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1,2-Dipolar addition model for the cytoprotective activity of selected α,β-unsaturated compounds with C=O functionality: an ab initio study

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Abstract

The mechanism of the addition of a nucleophile (an alkylthiol group) to a double bond of α,β-unsaturated systems in the gas phase was explored. In this study, intermediates of the reaction were also investigated using ab initio calculations (RHF/6-31G* and MP2/6-31+G*). Our results indicate that direct dipolar attack of the S–H group of an alkylthiol on the C=C double bond is a reasonable reaction path. The present results represent, therefore, additional support for our hypothesis. This suggests that the mechanism of cytoprotection might be mediated, at least in part, by a reaction between the olefinic acceptor and the sulfhydryl-containing groups of the mucosa.

Keywords: 1,2-Dipolar addition model; Cytoprotective activity; α,β-Unsaturated compounds; Ab initio calculations

1. Introduction

We have previously demonstrated that sesquiterpene lactone molecules helenalin and dehydroleucodin (Scheme 1) significantly prevent the formation of gastric lesions induced by various necrotizing agents [1,2].

An interesting development is the discovery that α-methylene-γ-butyrolactone [**1**] and 2-cyclopentenone [**2**] are also potent cytoprotective agents [3] (Scheme 2). From these results, we focused our attention on these isolated reactive centers and other structurally related compounds. The precise action mechanism of these compounds at the molecular level is still unknown. However, we attributed the cytoprotective activity to the presence of a non-hindered olefinic acceptor in the molecules and suggested that the mechanism of protection might be mediated, at least in part, by a reaction between the olefinic acceptor and the sulfhydryl-containing groups of the mucosa (acting as the nucleophilic partner) (Scheme 2).

We have demonstrated previously that the presence of an α,β-unsaturated carbonyl system is a structural requirement but it is not sufficient for cytoprotection [3]. Also, our results support the establishment of important facts connecting chemical structure with cytoprotective effect. Firstly, an adequate molecular accessibility appears to be necessary to produce the biological response, and secondly, the olefinic

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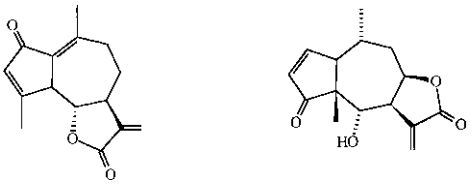


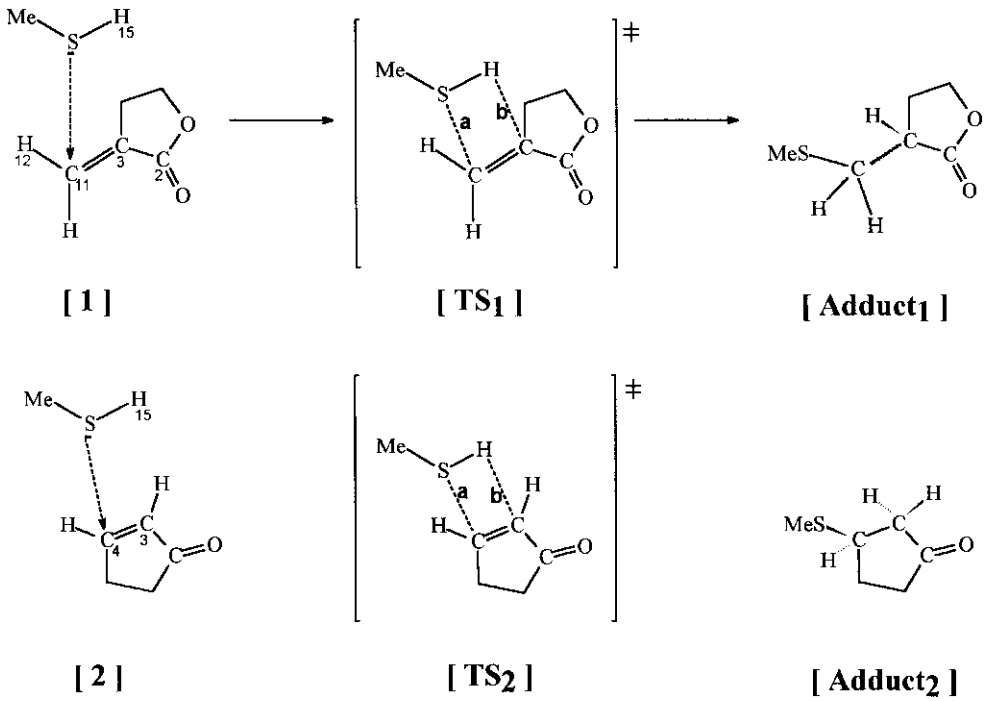
Scheme 1.

acceptor has to be included in a cyclic structure, or at least, in the proximity of a cyclic system. This result is not unexpected because, in general, cyclic derivatives, e.g. cycloalkenones, are more reactive than their acyclic counterparts, e.g. acyclic alkenones [4].

Several theoretical studies have been performed in order to shed light on the stereoselectivity of addition to C=C double bonds [5–7] as well as reactivity order of the activating groups [8–10]. However, compared with some other classes of conjugate acceptors, α,β-unsaturated lactones have received relatively little attention. Thus, although there are interesting features of the sulfhydryl group addition at the α,β-unsaturated system of lactones, this reaction has not been examined in terms of ab initio molecular orbital (MO) calculations.

The theoretical study of nucleophilic reactions that take place inside the active sites of biological systems with unknown three-dimensional (3D) structures can be quite challenging. Reduced glutathione, a tripeptide with 37 atoms in its neutral or zwitterionic form, demonstrates the increasing difficulties involved in such theoretical studies. Though this tripeptide may well be the biologically active agent, it is too large for accurate computational study. This fact makes it difficult to carry out ab initio study of such systems and their reactions. The use of downsized simulative models of glutathione is therefore desirable.

In the present report, we evaluate the mechanism of the addition of a nucleophile (an alkylthiol group) to an unsaturated double bond of compounds **1** and **2**, in terms of the molecular properties that are responsible for the reactivities of these compounds. In this study, intermediates of the reaction were investigated by use of MO calculations at the MP2 level of



Scheme 2.

theory. We have performed experiments with the same molecular systems of which calculations are reported here [3].

2. Methods of calculation

Geometries of reactants, products and transition states were fully optimized at the RHF/6-31G* and MP2/6-31+G* levels of theory. Ab initio calculations were carried out using the GAUSSIAN 94 system of programs [11]. The basis sets for these calculations were chosen from those incorporated in the GAUSSIAN 94 package. Geometry optimizations were performed with the optimization procedures in the GAUSSIAN 94 system of programs that is based on analytical calculation of the first derivatives of energy at the Hartree–Fock level. The electron correlation correction was carried out by the application of the Møller–Plesset (MP2) perturbational approach at the second order of theory [12–14].

The transition state (TS) geometries have been found according to the following procedure:

Firstly, a geometry of relative maximum energy has been found between the geometries of adduct molecules and reactant molecules separated at a distance of 8 Å. This geometry has been found at the AM1 [15] level by using the SADDLE technique described by Dewar et al. [16] as implemented in the MOPAC program [17].

We use this geometry as the starting point to optimize a TS in its neighborhood at AM1 level. The optimized reported geometries correspond to true TS stationary points because they have one unique imaginary vibrational frequency.

Next, starting with the AM1 optimized TS geometries, we have found the optimized TS geometries at the ab initio RHF and MP2 levels of theory. The ab initio geometries so found were proven to be true TS stationary points because there was only one imaginary vibrational frequency.

All ab initio energies were corrected for the zero-point and thermal energies to compare them with experimental results of parent compounds. The unscaled vibrational frequencies were used for calculating the zero-point and thermal energy corrections.

3. Results and discussion

*3.1. Potential energy surface (PES) representation for the alkylthiol attack on the C=C double bond of compounds **1** and **2***

In principle, a complete description of a reaction can be obtained if the potential energy surface is known [18–21].

For an n-atomic molecule, $3n$ coordinates (i.e. x, y and z for every atom) are needed to describe the position of the n atoms. If 3 coordinates are reserved for the translational mode (i.e. the x, y and z are coordinates for the center of mass) and 3 coordinates are used to describe the rotation of the molecule in space about its three principal axes of inertia, then $3n-6$ internal coordinates are required to define the structure and distortion of the molecule in question.

During a chemical reaction, all $3n-6$ internal coordinates change. Thus, the potential energy hypersurface may be regarded as a multivariable function

$$E = f(\chi_1, \chi_2, \chi_3, \ldots, \chi_{3n-6})$$

However, we can single out 2 of the $3n-6$ coordinates that change more drastically than the others during the chemical reaction [22]. For the reactions studied, the distances a(S···C) and b(H···C) (Scheme 2) were chosen to calculate the corresponding PESs (Figs. 1 and 2).

Figs. 1 and 2 depict the energy surfaces obtained from the AM1 calculations to the process **R** (reactants) → **TS** (transition state) → **P** (products).

The surfaces (Figs. 1 and 2, upper zones) exhibit minima for **R** and **P**, and they are equivalent to, but more informative than, the contour plots (Figs. 1 and 2, bottom), in which the viewer is positioned above the 3D surfaces. The reaction coordinates, i.e. the minimum energy reaction paths for the process **R** → **TS** → **P**, are shown by an arrow in Figs. 1 and 2. In both figures, there is a saddle point at the high energy region on the path between **R** and **P**. These saddle points are known as transition states (**TS**).

AM1 calculations appear to be particularly successful when they are applied to the evaluation of the alkylthiol attack on the C=C double bond of compounds **1** and **2**. It should be noted that since this method is based on the calculation of

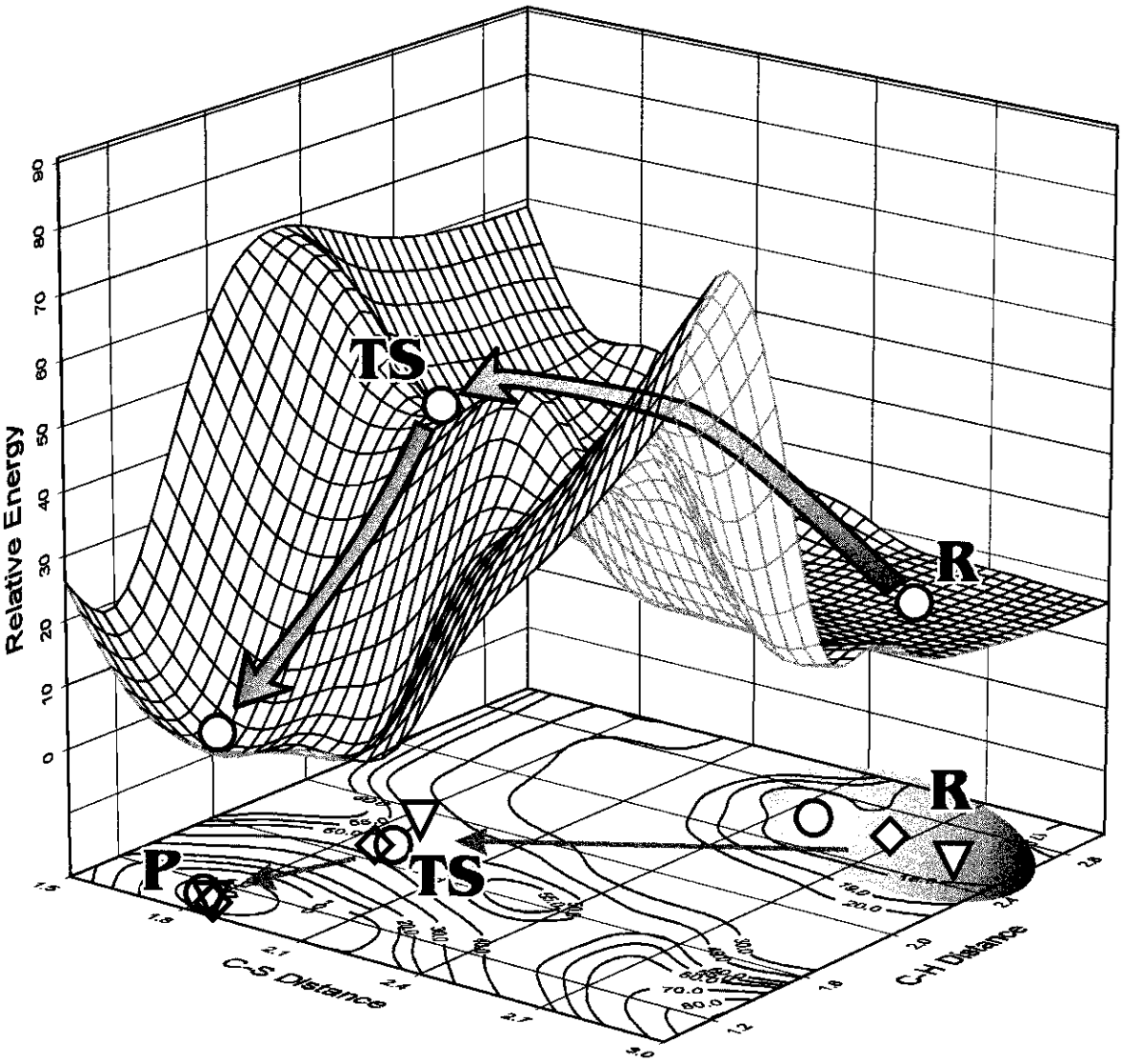


Fig. 1. Potential energy surface (PES) for the reaction: compound **1** + HS–CH_3 → [$Adduct_1$] (upper). Contour plot that corresponds to the PES (bottom). The critical points **R**, **TS** and **P** obtained at different levels of theory are represented as follows: (○) AM1; (▽) RHF/6-31G*; (△) MP2/6-31+G*. The reaction path is shown by an arrow.

electronic wavefunction, it can be applied to reactions controlled by electronic effects. However, one should be cautions in the interpretation of the results, because the parametrization is based upon properties of energy minima rather than transition structures.

In order to confirm the semiempirical-AM1 results, we perform ab initio (RHF/6-31G* and MP2/6-31+G*) calculations for each critical point obtained in the PESs shown in Figs. 1 and 2.

3.2. Ab initio (RHF/6-31G* and MP2) calculations

The concerted mechanism of addition has been investigated for both cases (**1** and **2**) which are envisaged to proceed by *syn* addition. In these cases, the

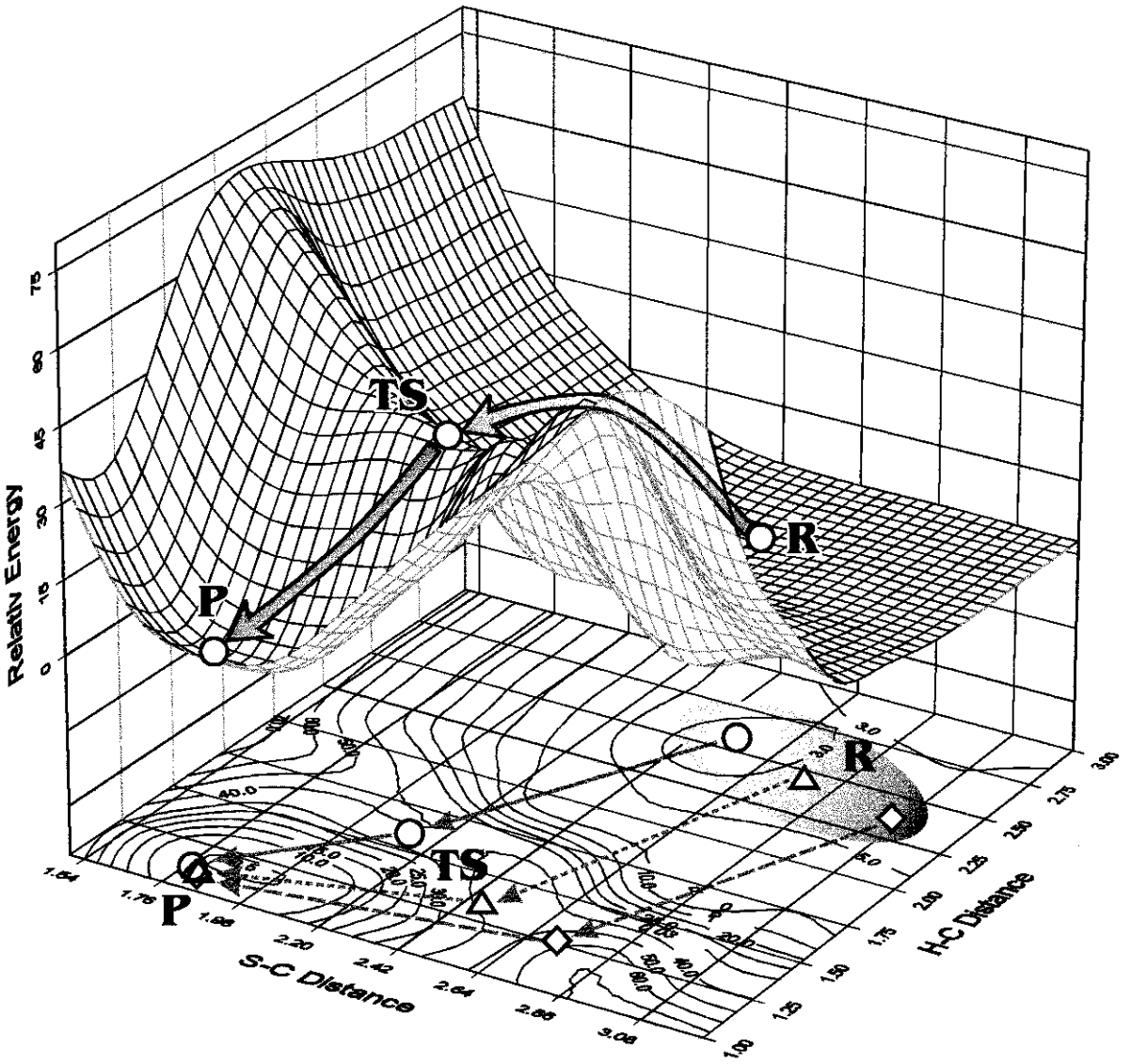


Fig. 2. Potential energy surface (PES) for the reaction: compound **2** + HS–CH_3 → [$Adduct_2$] (upper). Contour plot that corresponds to the PES (bottom). The critical points **R**, **TS** and **P** obtained at different levels of theory are represented as follows: (○) AM1; (▽) RHF/6-31G*; (△) MP2/6-31+G*. The reaction path is shown by an arrow.

nucleophilic sulfur of MeSH attacks at the β-position (Scheme 2).

Fig. 3 shows the reaction profile for the alkylthiol attack on the C=C double bond of **1** and **2** obtained from RHF/6-31G* calculations. The course of the nucleophilic addition at compound **1** is characterized by an incident angle of 100.4° with the S–C_{11} bond almost perpendicular to the H_{12}–C_{11}–C_3–C_2 plane (Scheme 2). These results are in agreement with our previous results [1,2,23] and in good accordance with our chemical intuition. Further approach of the alkylthiol to C_{11} along the angle of incidence, raises the energy of the complex until it reaches a transition state (TS_1) at an S–C distance of 1.86 Å. Along this path of approach, this state has the characteristics of a real transition state, with a single negative eigenvalue

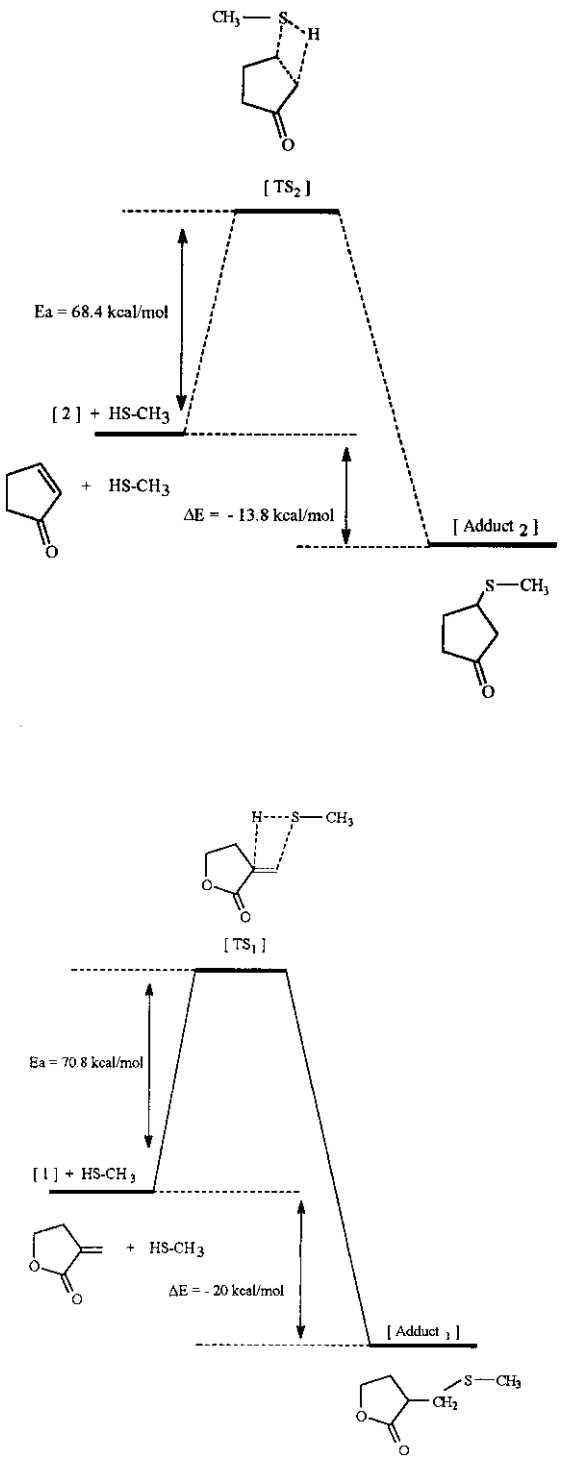


Fig. 3. Reaction profile for: (bottom) compound **1** + HS–CH_3 → [$Adduct_1$], from RHF/6-31G* calculations (bottom); and (top) compound **2** + HS–CH_3 → [$Adduct_2$], from RHF/6-31G* calculations.

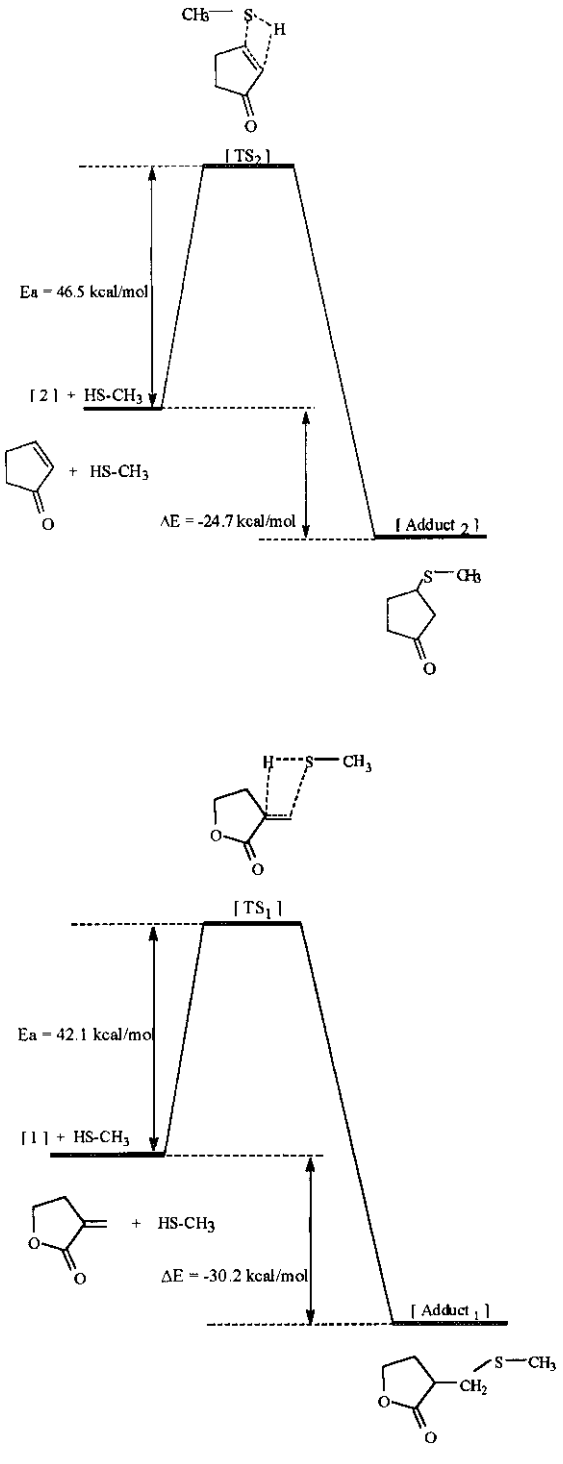


Fig. 4. Reaction profile for: (bottom) compound **1** + HS–CH_3 → [$Adduct_1$], from MP2/6-31 + G* calculations; and (top) compound **2** + HS–CH_3 → [$Adduct_2$], from MP2/6-31 + G* calculations.

in the Hessian matrix of force constants. The major contribution to the corresponding eigenvector originates from the S–C internal coordinate. From this transition state the complex moves down the potential energy surface to produce $Adduct_1$ which is the product of the addition of the S–H moiety across the double bond (Fig. 3). These results indicate that direct nucleophilic attack of alkylthiol on the C=C double bond is a reasonable reaction path.

All frequencies have imaginary values, confirming that the reported geometries for the adduct molecules correspond to true minima.

It should be noted that the set of results obtained strongly suggests that the alkylthiol attack at the C=C double bond of **1** is a highly exothermic process. It occurs if the reagent (MeSH) can assume the proper orientation. These results are in agreement with those previously reported for structurally related compounds and with experimental data [1].

The proposed reaction mechanism with a four-center TS that has been characterized as described previously is a true reaction path that shows it to be reasonable according to the estimated activation energies.

Fig. 3 also shows the results from RHF/6-31G* calculations for the alkylthiol attack on the C=C double bond of compound **2**. It should be noted that the RHF/6-31G* results suggest that in the case of compound **2**, the reaction mechanism is closely related to that obtained for compound **1**.

Theoretical calculations indicate that both reactions reported here are exothermic. However, ab initio RHF/6-31G* computations predict that the alkylthiol addition at **2** is less exothermic in comparison to the addition to compound **1**. Thermodynamic control thus favors the addition reaction between alkylthiol and α-methylene-β-butyrolactone [**1**].

These theoretical results are in good agreement with our experimental results previously reported [1,3]. However, it must be pointed out that the reliability of ab initio calculations may be affected by the omission of correlation energy, which could dominate the interaction between reactant units in the transition-state complex. Therefore, the calculated RHF/6-31G* activation energies are overestimated because Hartree–Fock methods do not consider the important correlation effects that occur in four-center TS molecules.

Table 1
Geometrical parameters of the dipolar addition reaction for compound **1** (lactone) and compound **2** (cyclopentenone) obtained at the RHF/6-31G* and MP2/6-31+G* levels of theory

	RFH/6-31G*		MP2/6-31G*	
	Interatomic distance (Å)	Bond angle (degrees)	Interatomic distance (Å)	Bond angle (degrees)
Lactone [TS_1]				
$S–C_{11}$	1.86		1.84	
$S–H_{15}$	1.44		1.43	
$C_3–H_{15}$	1.71		1.94	
$H_{15}–S–C_{11}$		76.3		80.7
$S–C_{11}–C_3$		100.4		101.6
Cyclopentenone [TS_2]				
$S–C_4$	2.71		2.46	
$S–H_{15}$	1.78		1.68	
$C_3–H_{15}$	1.27		1.35	
$H_{15}–S–C_4$		48.7		49.6
$S–C_4–C_3$		79.3		80.5

The Møller–Plesset perturbation theory is widely used as an efficient dynamic correlation correction beyond the Hartree–Fock level because it provides a size-consistent description of electron correlation effects [24]. Thus, in order to improve the above results, we performed calculations at MP2 level of theory. These results are shown in Fig. 4.

As expected, the values of activation energies obtained from MP2 computations are lower than those obtained from RHF/6-31G* calculations. The transition state for compound **1** (TS_1) is characterized by an S···C distance of 1.84 Å. A comparison between the most characteristic parameters of TS_1 and TS_2 obtained at the RHF/6-31G* and MP2/6-31+G* levels of theory are summarized in Table 1.

A spatial view for TS_1 and TS_2 obtained from MP2/6-31+G* calculations is shown in Fig. 5.

From Figs. 1 and 2, it should be noted that the spatial positions of critical points (**R**, **P** and **TS**) obtained using the different levels of theory are comparable. The most significant difference was observed for the transition state (TS) spatial positions obtained for compound **2** using the different levels of theory. Nevertheless, it is reasonable to expect this kind of difference, taking into account that the potential energy surfaces might be quite different from the semiempirical, Hartree–Fock and MP2 calculations.

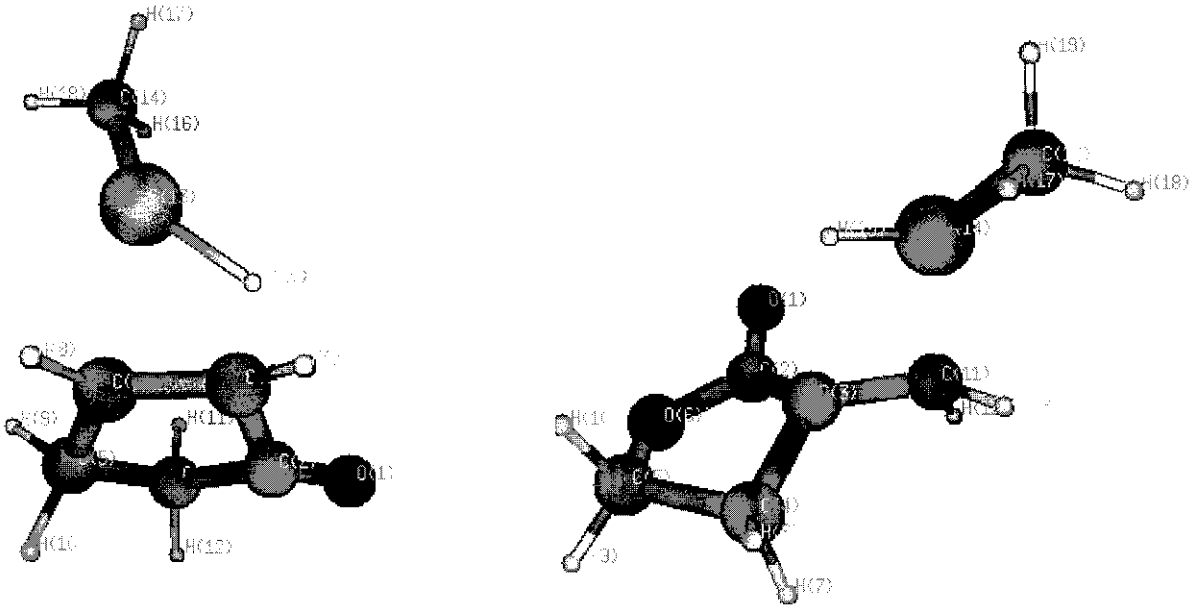


Fig. 5. Spatial view of the transition states [TS_1] and [TS_2] obtained from MP2/6-31+G* calculations.

4. Conclusions

On the basis of our results given by ab initio calculations using RHF/6-31G* and MP2/6-31+G* levels of theory, it became clear that theoretical computations at the semiempirical (AM1) molecular orbital (MO) calculations can provide useful information concerning the main features of energy surface. Of course, such information can be employed not only to complement experimental results, but also to develop trends and interpretations that are not directly accessible by experiment. Semiempirical calculations appear to be particularly successful. They provide a first approximate and an exploratory way to evaluate the potential energy surface. Ab initio RHF/6-31G* calculations are qualitatively good enough to find and characterize the different critical points (**R**, **TS** and **P**) on the energy surface. However, MP2 calculations indicate that the inclusion of electron correlation energy is absolutely necessary to provide quantitatively correct energy values for the different critical points on the potential energy surface describing the reaction mechanism.

On the other hand, our results are an additional support for our previous hypothesis suggesting that the molecular mechanism of cytoprotection might be mediated, at least in part, by a reaction between the olefinic acceptor and the sulfhydryl-containing groups of the mucosa. However, from the point of view of medicinal chemistry, one must be cautions in such interpretations because the theoretical calculations were performed in vacuo and we expect that solvent effects or biological environment effects will modify these results to a certain extent.

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