# Experimental and theoretical assessment of the mechanism involved in the reaction of steroidal ketone semicarbazone with hydrogen peroxide

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## ARTICLE INFO

## ABSTRACT

3β-Acetoxy-5α-cholestan-6-one semicarbazone **1** on reaction with hydrogen peroxide affords selectively 3β-acetoxy-5α-cholestan-6-spiro-1′,2′,4′-triazolidine-3′-one 2. The structural assignment of the product was confirmed by spectral data and elemental analysis. A free radical mechanism of the present reaction was described successfully by calculating theoretical models of **1**, **A**, **B** and **2**, using DFT with B3LYP/6-31G* basis set. It was found that the reaction undergoes through the formation of two radical intermediates and the only one isomer of the product in which –NH–CO– group is *cis* with respect C5α-H, was selectively obtained. Frontier molecular orbital, spin electronic density, electrostatic potential and atomic charges were discussed.

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## 1. Introduction

The establishment of an efficient method for synthesizing tria-zolidinones, the nitrogen-containing heterocyclic compounds has received great attention for drug design in the development of pharmaceutical and agricultural field. It has been reported that triazolidinone has the ability to increase the yield of the plant food, which is contained in the plant storage organs like potatoes [1]. It is also reported that triazolidinone does activate the human adrenergic receptor [2]. Krenzer has emphasized on synthesis and herbicidal activities of different type of triazolidinone [3,4]. Various research workers have adopted different approach towards the synthesis and structural determination of triazolidinones [5–9]. However, Schantl and Gstach have reported the synthesis of tria-zolidinone from arylazoalkylisocynate using Grignard reagent *via* nucleophilic addition [10].

In recent years, density functional theory (DFT) has become a shooting star in molecular quantum mechanics for explaining the reaction mechanisms. The main idea of DFT is to describe an interacting system of fermions *via* its density and not *via* its many-body wave function. Thus the development of better and better exchange-correlation functional made it possible to calculate many molecular properties with comparable accuracies to traditional correlated DFT methods, with more favorable computational costs [11]. According to the literature, it has been proved that DFT has

a great accuracy in reproducing experimental data of molecular structural properties, IR frequencies, intensities, etc. [12–14].

In continuation of our previous work on synthesis and theo-retical investigation [15–19] of biologically interesting steroidal compounds, herein we present a novel and convenient method of formation of steroidal spiro-triazolidinone. Its structure is estab-lished by physical, elemental, spectral and computational data. A free radical reaction mechanism of the present reaction is also described implying the density functional theory.

## 2. Materials and methods

### 2.1. General

The IR (KBr) spectrum recorded on a Perkin-Elmer 782 infrared spectrophotometer. ^{1}H NMR spectra was recorded on a Bruker BZH-200 instrument. Chemical shifts are reported in parts per million downfield from tetramethylsilane. Multiplicities of proton reso-nance were designated as singlet (s) and multiplet (m). Melting point reported is uncorrected. Chloroform and hydrogen perox-ide were purchased from the Aldrich Chemical Company. Light petroleum ether and ethyl acetate were supplied by Merck.

## 3. 3β-Acetoxy-5α-cholestan-6-spiro-1′,2′,4′-triazolidine-3′-one 2

The solution of 3β-acetoxy-5α-cholestan-6-one semicarbazone **1** [20] (1.05 g, 2.10 mmol) in chloroform (25 mL) was treated with

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excess of $H_2O_2$ (30%, 4 mL) and stirred for 2.5 h at 0 °C. The progress of the reaction was monitored by TLC. As the reaction proceeds the solution turned yellow in color. The organic layer was separated, dried over anhydrous sodium sulfate and then evaporated to dryness *in vacuo*. The resultant crude residue was purified over silica gel column (light petroleum ether–ethyl acetate; 4:1), gave the title compound **2** (81.5% yield, mp 166–167 °C) as a square shaped, white crystals. IR $\nu_{max}$/cm$^{-1}$ (KBr): 3340 (N–H), 1700 (C=O), 1480 (C–N) and 1040 (C–O). $^1$H NMR (200 MHz; CDCl$_3$; Me$_4$Si): $\delta$ 7.85 (2H, s, N*H*CON*H*), 6.7 (1H, s, N*H*), 2.04 (3H, s, OCOC*H*$_3$) and 4.24 (1H, m, *W1/2* 18 Hz, C$_3\alpha$-*H*). Analysis. Calculated for C$_{30}$H$_{51}$N$_3$O$_3$: C, 71.81; H, 10.24; N, 8.37. Found: C, 71.86; H, 10.31; N, 8.48%.

### 3.1. Computational method

The molecules calculated in this article are 3β-acetoxy-5α-cholestan-6-one semicarbazone **1**, radical intermediates (**A** and **B**) and 3β-acetoxy-5α-cholestan-6-spiro-1′,2′,4′-triazolidine-3′-one **2**. The structure **3**, where NH–CO– group is *trans* with respect to C5α-H is also calculated. All molecules were fully optimized in order to describe the reaction mechanism of the present reaction and to support the stereochemistry of the product.

The lower energy conformation of all the structures was obtained by using *ab initio* theory. However, the molecular calculations started with the fully optimized, semiempirical PM3 level of theory, followed by the Hartree–Fock (HF) model with minimal STO-3G basis set. The resulting wavefunction, Hessian matrix and the geometry of molecules obtained were used to calculate HF with next level of calculation that is split-valence basis set 3-21G(*). The obtained geometry was further subjected for the calculation with higher level of basis set, HF/6-31G*. The procedure was further applied for a final calculation that is DFT with B3LYP/6-31G* method. The "d" polarized functions were added for carbon, oxygen and nitrogen atoms. Stability and feasibility of all the optimized structures were supported by calculating their fundamental frequencies and assigned as local minima (all real frequencies).

Total energy, atomic charges, electrostatic potential, spin electronic density, dipole moment and frontier molecular orbitals (FMO) were calculated. The energy of highest occupied molecular orbital (HOMO) and lowest unoccupied molecular orbital (LUMO) was used for calculating the hardness as an index of molecular reactivity [21,22]. The molecules studied in this paper were built with Spartan'04 Windows [23], a graphical software for quantum chemical calculations.

## 4. Results and discussion

The synthesis of requisite 3β-acetoxy-5α-cholestan-6-spiro-1′,2′,4′-triazolidine-3′-one **2** from 3β-acetoxy-5α-cholestan-6-one
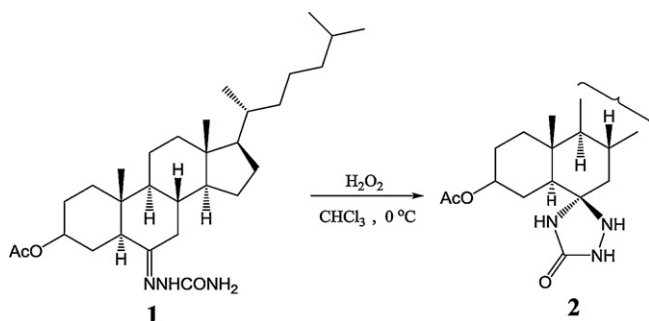
semicarbazone **1** on reaction with hydrogen peroxide was accomplished with good yield, as summarized in Scheme 1.

### 4.1. Spectroscopic data

Selected diagnostic bands of infrared spectrum of β-acetoxy-5α-cholestan-6-spiro-1′,2′,4′-triazolidine-3′-one **2**, provided vital information for determining its structure. The IR spectrum exhibited characteristic absorption bands at 3340 for N–H, 1480 for C–N and 1040 cm$^{-1}$ for C–O. The presence of amide carbonyl stretching frequency at 1700 cm$^{-1}$, which is the evidence of 5 membered cyclic amide ring, also supports the structure **2**. Besides, $^1$H NMR spectrum showed a two-proton broad singlet at $\delta$ 7.85 (N*H*CON*H*), a three-proton singlet at 2.04 (OCOC*H*$_3$), one-proton multiplet at 4.24 (C$_3\alpha$-*H*) and one-proton singlet at 6.7 (N*H*) (exchangeable with deuterium). All the spectral data are in good agreement with the desired structure **2**.

### 4.2. Stereochemistry

The spiro-cyclization of steroidal ketone semicarbazone forms respective spiro-1′,2′,4′-triazolidine-3′-one. It is proposed that there is a considerable amount of steric hindrance to the ring-closure from one side of the ring at C-6, which might be explained on the basis that amide (–NH–CO–) group is more bulky than –NH–NH– group. Thus, the triazolidinone ring closes at C-6 by the attack of –CO–HN• (being a radical) of semicarbazone moiety, preferably in the way that –NH–CO– group became equatorially (α) attached to the C-6 position to avoid 1,3-diaxial interaction due to C10β–CH$_3$, the angular methyl group. Accordingly, only one isomer of this reaction **2**, in which –NH–CO– group is *cis* with respect to the C5α-H, was selectively obtained. Further, formation of this
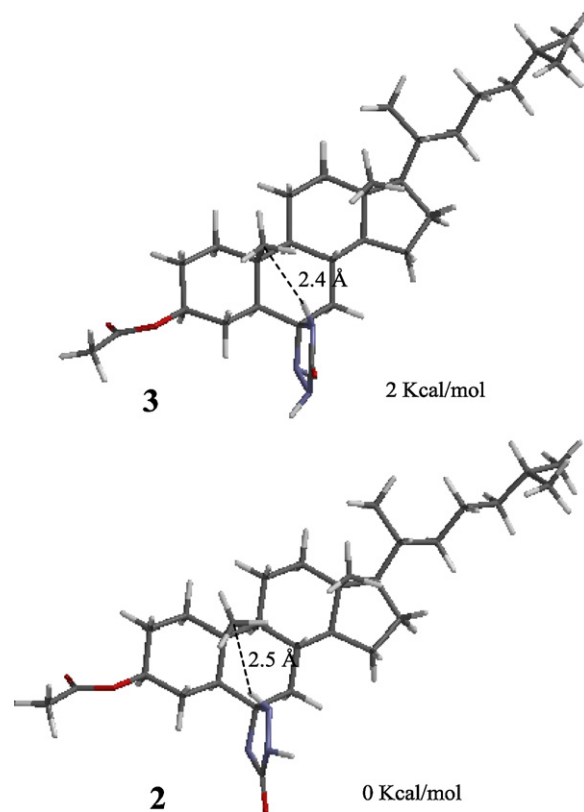


**Fig. 1.** Calculated molecular structure of **2** and **3** is shown. In structure **2**, –NH–CO– group is a *cis* with respect to C5α-H. In structure **3**, –NH–CO– group is a *trans* with respect to C5α-H.



**Scheme 1.** Synthetic scheme of formation of steroidal 6-spiro-1′,2′,4′-triazolidine-3′-one.