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Structural changes on citronellol induced by gamma radiation

Diana Rodríguez-Linares[a]*, Edelsys Codorniu-Hernández[a], Yuri Aguilera-Corrales[a], Manuel Rapado-Paneque[b], Rolando Quert-Alvarez[a] and Noel Ferro[c]

Structural changes in pure citronellol after exposure to different doses of gamma radiation were elucidated by spectroscopic techniques and molecular modelling methods. The citronellol samples were irradiated in an MPX-γ30 installation (^{60}Co, $E = 1.25$ MeV), absorbing in a dose range from 1.45 to 10.02 kGy. Citronellal and hydroxycitronellal compounds were detected in the irradiated samples. Some mechanisms of radiolytic transformations for citronellol were proposed and modelled at semiempirical quantum chemical level. These results provide the basis for the possible development of new synthetic pathways for close structurally related compounds, based on the use of gamma irradiation. Copyright © 2009 John Wiley & Sons, Ltd.

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Keywords: gamma radiation; citronellol; monoterpenes; organic compounds; radiolysis

INTRODUCTION

In the past decade, significant progress has been made in the analysis of natural product biosynthesis pathways of terpenoids.[1,2] In addition, many investigations have been made on its physical and chemical behavior[3] as well as on obtaining structurally related terpenic compounds with certain applications in the agrochemical and pharmaceutical industry such as rose oxide[4] and hydroxycitronellal.[5] Citronellol is a typical acyclic monoterpenoid including one double bond and one alcoholic group (Fig. 1a). It is recognized as a fragrant ingredient of oils of citronella,[6] damascene rose,[7] and geranium[8] as well as the second component from the essential oil of *Eucalyptus citriodora* Hook.[9] Therefore, it has been extensively applied in food, perfume, cosmetic industry, and as an insect repellent.[1–3,5–10]

However, no attention has been given to the use of ionizing radiation as a pathway in the synthesis of citronellol-related compounds. Some results about the irradiation of orange juice[11] and about the study of gamma radiolysis of chiral molecules have been published.[12] Nevertheless, to determine which radicals in these complex systems have the major influence in the chemical transformations is a critical endeavor that remain to be seen. We focused the present work on the effect of gamma radiation on isolated acyclic monoterpenes. Structural changes of citronellol after exposure to different doses of gamma radiation were assessed by spectroscopic techniques and molecular modeling methods. It would allow a better understanding of the natural mixtures irradiation. This proposal could be used as the basis for the design of new synthetic pathways, saving reactants and time.

MATERIALS AND METHODS

Citronellol, natural compound used in the present research, has a purity of 97% (Aromáticos Gama, S.A. de C.V.).

Gas chromatograph coupled mass spectrometry (GC-MS)

CG-MS analysis was performed using a Crom 6890 EM 5973 (Agilent Technologies, US) equipped with a SPB-5 column (15 m × 0.25 mm i.d.; 0.10 μm film thickness). Detector and injector temperature were set at 230 and 250 °C, respectively. The following oven temperature regime was used: first, stabilized at 60 °C during 2 min, next increased to 100 °C at 4 °C/min, and later at 10 °C/min up to 200 °C (total oven program = 22 min). Carrier gas used was helium with a constant flow of 1 ml/min. The ionization energy was set at 70 eV. Each component of samples A, B, C, and D were identified by using the NISTASCI database of the GC-MS system. The percentage composition was computed from the GC peak areas according to the 100% method without any correction factors.

Infrared spectroscopy with Fourier transformed (FT-IR)

An infrared spectrometer with Fourier transformed (FT-IR) Magna 550 (Nicolet Instrument Corp., Madison, WI) with software Nicolet

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* *Correspondence to: D. Rodríguez-Linares, Department of Molecular Design and Synthesis, Higher Institute of Technologies and Applied Sciences, Ave. Salvador Allende y Luaces, Quinta de los Molinos, Plaza de la Revolución, Ciudad Habana, CP 10600, AP 6163, Cuba.*
E-mail: dira@instec.cu

a *D. Rodríguez-Linares, E. Codorniu-Hernández, Y. Aguilera-Corrales, R. Quert-Alvarez*
Department of Molecular Design and Synthesis, Higher Institute of Technologies and Applied Sciences, Ciudad Habana, Cuba

b *M. Rapado-Paneque*
Center of Nuclear Development Applied Studies, Ciudad Habana, Cuba

c *N. Ferro*
Institute for Plant Genetic, Group Molecular Biochemistry, University of Hannover, Hannover, Germany

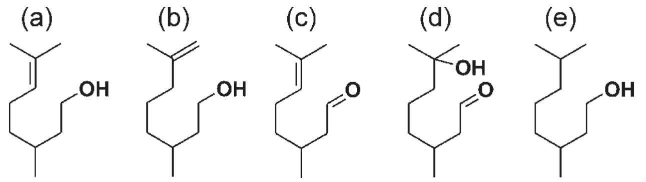

Figure 1. Molecular structure of citronellol and related monoterpenes: (a) (citronellol, cas [106-22-9]); (b) (rhodinol, cas [6812-78-8]); (c) (citronellal, cas [106-23-0]); (d) (hydroxycitronellal, cas [107-75-5]); (e) (dihydrocitronellol, cas [106-21-8])

OMNIC 2.1 was used. Citronellol samples (10 μl) were placed inside the cell, in their usual oleaginous liquid state. The spectra were accumulated from 64 scans with a spectral resolution of 4 cm^{-1}.

Irradiation of citronellol samples

In order to carry out the irradiation process, 2 ml of citronellol pattern was introduced in glass ampoules of 2.5 ml and sealed with paraffin. Therefore the samples were placed in the irradiator; model MPX-γ30, with a dose rate of 1.83 kGy/h. The irradiation time inside the irradiator was measured with a chronometer with a precision of ± 0.1 min. To verify the dose rate a filmic dosimeter PERPEX analyzed with CALIMBA software was used.

Calculation of absorbed dose

Absorbed dose was calculated using the irradiation time in hours and the dose rate (8.71 kGy/h). The irradiated samples of citronellol were called B (1.45 kGy), C (6.07 kGy), and D (10.02 kGy) for a better understanding of experimental results. Sample A was used to identify the citronellol pattern.

COMPUTATIONAL DETAILS

In order to perform a theoretical study about the reaction mechanisms that take place in citronellol samples after different doses of gamma radiation two general steps were followed:

1. The exploration of the potential energy surface (PES) of reactants and products of the proposed mechanisms by quantum mechanical methods.
2. The calculation of the energy profile for the proposed reaction mechanisms, including the transition state (TS) structures.

Citronellol, citronellal, and hydroxycitronellal molecules (Fig. 1a, c, and d) present a large conformational variability due to their acyclic forms and several simple bonds with free rotation. The PES of each molecule was explored by a conformational analysis with PC Spartan Pro software.[13] Molecular mechanics (MM) methods as well as semiempirical Hamiltonians AM1[14] and PM3[15] were used to obtain the conformational analysis results.

The most stable conformers of each distribution were reoptimized through semiempirical Hamiltonians AM1,[14] PM3,[15] and PDDG/PM3[16] using the semiempirical package MOPAC version 6.0.[17] The structural changes observed in the irradiated citronellol spectra were used to propose different mechanisms of radiolysis transformations for citronellol. These mechanisms were modeled using the minimum structures from the conformational analysis as starting geometries. The energy profiles of the proposed reaction mechanisms were modeled using PC Spartan Pro.[13] It was designed for the study of chemical reactions. AM1 semiempirical Hamiltonian was used for obtaining the stationary point and the TS structure.

RESULTS AND DISCUSSION

Spectroscopic characterizations

GC-MS have been the most widely used technique in the analysis of terpenic compounds in natural mixtures; instead of many other chromatographic methods that have been exhaustively studied.[1–10] Each component detected in samples A (citronellol pattern), B (1.45 kGy), C (6.07 kGy), and D (10.02 kGy) were identified comparing the mass spectra obtained (picks presented and intensity rates) with those from the NISTASCI database of the GC-MS system. The percentage composition reported in the present paper was computed from the GC peak areas according to the 100% method without any correction factors. In spite of these percents are semiquantitative values they offer a first approach to identify the structural changes in the irradiated citronellol samples. The chromatographic data are presented in the Table 1. (All the spectra are provided as supplementary information.).

Citronellol pattern

Citronellol pattern shows a purity higher than 97%. A corresponding analysis of impurities of citronellol samples was carried out by GC-MS. The results show two main impurities: rhodinol (2.34%) and dihydrocitronellol (0.14%) and these are structurally related with citronellol. All the signals observed in the FT-IR spectrum of citronellol pattern are consistent with the molecular structure of citronellol. These analysis of citronellol

Table 1. Percent composition of compounds in the citronellol pattern (sample A) and irradiated samples B, C, and D

Signal	Compounds (%)	$t_{\text{retención}}$ (min)	Sample A (0 kGy)	Sample B (1.45 kGy)	Sample C (6.07 kGy)	Sample D (10.02 kGy)
1	Citronellal	4.24	—	0.50	0.55	1.27
2	Dihydrocitronellol	5.34	0.14	1.61	1.75	1.75
3	Rhodinol	5.87	2.34	0.75	0.76	0.85
4	Citronellol	6.17	97.52	95.09	94.77	86.95
5	Nid1	8.84	—	1.02	1.06	3.21
6	Hydroxycitronellal	9.70	—	1.03	0.95	3.36
7	Nid2	13.62	—	—	—	2.61

pattern (sample A) spectra corroborated that this sample is appropriate to carry out the irradiation study.

Irradiated citronellol samples

The signals in the chromatograms of the irradiated samples B, C, and D were identified by MS as terpenes which are structurally related with citronellol: citronellal, hydroxycitronellal, rhodinol, and dihydrocitronellol (Fig. 1c, d, b, and e).

In chromatograms of samples B and C three new signals are shown with respect to the chromatogram of sample A: signal **1** corresponds to citronellal (retention time of 4.24 min), and signal **6** corresponds to hydroxycitronellal (retention time of 9.07 min). In addition, the signal identified with number **5** is a non-identified compound on the used database (retention time of 8.85 min). This was called non-identified compound 1 (Nid1) and that will be used in later analysis.

It was noted that any chromatographic signal disappears with the increase of the absorbed dose. Figure 2 shows the variations in the percentage values of compounds in the irradiated samples regarding sample A, at absorbed doses of 1.45 kGy (sample B), 6.07 kGy (sample C), and 10.02 kGy (sample D). The percent of each component does not show differences between samples B and C. However in sample D (highest dose), there is a large variation of the percentage values for some components (Fig. 2). This suggest that there are some complex regeneration mechanisms in the dose range 1.45–6.07 kGy (B-C),[19] which are displaced at the highest dose.

One of the oxidation products of citronellol is citronellal (**1**), following the logical pathway of oxidation as the main transformation under the irradiation of organic samples.[18,19] The percentage of citronellal is lower than hydroxycitronellal in the range of the studied absorbed dose. This fact indicates that citronellal is formed in the irradiation process and its radiolysis[18,19] favored the hydroxycitronellal formation. This process (**6**) can be explained by a mechanism in two steps: (1) the attack of a hydrogen radical to the double bond, forming a tertiary radical (the most stable of the two radicals that can be formed),[18,19] followed by (2) its interaction with a hydroxyl radical with the corresponding elimination of the double bond (which is in citronellal molecular structure at position 6, 7).

Rhodinol percent decreases in sample B (0.75%) with respect to the sample A (2.34%). This behavior is similar to citronellol but in sample C and D increased up to 1% lightly. This indicates the occurrence of the regeneration of this compound. But such analyses are beyond another scientific report and will not be further discussed here.

Dihydrocitronellol percent increases from sample A to B, until 1.61%, and then at 1.75% in sample C, being constant in sample D. This supports the fact that radical $\dot{H}$ participates in the radiolytic transformations of citronellol,[20,21] because dihydrocitronellol is the product of citronellol's hydrogenation, which could be performed by hydrogen radical. It must be pointed out that the most abundant compounds formed from citronellol by the action of gamma radiation were Nid1 and hydroxycitronellal in the sample D (3%). Nid2 is only present at high dose in this sample.

The FT-IR spectra of B, C, and D samples do not show differences. Signals related to hydroxyl group and double bond carbon-carbon are observed in FT-IR spectra of samples B, C, and D. These bands can also be seen in the FT-IR spectrum of sample A. They also present the signals corresponding to the ketone group.

Citronellol, rhodinol, dihydrocitronellol, citronellal, and hydroxycitronellal were identified in the irradiated samples by GC-MS, and their presences were confirmed by the FT-IR spectra. According to this information and based on the fundamental mechanisms of radiolysis in pure organic compounds, we propose different reaction mechanisms for the citronellol radiolytic transformations.

Mechanisms of citronellol radiolytic transformations

Degradation (oxidation) and polymerization are the most common process induced by the action of ionizing radiation in organic compounds, taking place by increasing the absorbed dose.[18,19] In the absorbed dose range of the present study (1.45–10.02 kGy) the main expected process is the oxidation, because polymerization process had not been observed in similar studies.[11]

There are many charged and/or radical species that can arise in the relaxation process of a molecule excited by the ionizing radiation. However, in non-polar media, these charged species

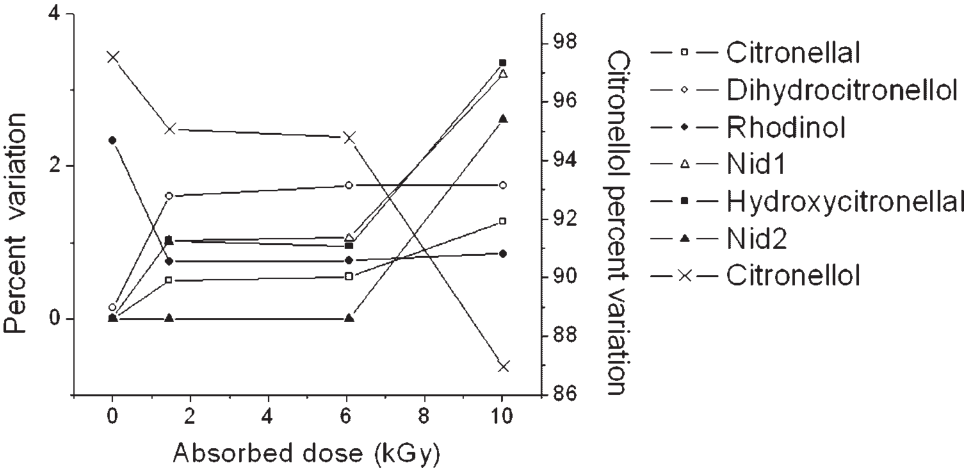

Figure 2. Percent variation of each compound with the absorbed dose

are less solvated, favoring recombination to the initial excited molecule. This is not the case of radical formation, where the recombination process has lower trend. For that reason, we considered only radicals in the studied mechanisms.

Chemical processes of degradation (citronellol) and formation (citronellal and hydroxycitronellal) are the main points in this discussion. Citronellal and hydroxycitronellal should be stable products of citronellol radiolysis because they were not presented in chromatogram of sample A and their percentages increase with the absorbed dose.

Compounds that keep constant their relative percent should degrade and regenerate in the same magnitude or are stable to exposition of gamma radiation. This is the case of rhodinol and dihydrocitronellol in the dose range of 1.45–10.02 kGy.

The initial assumption for the evidence of $\dot{OH}$ radical intervention in the radiochemical processes induced by gamma irradiation was that the radicals were produced trough the scission of the hydroxyl group of the citronellol molecule. As it is well known, functional groups are more exposed to suffer the effects of radiolysis than the rest of the backbone. Therefore, the product corresponding to this structural change (2,6-dimethyl-2-octene) was not identified by MS between the final products of radiolysis, and theoretical studies show that this transformation is not thermodynamically favored.

Next, it is necessary to take into account that as a consequence of the distillation procedure to obtain citronellol the final product contains 0.2% of water (as it is was informed by the supplier). Therefore, $\dot{H}$ and $\dot{OH}$ radicals participate in these transformations as consequence of the water radiolysis. In the proposal of the citronellol radiolysis mechanisms it was assumed that $\dot{H}$ is the most abundant radical, because $\dot{H}$ has many possibilities of formation by C—H bond rupture. In fact, it has been reported as one of the most abundant radical in the radiolysis of pure organic compounds.[18]

The structural changes involving $\dot{H}$ radical have to be related to the attack of citronellol molecule in C=C or the oxygen hydroxyl group. It is in agreement with previous reports that ionizing radiation acts preferably on the functional groups of the organic compounds.[20,21] In addition, the stability rules of radicals (tertiary > secondary > primary) was considered, as the most stable radicals have more probabilities of participating in the formation of final products of radiolysis.[18–21]

Oxidation mechanism of citronellol

Previous theoretical studies about citronellol oxidation to citronellal were not found, neither of similar compounds. However, two mechanisms are proposed for the formation of an aldehyde starting from the corresponding primary alcohol, induced by ionizing radiations.[19] The mechanism proposed in this research has a single step that consists of a rearrangement of citronellol molecule where a hydrogen molecule is removed (Fig. 4 A), based on the arguments mentioned above (Fig. 3A).

The structure of the TS obtained for the oxidation of citronellol is shown in the mechanism energy profile (Fig. 4). Only one imaginary frequency was obtained, corresponding to H—H bond vibration in the formation of citronellal molecule when a H_2 molecule is removed from citronellol. That confirms that there is a TS corresponding to citronellal molecule formation starting from citronellol. This transformation is exothermic ($E = -17.04$ kcal/mol) and the energy barrier is of 74.77 kcal/mol.

Mechanism of hydroxycitronellal formation

The mechanism of hydroxycitronellal formation starting from citronellal is very specific. On the one hand, the presence of hydroxycitronellal in the irradiated citronellol samples was confirmed experimentally for GC-MS (MS comparison with database), and in the same way we realized that this is the most abundant identified compound formed by gamma radiation action (GC area peaks). On the other hand, citronellal is formed in relatively small quantities that only rise above the 1% at maximum dose, what is a non-common issue, considering that citronellal is the primary product of citronellol radiolysis. We also

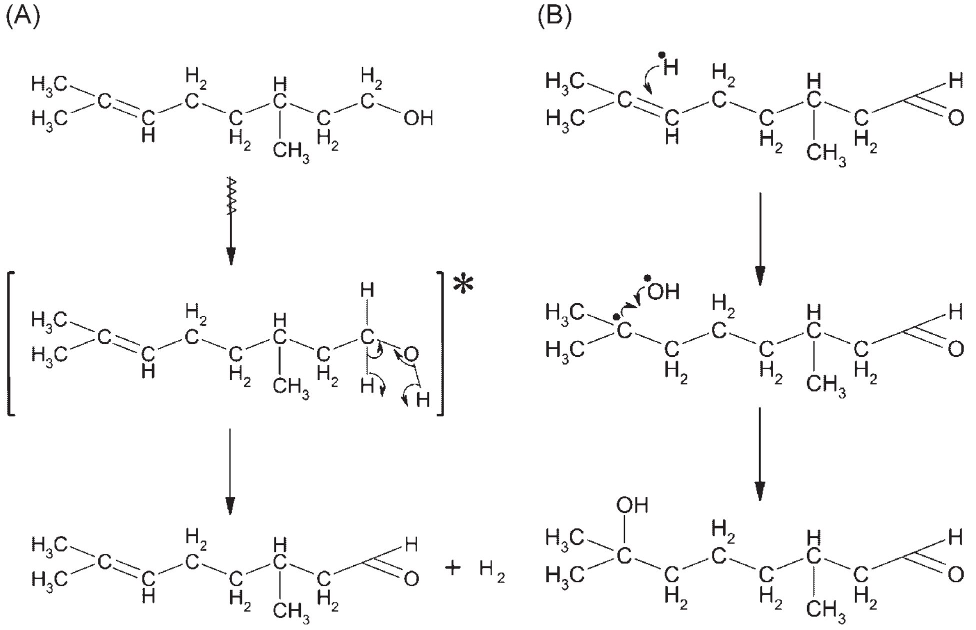

Figure 3. Mechanisms proposed induced by gamma radiation. Citronellal formation (A), hydroxycitronellal formation starting from citronellal (B).

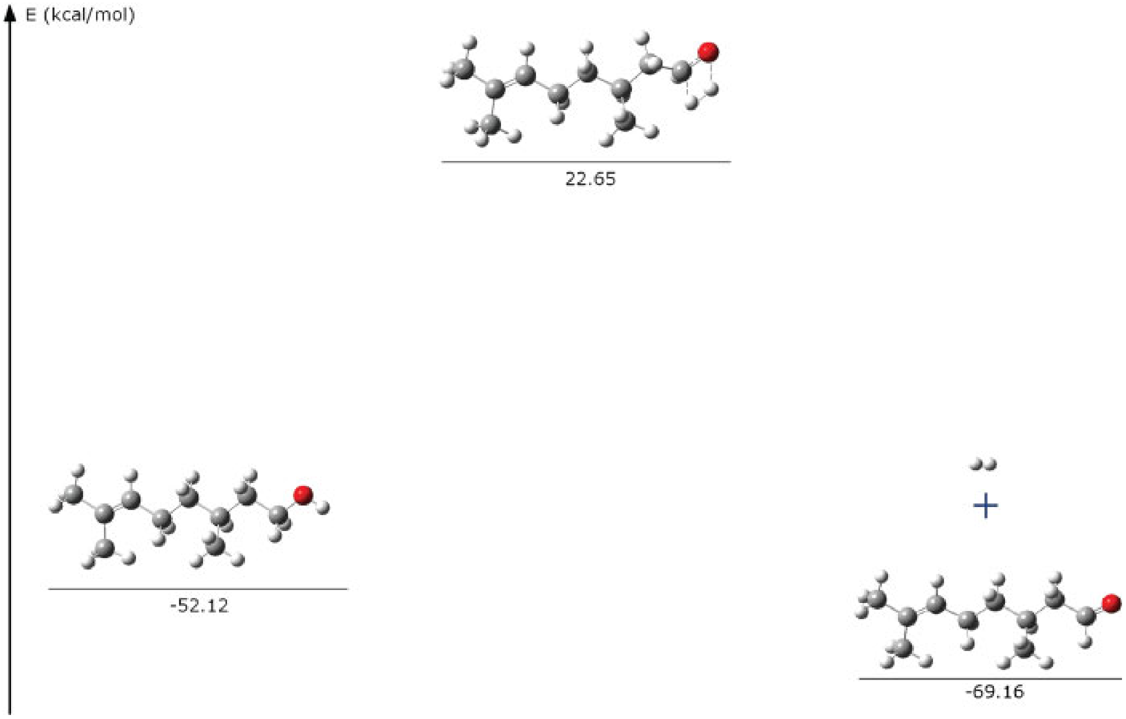

Figure 4. Energy profile of mechanism proposed for citronellal formation

know that the final products of organic compound radiolysis are exposed to suffer radiolysis.[18] Based on this information we propose the mechanism of Fig. 3B.

This mechanism for the formation of hydroxycitronellal starting from citronellal explain the fact that citronellal is presented in smaller quantity than hydroxycitronellal in the studied dose range (Fig. 5), in spite of being the major product of the citronellol radiolysis. The first step of this mechanism is the attack of an $\dot{\mathrm{H}}$ to the double bound C═C of the citronellal molecule, forming the most stable radical (tertiary) of the two possible radicals. In the second step, the tertiary radical combines with an $\mathrm{O}\dot{\mathrm{H}}$ to form the hydroxycitronellal molecule.

The TS structure obtained is shown in the energy profile (Fig. 5) and has an imaginary vibration frequency regarding to hydroxycitronellal formation. It confirms the proposed structure as a TS. This is an exothermic transformation ($E = -67.83$ kcal/mol) and energy barrier is only 2.82 kcal/mol. It would explain why citronellal is presented in an abnormal very low percent in the whole studied dose range, in spite of citronellal is the primary product of citronellol radiolysis; since process of hydroxycitro-

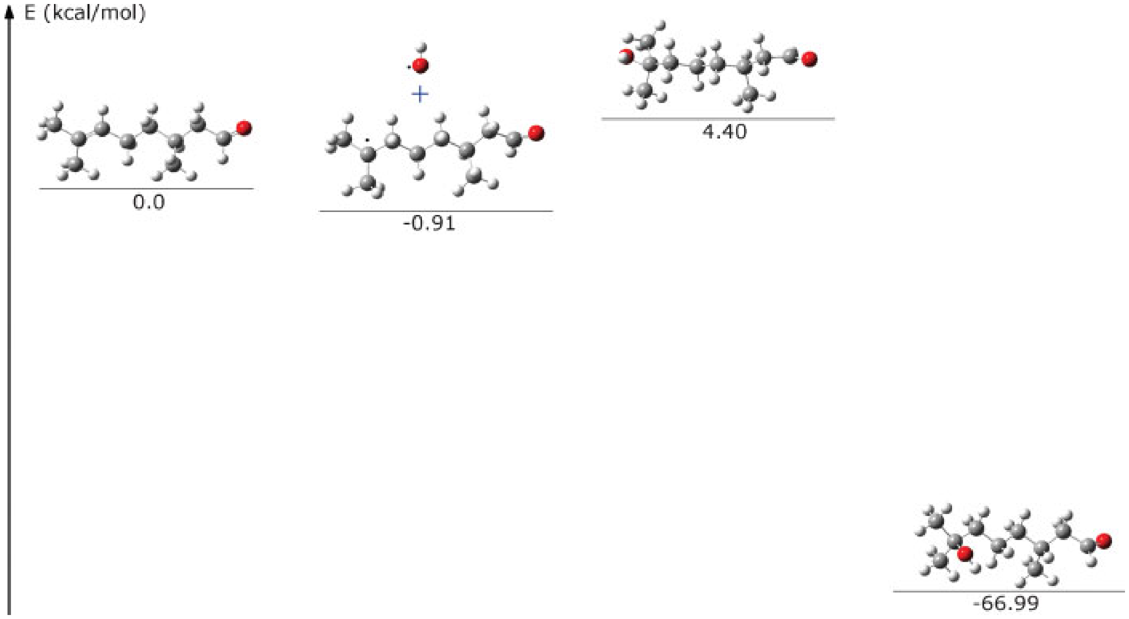

Figure 5. Energy profile of mechanism proposed for hydroxycitronellal formation starting from citronellal

nellal formation starting from citronellal is thermodynamically favored. The total energy variation of hydroxycitronellal formation starting from citronellol, being obtained citronellal like intermediate, is exothermic $E = -79.05$ kcal/mol, so the process is also thermodynamically favored.

The results presented in this paper, both experimental and theoretical indicate that gamma irradiation could be used to obtain structurally related compounds, of much more interest (hydroxycitronellal) than the substrate (citronellol). These results provide the basis for new synthetic pathways of other structurally related compounds of citronellol by gamma irradiation. Other factors such as the presence of water have to be considered. Its presence guarantee the OH radicals needed to obtain the hydroxycitronellal as a final product.

CONCLUSIONS

Citronellal and hydroxycitronellal were identified by mass spectroscopy and recognized as products of the radiolysis of pure citronellol after the gamma irradiation with 1.45 kGy. The variation of the composition has a nonlinear relation with the absorbed dose, where hydroxycitronellal was one of the major radiolytic products. The action of the gamma radiation in citronellol is more complex than a simple degradation and it could be used to obtain different structurally related compounds. The theoretical results obtained by AM1 semiempirical Hamiltonian are in agreement with experimental evidences, and explain the transformations of citronellol induced by gamma radiation. All these results could be used in future researches, to propose a new synthetic tool for compounds derived from citronellol with many important applications, such as hydroxycitronellal and rose oxide.

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