hydrolysis using PM3 method.

CONCLUSIONS

From the above discussions, the following conclusions are obtained:

(I) The hydrolysis of khellin gives ω aceto khellinone and khellinone in alkaline media. The two mechanisms pass by closed enolate intermediate which opened in acidic media by

Table 1. Calculated Electronic Energies (ΔE), Including ZPE Corrections and Gibbs Free Energies (ΔG), of Various Minima and Transition States for the Complete Hydrolysis of Khellin

	ΔE	ΔG
1+OH ⁻	-14,635	-43,276
TS1	-4,648	-32,388
2	-15,549	-46,289
2'	-13,606	-43,352
TS2	28,347	-1,0831
3	-18,655	-51,156
TS3	1,469	-30,015
4	-38,248	-70,375
TS4	9,2613	-22,016
4'	-35,841	-68,750
TS5	-27,642	-59,782
2 + OH ⁻	-23,965	-57,005
Acid + 5	-49,653	-83,379
6	-3,336	-31,883

giving ω acetokhellinone.

(II) In alkaline media, the closed enolate intermediate gives molecular ion which produces khellinone by intramolecular rearrangement.

(III) This work highlights the crucial role of the media and we are doing know extensive calculations including the solvent effects on this type of calculations.

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