

Acid-Catalyzed *Z-E* Isomerization of γ -Alkylidenebutenolides: An Experimental and DFT Study

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The *Z-E* isomerization of γ -alkylidenebutenolide analogues to natural nostocloides and other natural butenolides was investigated using ¹H nuclear magnetic resonance (NMR) and high performance liquid chromatography (HPLC) data as well as density functional theory (DFT) calculations at the ω B97x-D/6-31G(d,p) level, including solvent effects with the polarizable continuum solvation approach. The experimental data supported the *Z* to *E* isomerization of γ -alkylidenebutenolides under acid catalysis. Newly prepared samples have predominantly *Z* configuration, which partially isomerizes to the *E* isomer under acidic conditions. Density functional theory studies corroborate the experimental findings. While neutral γ -alkylidenebutenolides are more stable in the *Z* form, protonation of the γ -lactone carbonyl group results in preferential stabilization of the *E* isomer.

Keywords: nostoclide, *Z-E* isomerization, butenolides, γ -lactone, DFT calculations, acid catalysis

Introduction

γ -Alkylidenebutenolides constitute a class of compounds characterized by an α,β -unsaturated γ -lactone core bearing an alkylidene substituent at the gamma position (Figure 1).¹⁻³ This structural moiety is found in a large array of bioactive natural compounds, and has been regarded as a key feature for their biological properties, including antitumoral,⁴ cytotoxic,⁵ anti-inflammatory,⁶ antibiotic,⁷ antihistaminic,⁸ anti human immunodeficiency virus (HIV),⁹ human aldose reductase inhibition,¹⁰ and phytotoxic activities.¹¹⁻¹⁶

In natural γ -alkylidenebutenolides, the exocyclic double bond of the benzylidene moiety can be found in both *Z* and *E* configuration. In some cases, the compounds have been isolated as *Z/E* diastereoisomeric mixtures.¹⁷⁻¹⁹ Lee and Brown¹⁸ reported the isolation of the diastereomers

maculalactones B (**11-Z**) and C (**11-E**) (Figure 1) from the marine cyanobacterium *Kyrtuthrix maculans*. They found that, after three weeks in deuterated chloroform (CDCl₃), maculalactone B underwent about 50% conversion to maculalactone C, but the reverse was not observed for a solution of maculalactone C. Also, both *Z* and *E* diastereomers of rubrolide A (**12**) (Figure 1) were isolated from an ascidian of the genus *Botryllus*.¹⁷ In this case, ¹H nuclear magnetic resonance (NMR) analysis of a *Z/E* mixture of rubrolide A in methanol-*d*₄ showed a *Z/E* proportion of 3:1, but the amount of the *E*-isomer decreased when the NMR was taken in dimethylsulfoxide-*d*₆.¹⁷ More recently, Wang *et al.*¹⁹ reported the isolation of cadiolide F (**10**) and rubrolides A (**12**) and Q (**13**) (Figure 1) from the tunicate *Pseudodistoma antinboja*. A ¹H NMR study in acetone-*d*₆ showed that all these compounds existed as mixtures of *Z/E* isomers, with the *Z* form predominating in all cases. The authors reported that residual trifluoroacetic

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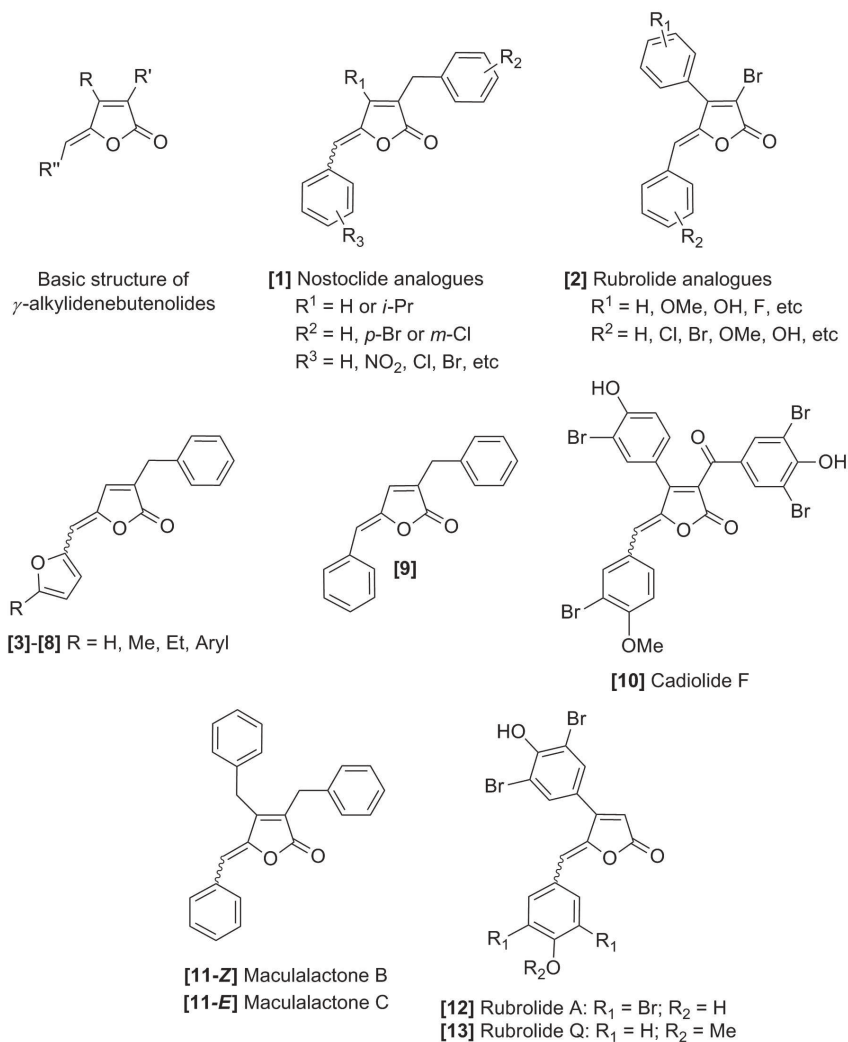


Figure 1. Chemical structures of some synthetic and natural occurring γ -alkylidenebutenolides.

acid in the NMR solutions was the cause of changes in both, the color and NMR spectra, of these compounds.

In view of promising pharmacological and agrochemical activities of butenolides, our research group has been engaged in preparing several analogues of natural γ -alkylidenebutenolides in the search for even more bioactive molecules.^{11-16,20-23} Until now, we have reported the synthesis, structural and theoretical studies and evaluation of biological activities of over a hundred analogues to nostoclide and rubrolides, two classes of natural butenolides (Figure 1, compounds **1** and **2**).^{11-16,20-23} In our studies, almost all compounds were obtained in the *Z* configuration. However, among 11 analogues of nostoclide presenting a (furan-2-yl)methylene (Scheme 1, compounds **3-8**),¹¹ only **3** was isolated as pure *Z* isomer, all the others were obtained as *Z/E* mixtures.¹¹ After purification, we observed that some of these compounds undergo *Z/E* isomerization during NMR analysis in CDCl_3 . Similarly, other studies on the synthesis of

γ -alkylidenebutenolides also led to *Z/E* mixtures of diastereomers, regardless the synthetic strategy used.²⁴⁻²⁷ For example, Kar *et al.*²⁷ observed *Z/E* isomerization of maculalactones B and C after 8 days in CDCl_3 .

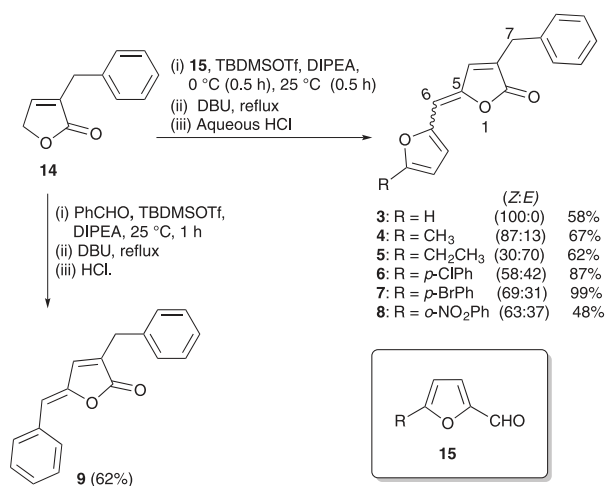
Despite all these reports on the synthesis and isolation of γ -alkylidenebutenolides, we are not aware of any detailed investigation on the *E/Z* isomerization of such compounds. In the present paper, aiming to get a better understanding on the *E/Z* isomerization process, we report an experimental (high performance liquid chromatography (HPLC) and ^1H NMR) and theoretical (density functional theory (DFT) calculations) study on the isomerization of the γ -alkylidenebutenolides **3-9**, and theoretical study of cadiolide F (**10**),¹⁹ maculalactones B (**11-Z**) and C (**11-E**),^{18,27} and rubrolides A (**12**) and Q (**13**).^{17,19} Based on the results, the possible causes for the low diastereoselectivity found in the synthesis of these compounds are discussed. Furthermore, calculations allowed us to propose a theoretical explanation for the relative stability and isomerization between the *Z* and *E* forms of

natural compounds **10-13**. These results could assist in future works on synthesis and isolation of this class of compounds.

Results and Discussion

General considerations

The nostoclide analogues **3-9** were prepared via a vinylogous aldol reaction between 3-benzylfuran-2(5*H*)-one (**14**) and several aromatic / heteroaromatic aldehydes (**15**), followed by an elimination reaction (Scheme 1).^{11,14} Compounds **3** and **9** were obtained only in the *Z* configuration, while compounds **4-8** were obtained as mixtures of *Z* and *E* diastereomers. Purification of the diastereomeric mixtures through silica-gel flash column chromatography was unsuccessful, even though a thin-layer chromatography (TLC) analysis of the reaction mixture revealed two separated spots for *Z* and *E* isomers. The compounds were then purified by preparative HPLC using a silica column, followed by recrystallization. The purity of all isomers was confirmed by analytical HPLC analysis.¹¹ For structural characterization, pure compounds were dissolved in CDCl₃ for ¹H NMR analysis and, surprisingly, signals characteristic of both diastereomers were observed in the spectra of almost all samples, with the exception of those associated to compounds **3** and **9**.



Scheme 1. Synthesis of nostoclide analogues **3-9**.

Thus, to better understand the isomerization of these compounds, we carried out a series of ¹H NMR and HPLC analysis as discussed in the following section.

¹H NMR analysis

The isomerization of compounds **3-9** was investigated by ¹H NMR analysis by measuring the change in the areas

of signals corresponding to hydrogens H-7 (benzylic methylene) and H-6, the exocyclic double bond, at around 3.3-3.8 ppm and 5.4-6.3 ppm, respectively.

Initially, the ¹H NMR spectrum of compound **3**, dissolved in CDCl₃, was obtained and the sample was kept in a refrigerator at 5 °C for three months. After this period, a new NMR spectrum was obtained with the sample. From this experiment, it was observed that in the first spectrum the signals for H-6 and H-7 appeared at 3.72 and 5.91 ppm for the *Z* isomer and no signal for the *E* isomer was observed. After three months the ¹H NMR spectrum revealed an increase in the signals corresponding to the *E* isomer and the calculated ration of *Z*:*E* was 60:40.

Based on these results, ¹H NMR spectra for compounds **3-4** and **6-9** were systematically obtained in CDCl₃ at intervals of 0, 1, 4, 8, 12 days. All diastereomeric mixtures of compounds **3-4**, **6-8** contained excess of the *Z* isomer. To illustrate this experiment, representative spectra of compound **7** (initially as 82:18 *Z*:*E* mixture) are presented in Figure 2. The isomerization was confirmed by increase of the signal at 6.28 ppm, corresponding to the hydrogen atom H-6 of **7-E**, and concomitant decrease in the signal of this same hydrogen atom of **7-Z**, at 5.94 ppm. As observed from Figure 2, after 12 days in CDCl₃ an inversion of the relative proportions of *Z* and *E* diastereomers occurred, and **7-E** became the major isomer (43:57 mixture *Z*:*E*). These results confirmed that although the *Z* diastereomer of compound **7** was the major isomer obtained, under the experimental conditions in CDCl₃ it partially isomerizes to the *E* diastereomer.

The same behavior described for compound **7** was observed for compounds **4** and **6** and their initial and final composition in terms of *Z*:*E* isomers is illustrated in Figure 3. For compounds **3**, **8** and **9**, no isomerization was observed at the assessed period and conditions. It should be noted that the sample of compound **3** used in this experiment was the one kept at -5 °C for three months.

Based on the results just described, and looking for a rationalization of the observed isomerization, we hypothesized that the residual acidity of deuterated chloroform might account for the isomerization process through a protonation of the carbonyl oxygen, according to the mechanism proposed in Scheme 2.

The protonation, followed by enolization would result in the carbocation (**II**) that could then suffer a rotation around the C5-C6 bond as indicated in Scheme 2. This hypothesis is supported by the longer calculated C=C bond between C5/C6 in the protonated species (average 1.352-1.375 Å) in comparison with the neutral compounds (average 1.340-1.344 Å). This rotation could result in a thermodynamic equilibrium mixture of both isomers. Note that resonance with the π electrons from the furan ring

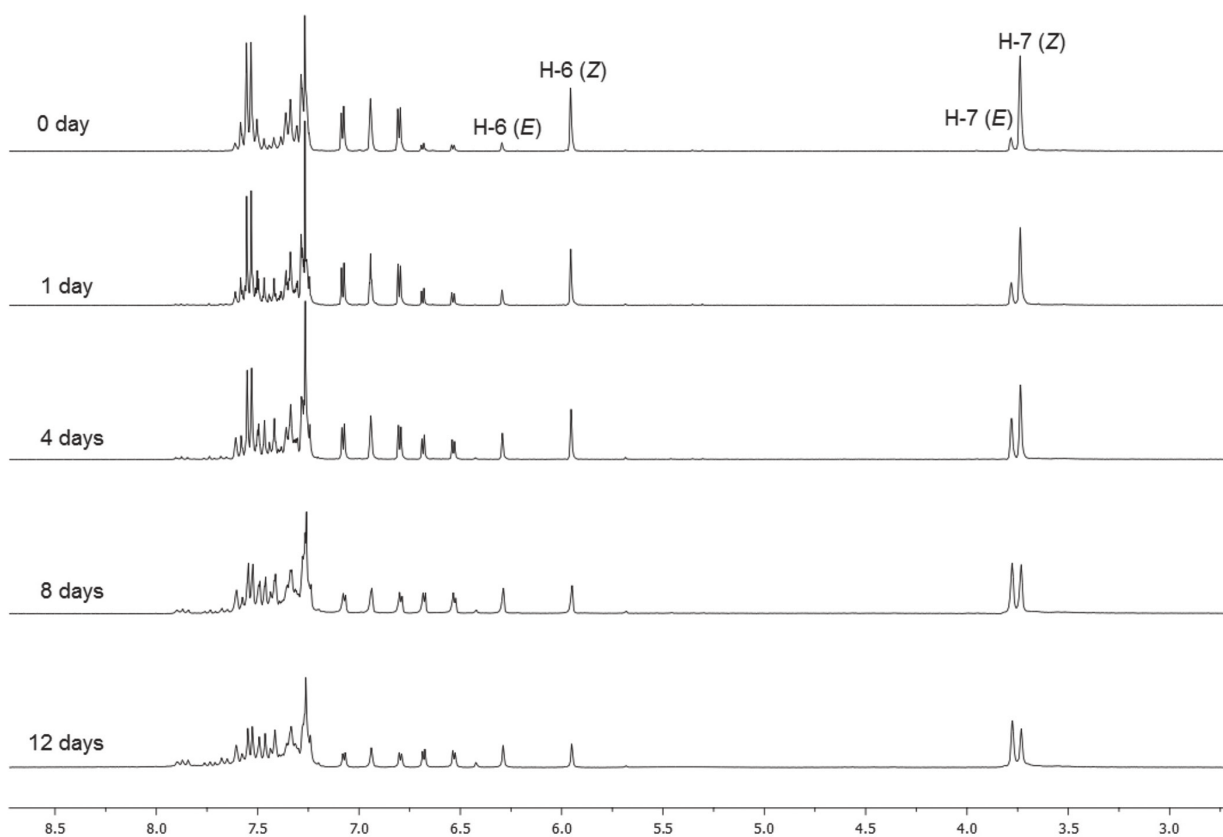


Figure 2. ^1H NMR (300 MHz) spectra of compound **7** in CDCl_3 submitted to isomerization experiment after regular time intervals.

would stabilize further the intermediate cation, weakening the C5-C6 bond.

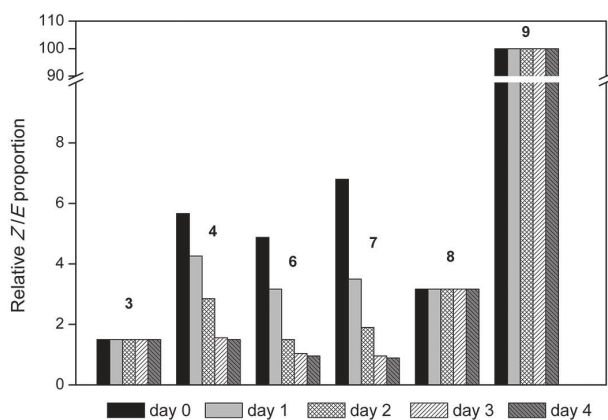
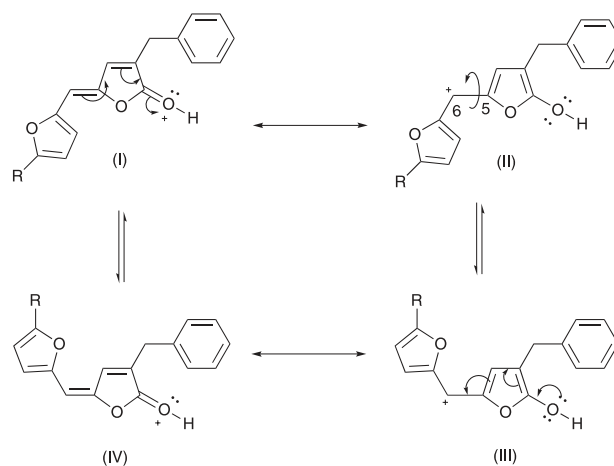


Figure 3. Relative proportions between *Z* and *E* diastereomers in CDCl_3 obtained by ^1H NMR analysis.

Based on this hypothesis, we envisaged that the isomerization also could take place during the purification process by silica-gel column chromatography. So, if the silica is able to catalyze the isomerization, this could explain the initial failure in the separation of the compounds by chromatography. Indeed, we found that the diastereomers of

compounds **4-8** could only be purified by HPLC, followed by recrystallization. The same isomerization process was reported by Lee and Brown¹⁸ and Brown and Wong²⁶ during the separation of maculactones (**11**).



Scheme 2. Proposed intermediates for acid-catalyzed *Z/E* isomerization of nostoclide analogues **4-8**.

To prove or not this hypothesis, we performed a series of isomerization experiments with compounds **3-9**, and analyzed the mixtures by HPLC as described below.

Isomerization experiments and HPLC analysis

For this experiment, the sample of compound **3** used was the one that had been kept in CDCl_3 (pH = 4) for three months and had already suffered some isomerization as described above. We envisaged whether this sample would suffer further isomerization under the new experimental conditions. Initially, compound **5** was obtained at the *Z:E* ratio of 30:70. During the storage period (approximately 3 months), this compound spontaneously isomerized to the *Z* isomer, reaching the *Z:E* ratio of 95:5, and this new diastereomeric mixture was used in the present study. Since such isomerization during storage was not observed for the other compounds, the only plausible explanation could be the presence of some residual acid in this specific sample.

For the mixtures of **4-9**, the *Z* isomers also predominate and their initial compositions were as observed in Figure 4 (day zero). In all cases, the samples were dissolved in dichloromethane/hexane 1:1 (v/v), followed by addition of silica gel. The mixture was then kept under magnetic stirring for 12 days. Aliquots of the suspensions were taken at regular time intervals and analyzed by HPLC. The results from these experiments are presented in Figure 4 and representative chromatograms for compound **7** are shown in Figure 5. From these chromatograms, a decrease in the amount of the *Z* isomer is accompanied by an increase in the *E* form, in agreement with the observed results from the NMR experiments.

As observed from Figure 4, in the case of compound **3**, there is no visible change in the *Z/E* ratio. This was somehow anticipated since the sample had already been subjected to a long period of equilibration in chloroform solution. For compounds **4-7** there was a dramatic reduction in the concentration of the *Z* isomer. In case of compounds **6** and **7**, the *E* isomer became the major constituent, corresponding to 63 and 62% of the final mixtures, respectively, and compounds **8** and **9** did not suffer any isomerization.

From these results, we observed that the furan derivatives **3-7** undergo isomerization, while **8** does not. This might be associated with the strong electron withdrawing effect of the nitro group that reduces the stabilization of intermediate (**II**) in **8** (Scheme 2).

With these experiments, we have demonstrated that *Z/E* isomerization in this class of compounds bearing an electron-rich furan group takes place on silica-gel, accounting for the difficulties encountered in isolating these compounds through silica-gel column chromatography.

In line with our results, other authors also reported *Z/E* isomerization during the isolation of some natural

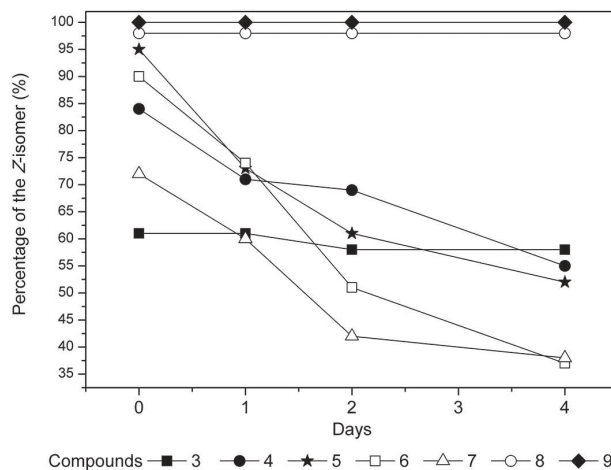


Figure 4. Relative proportions between *Z* and *E* diastereomers after exposition to silica-gel, obtained by HPLC analysis.

γ -alkylidenebutenolides.^{18,26,27} Based on the isomerization of the *Z*-maculactone **B** to the *E*-maculactone **C** in CDCl_3 (Figure 1), Lee and Brown¹⁸ proposed that the *E*-isomer is apparently the thermodynamically most stable isomer. This enhanced stability is possibly due to π - π stacking interactions between the benzyl and benzylidene rings, possible only in the *E*-isomer. On the contrary, in a further study, Brown and Wong²⁶ supposed that, because the synthesis of maculactone led only to the *Z*-isomer, maculactone **B** might be the thermodynamically more stable form, possibly due to steric interaction between the benzyl and benzylidene rings. In this same work, the authors reported that irradiating maculactone **B** with ultraviolet (UV) light led to maculactone **C**, the supposedly less stable diastereomer, affording a mixture of *E/Z* at 4:1. Kar *et al.*²⁷ found that, submitting a 1:1 mixture of maculactone **B** and **C** to heating at 300 °C for 3 h, maculactone **B** (*Z*) was exclusively obtained, leading the authors to propose that the *Z*-isomer is thermodynamically more stable than the *E*-isomer. However, when maculactone **B** was kept in CDCl_3 for 8 days, a 50% conversion to maculactone **C** occurred, which was rationalized as a function of the π - π stacking interactions present in the *E*-isomer.²⁷

In the present work, we found that nostoclide analogues **3-7**, but not **8** and **9**, underwent *Z/E* isomerization in both chloroform and silica-gel. To get more insight on the relative stability of the isomers and on the isomerization phenomena as well, we performed a set of theoretical calculations for both isomers of synthetic and natural γ -alkylidenebutenolides **3-13**.

Computational studies on the *Z/E* isomerization

To find a rationalization for the results reported above and to support the hypothesis that *Z/E* isomerization of

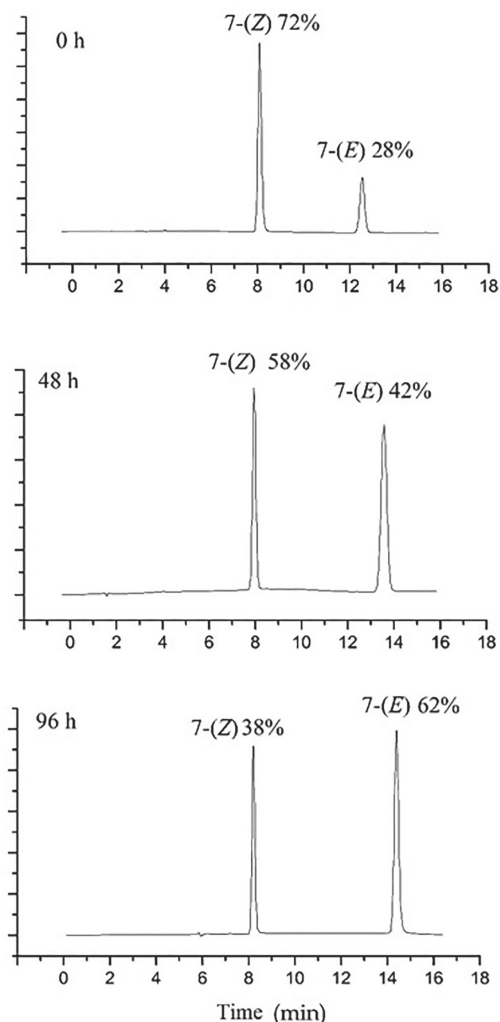


Figure 5. HPLC trace of the 7-*Z* and 7-*E* mixture at 0, 48 and 96 h after exposition to silica-gel at room temperature in hexane-dichloromethane 1:1 (v/v) mixture.

synthetic and naturally occurring γ -alkylidenebutenolides is favoured by the presence of acid, the relative energies of both stereoisomers of compounds **3-13** and their respective protonated forms were calculated. The ω B97x-D functional with the 6-31G(d,p) basis set was used, according to the methodology described. The relative Gibbs free energies ($\Delta G = G(Z) - G(E)$, a negative value meaning the *Z* isomer more stable) for the most stable conformer of each compound, in gas phase and including solvation energy in chloroform, are given in Table 1. To investigate the effect of an acidic medium on the relative energies, we also computed the protonated form of each compound, by adding a proton to the carbonyl oxygen atom of the lactone ring, according to Scheme 2.

In the analysis of the data presented in Table 1, we will focus first on compounds **3-9**, due to their similarities in terms of lacking a substituent group at carbon β of the lactone ring. For this group of compounds, the gas phase

relative energies for the *Z* and *E* neutral forms indicate the *Z* isomer as the most stable, except for **8**. As can be observed from Table 1, ΔG values of the protonated forms in the gas phase are more positive compared with the corresponding ΔG values for the neutral species. Such results clearly indicate that protonation of the lactone ring for compounds **3-8** helps stabilize the *E* isomer, thereby favouring isomerization. For compound **8** the *E* is the most stable isomer under neutral and protonated forms ($\Delta G > 0$ in both cases). So, the preferential formation of the *Z* isomer (76%) in this case is associated to the kinetics of the reaction. In the case of compound **9** the higher negative value for ΔG in comparison with those of **3-7** is in agreement with the experiment, that lead to isolation of only the *Z* isomer and no conversion into *E* in the isomerization experiments carried out (Figures 3 and 4).

Some geometric data reported in the Supplementary Information (SI) section (Table S2) confirms that protonation of the lactone ring may facilitate the isomerization process. For example the $C_5=C_6$ double bond distance in the protonated form is on average 0.023 Å longer than in the neutral forms. The γ -lactone and furan rings are almost coplanar in each derivative (measured through the dihedral angle around the $C_5=C_6$ double bond).

Table 1. Calculated Gibbs free energy difference (ω B97x-D/6-31G(d,p)) for the *Z/E* diastereomers of **3-13** and their protonated forms in the gas phase and in chloroform

Compound	Neutral form		Protonated form	
	ΔG^a gas phase / (kcal mol ⁻¹)	ΔG^b chloroform / (kcal mol ⁻¹)	ΔG gas phase / (kcal mol ⁻¹)	ΔG chloroform / (kcal mol ⁻¹)
3-Z/3-E	-1.14	-1.09	0.86	0.65
4-Z/4-E	-0.72	-0.68	0.12	0.25
5-Z/5-E	-1.03	-1.02	-0.20	0.08
6-Z/6-E	-1.04	-0.99	-0.60	0.09
7-Z/7-E	-0.61	-0.48	-0.28	0.30
8-Z/8-E	0.97	0.17	2.62	0.29
9-Z/9-E	-1.74	-1.74	-2.10	-1.76
10-Z/10-E	-0.71	-0.16	-1.96	-2.79
11-Z/11-E	-3.50	-3.88	-4.17	-4.17
12-Z/12-E	3.38	1.74	0.60	0.51
13-Z/13-E	-1.25	-2.81	-3.41	-3.95

^a ΔG : difference in the Gibbs free energy at 298 K [$\Delta G = G(Z) - G(E)$];

^bthe solvent effect was computed in a single-point calculation, using the solvation model based on density and chloroform as the solvent, and was added to the relative energies obtained in the gas-phase.

The contribution of solvation to the relative stability of the *E* and *Z* isomers is also included in Table 1. In general,

the solvent effect is negligible. For example, for the neutral forms of the derivatives **3-7** and **9** it is below 0.1 kcal mol⁻¹. Even for the protonated species, where electrostatic contribution might play some role on the relative solvation energy, it is below 1.0 kcal mol⁻¹. The consequence is that both isomers become even more isoenergetic. The last column in Table 1 gives the final relative energies, including the electronic and solvation contributions. It shows that for protonated forms of the derivatives **3-8** the differences are below 1.0 kcal mol⁻¹, indicating the presence of both isomers in equilibrium. For derivative **9** and the natural products **10-13** the final results including solvation follow the same trend as the result for the gas-phase, with the *Z* isomer being the most stable form, except for the rubrolide A (**12**), where the *E* isomer is slightly more stable.

The higher energy difference between the *Z* and *E* isomers found for the natural products (**10-13**) may be attributed to the substituent in the β -carbon of the lactone ring. For compounds **10-13**, the presence of the substituent in the β -carbon results in strong steric interaction for the *E* isomer, but not for the *Z* isomer, making the last much more stable, being it the only species observed in the experiment, or at least in higher proportion when obtained as a mixture. The natural cadiolide F (**10**) has an additional carbonyl group in the benzylic ring which might also undergo protonation. Protonation of this carbonyl group instead of the lactone carbonyl also gives the *Z* isomer as the most stable form (by 3.7 kcal mol⁻¹).

The much lower energy differences computed for the nostoclide analogues **3-9** allow them to be found as a mixture with changeable composition, depending on the experimental conditions, with an acidic medium clearly favouring the *E* isomer.

Conclusions

Experimental ¹H NMR and HPLC data showed clear evidences for the *Z/E* isomerization of synthetic γ -alkylidenebutenolides under acid catalysis. Changes in HPLC and ¹H NMR signals were observed after sometime during the experiments, in tests carried out at regular time intervals showing the occurrence of isomerization. In general, the percentage of the *Z* isomer in the samples decreased whereas the amount of the *E* isomer increased over time.

Theoretical investigations at the ω B97x-D/6-31G(d,p) level reinforced the experimental observations. From computed relative energies and thermochemical data it was shown that the *Z* form is more stable than the *E* isomer. However, when protonated in the carbonyl group of the lactone ring the *E* form is preferentially

stabilized, favouring their interconversion and increasing the percentage of the *E* isomer in the diastereomeric mixture. Calculations also helped to understand the relative stability and conversions between *Z* and *E* isomers of the natural butenolides rubrolide A and Q, cadiolide F, and maculolactones A and B.

Experimental

General procedures

Analytical thin-layer chromatography (TLC) was performed on silica gel 60 F₂₅₄ 0.2 mm thick-plates (Merck, Rio de Janeiro-Brazil). Flash column chromatography fractionations were performed using silica gel 230-400 mesh. Purification by HPLC was performed on Shimadzu LC-10A equipment using ultraviolet (UV) detector at 292 nm and Shim-Pack 20 \times 250 mm (15 μ m) preparative column. After HPLC separation, the compounds were purified by recrystallization using a mixture of dichloromethane and hexane. Infrared spectra were recorded on a PerkinElmer Paragon 1000 FTIR spectrophotometer or Varian 660-IR FTIR equipped with a PIKE Gladi ATR accessory. Mass spectra were recorded on a Shimadzu GCMS-QP5050A instrument by direct insertion, using electron ionization (EI) mode (70 eV). The ¹H and ¹³C NMR spectra were recorded on a Varian Mercury 300 spectrometer at 300 and 75 MHz, respectively. The solvent used was deuterated chloroform (CDCl₃); tetramethyl silane (TMS) was the internal reference, unless otherwise stated. Melting points were measured with a MQAPF-301 apparatus and were not corrected. Reagents were procured from Sigma-Aldrich (Milwaukee, Wisconsin, USA) and were used without any purification. Analytical grade solvents were purified by the reported procedures.²⁸ A required 3-benzylfura-2-(5*H*)-one (**14**) was available in the laboratory and can be prepared from furfuraldehyde according to a procedure reported in the literature.²⁹

Synthesis

Compounds **3-8** were previously reported by our group¹¹ but most of these compounds were newly prepared for the current study. Compound **9** was prepared as previously reported.¹⁴ The general synthetic procedure was as following:

A 25 mL two-necked round bottomed flask was charged with 3-benzylbutenolide **14** (100 mg, 1 equiv) solution in dichloromethane (3 mL), trimethylsilyl trifluoromethanesulfonate (TBDMSOTf) (1.3 equiv),

N,N-diisopropylethylamine (DIPEA) (2.0 equiv) and aldehyde (1.2 equiv). The resulting mixture was stirred at 0 °C for 0.5 h and room temperature for 0.5 h. The reaction mixture was warmed at 40 °C and 1,8-diazabicyclo [5.4.0]undec-7-ene (DBU) (2.0 equiv) was added. After 1 h under reflux, dichloromethane (70 mL) was added. The resulting organic layer was washed with HCl aqueous solution (3 mol L⁻¹; 2 × 25 mL) and brine (25 mL). The organic layer was dried over MgSO₄, filtered, and concentrated under reduced pressure. The crude residue was purified by flash column chromatography on silica-gel using dichloromethane (DCM) / hexane (2:1 to 1:2 v/v) as eluent.

For all compounds the spectroscopic and physical data are in agreement with previous report and are not listed here.^{11,14}

Compound 3

Yellow solid; yield 58%; mp 76.1-77.0 °C; Rf = 0.46 (dichloromethane / hexane 2:1, v/v); obtained as a simple *Z* isomer, but for the purpose of the isomerization studies, a sample of **3** was kept in chloroform for three months and the resultant mixture of *Z:E* isomers (64:36) was employed.

Compound 4

Yellow solid; mp 87.4-87.7 °C; isolated in 67% yield as a mixture of 87:13 *Z/E* isomers; **4Z**: Rf = 0.46 (dichloromethane/hexane 1:2, v/v); **4E**: not isolated; Rf = 0.41 (dichloromethane/hexane 1:2, v/v).

Compound 5

Orange amorphous solid; isolated in 62% yield, as a mixture of 30:60 *Z/E* isomers; **5Z**: Rf = 0.30 (dichloromethane/hexane 1:1, v/v); **5E**: orange amorphous solid; Rf = 0.21 (dichloromethane/hexane 1:1, v/v).

Compound 6

Isolated in 87% yield as a mixture of 58:42 *Z/E* isomers; **6Z**: yellow solid; mp 156.5-158.1 °C; Rf = 0.31 (dichloromethane/hexane 2:3, v/v); **6E**: yellow solid; mp 202.3-204.0 °C; Rf = 0.22 (dichloromethane/hexane 2:3, v/v).

Compound 7

Isolated in 99% yield as a mixture of 69:31 *Z/E* isomers; **7Z**: yellow solid; mp 172.8-174.9 °C; Rf = 0.35 (dichloromethane/hexane 1:1, v/v); **7E**: yellow solid; mp 204.5-206.9 °C; Rf = 0.40 (dichloromethane/hexane 1:1, v/v).

Compound 8

Isolated in 48% as a mixture of 63:37 *Z/E* isomers;

8Z: yellow solid; mp 114.4-114.9 °C; Rf = 0.34 (dichloromethane/hexane 2:1, v/v); **8E**: dark yellow solid; mp 122.2-122.7 °C; Rf = 0.42 (dichloromethane/hexane 2:1, v/v).

Isomerization experiments, HPLC and NMR analyses

The progress of the isomerization was investigated by HPLC analysis. To this, 2 g of silica-gel 60 (230-400 mesh) and 12 mg of diastereoisomeric mixtures of compounds **3-9** (*Z:E* 64:36, 84:16, 95:5, 90:10, 74:26, 99.8:0.2 and 100:0, respectively) were dissolved in 15 mL of dichloromethane/hexane, 1:1 (v/v). The suspensions were continuously stirred while aliquots of 20 µL were collected periodically (0, 1, 2, 3, and 4 days). The samples were injected in a Varian Pro Star 210 HPLC system equipped with a diode array detector (DAD) (UV-292) using a silica si60 pre-packed column (4 × 250 mm, 5 µm) and eluting with hexane/dichloromethane 1:1 (v/v) at a flow rate of 0.8 mL min⁻¹. For ¹H NMR spectroscopy analyses, the samples **3-4** and **6-9** were dissolved in CDCl₃ (20 mg *per* 0.6 mL) and the spectra were recorded at regular intervals (1, 2, 3, 4, 8, and 12 days) on Varian Mercury-300 spectrometer. TMS was used as internal standard.

Theoretical calculations

The geometry of each compound (**3-13**) was fully optimized at the DFT level. To define the starting geometry for the DFT calculations, a conformational analysis of each isomer was carried out employing the AM1 semi-empirical method by means of the “conformer distribution” subroutine of the Spartan’06 software.³⁰ These preliminary calculations gave the most stable conformation for each isomer at the semi-empirical level.^{30,31} After that, DFT calculations were carried out in the gas phase and in chloroform, using the Gaussian 09 software³² to optimize the geometry and compute the thermochemical data of the isomers.³²⁻³⁵ The association of the well-known ωB97x-D functional with the 6-31G(d,p) basis set was used in the optimization process, employing the most stable conformations obtained from the AM1 calculations.³⁶⁻³⁸ Each successful geometry optimization was followed by calculation of the second order Hessian matrix (minimum energy structures were characterized by no negative eigenvalue in the Hessian matrix) and the results were analyzed in terms of the computed thermodynamic properties.

The polarizable continuum model (PCM) was employed to simulate the effect of the solvent chloroform in single point calculations, using as input the optimized geometries obtained for each isomer in the optimization process.³⁹ For

the computation of the solvation effects we had to modify the molecular cavity specifications from the default values in the Gaussian software. The overlap index between two interlocking spheres was taken as 0.80 and the minimum radius was set to 0.50 Å to run specific calculations to obtain the total solvation Gibbs free energy of the isomers.⁴⁰ The final relative energies reported are the Gibbs free energy computed at 298 K, corrected by the relative solvation energy.

To identify the effect of an acidic medium, all calculations were repeated with the γ -alkylidenebutenolides protonated in the carbonyl group of the lactone ring.

Supplementary Information

Supplementary information is available free of charge at <http://jbcs.sbq.org.br> as PDF file.

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References

- Rossi, R.; Lessi, M.; Manzini, C.; Marianetti, G.; Bellina, F.; *Curr. Org. Chem.* **2017**, *21*, 964.
- Negishi, E.; Kitora, M.; *Tetrahedron* **1997**, *53*, 6707.
- Brückner, R.; *Curr. Org. Chem.* **2001**, *5*, 679.
- Richardson, A. D.; Ireland, C. M.; *Toxicol. Appl. Pharmacol.* **2004**, *195*, 55.
- Bellina, F.; Anselmi, C.; Rossi, R.; *Tetrahedron Lett.* **2002**, *43*, 2023.
- Pearce, A. N.; Chia, E. W.; Berridge, M. V.; Maas, E. W.; Page, M. J.; Webb, V. L.; Harper, J. L.; Coop, B. R.; *J. Nat. Prod.* **2007**, *70*, 111.
- Miao, S.; Andersen, R. J.; *J. Org. Chem.* **1991**, *56*, 6275.
- Liu, G. Z.; Xu, H. W.; Chen, G. W.; Wang, P.; Wang, Y. N.; Liu, H. M.; Yu, D. Q.; *Bioorg. Med. Chem.* **2010**, *18*, 1626.
- Singh, S. B.; Jayasuriya, H.; Dewey, R.; Polishook, J. D.; Dombrowski, A. W.; Zink, D. L.; Guan, Z.; Collado, J.; Platas, G.; Pelaez, F.; Felock, P. J.; Hazuda, D. J.; *J. Ind. Microbiol. Biotechnol.* **2003**, *30*, 721.
- Manzanaro, S.; Salva, J.; de la Fuente, J. A.; *J. Nat. Prod.* **2006**, *69*, 1485.
- Barbosa, L. C. A.; Varejão, J. O. S.; Petrollino, D.; Pinheiro, P. F.; Demuner, A. J.; Maltha, C. R. A.; Forlani, G.; *ARKIVOC* **2012**, 15.
- Barbosa, L. C. A.; Demuner, A. J.; de Alvarenga, E. S.; Oliveira, A.; King-Diaz, B.; Lotina-Hennsen, B.; *Pest Manage. Sci.* **2006**, *62*, 214.
- Barbosa, L. C. A.; Rocha, M. E.; Teixeira, R. R.; Maltha, C. R. A.; Forlani, G.; *J. Agric. Food Chem.* **2007**, *55*, 8562.
- Teixeira, R. R.; Barbosa, L. C. A.; Forlani, G.; Piló-Veloso, D.; Carneiro J. W. M.; *J. Agric. Food Chem.* **2008**, *56*, 2321.
- Barbosa, L. C. A.; Demuner, A. J.; Maltha, C. R. A.; Teixeira, R. R.; Souza, K. A. P.; Bicalho, K. U.; *Z. Naturforsch.* **2009**, *64b*, 245.
- Teixeira, R. R.; Pinheiro, P. F.; Barbosa, L. C. A.; Carneiro, J. W. M.; Forlani, G.; *Pest Manage. Sci.* **2010**, *66*, 196.
- Smith, C. J.; Hettich, R. L.; Jompa, J.; Tahir, A.; Buchanan, M. V.; Ireland, C. M.; *J. Org. Chem.* **1998**, *63*, 4147.
- Lee, S. C.; Brown, G. D.; *J. Nat. Prod.* **1998**, *61*, 29.
- Wang, W.; Kim, H.; Nam, S. J.; Rho, B. J.; Kang, H.; *J. Nat. Prod.* **2012**, *75*, 2049.
- Teixeira, R. R.; Barbosa, L. C. A.; Carneiro, J. W. M.; Correa, R. S.; Ellena, J.; Doriguetto, A. C.; *J. Mol. Struct.* **2009**, *917*, 1.
- Teixeira, R. R.; Barbosa, L. C. A.; Santana, J. O.; Veloso, D. P.; Ellena, J.; Doriguetto, A. C.; Drew, M. G. B.; Ismail, F. M. D.; *J. Mol. Struct.* **2007**, *837*, 197.
- Varejao, J. O. S.; Barbosa, L. C. A.; Maltha, C. R. A.; Lage, M. R.; Lanznaster, M.; Carneiro, J. W. M.; Forlani, G.; *Electrochim. Acta* **2014**, *120*, 334.
- Barbosa, L. C. A.; Maltha, C. R. A.; Lage, M. R.; Barcelos, R. C.; Dona, A.; Carneiro, J. W. M.; Forlani, G.; *J. Agric. Food Chem.* **2012**, *60*, 10555.
- Boukouvalas, J.; Maltais, F.; Lachance, N.; *Tetrahedron Lett.* **1994**, *35*, 7897.
- Boukouvalas, J.; Beltrán, P. P.; Lachance, N.; Côté, S.; Maltais, F.; Pouliot, M.; *Synlett* **2007**, 219.
- Brown, G. D.; Wong, H. F.; *Tetrahedron* **2004**, *60*, 5439.
- Kar, A.; Gogoi, S.; Argade, N. P.; *Tetrahedron* **2005**, *61*, 5297.
- Perrin, D. D.; Armarego, D. L. F.; *Purification of Laboratory Chemicals*, 3rd ed.; Pergamon: Oxford, UK, 1988.
- Näsman, J. H.; *Org. Synth.* **1990**, *68*, 162.
- Spartan'06*; Wavefunction Inc.: Irvine, CA, USA, 2006.
- Dewar, M. J. S.; Zorbisch, E. G.; Healy, E. F.; *J. Am. Chem. Soc.* **1985**, *107*, 3902.
- Frisch, M. J.; Trucks, G. W.; Schlegel, H. B.; Scuseria, G. E.; Robb, M. A.; Cheeseman, J. R.; Montgomery Jr., J. A.; Vreven, T.; Kudin, K. N.; Burant, J. C.; Millam, J. M.; Iyengar, S. S.; Tomasi, J.; Barone, V.; Mennucci, B.; Cossi, M.; Scalmani, G.; Rega, N.; Petersson, G. A.; Nakatsuji, H.; Hada, M.;

- Ehara, M.; Toyota, K.; Fukuda, R.; Hasegawa, J.; Ishida, M.; Nakajima, T.; Honda, Y.; Kitao, O.; Nakai, H.; Klene, M.; Li, X.; Knox, J. E.; Hratchian, H. P.; Cross, J. B.; Bakken, V.; Adamo, C.; Jaramillo, J.; Gomperts, R.; Stratmann, R. E.; Yazyev, O.; Austin, A. J.; Cammi, R.; Pomelli, C.; Ochterski, J. W.; Ayala, P. Y.; Morokuma, K.; Voth, G. A.; Salvador, P.; Dannenberg, J. J.; Zakrzewski, V. G.; Dapprich, S.; Daniels, A. D.; Strain, M. C.; Farkas, O.; Malick, D. K.; Rabuck, A. D.; Raghavachari, K.; Foresman, J. B.; Ortiz, J. V.; Cui, Q.; Baboul, A. G.; Clifford, S.; Cioslowski, J.; Stefanov, B. B.; Liu, G.; Liashenko, A.; Piskorz, P.; Komaromi, I.; Martin, R. L.; Fox, D. J.; Keith, T.; Al-Laham, M. A.; Peng, C. Y.; Nanayakkara, A.; Challacombe, M.; Gill, P. M. W.; Johnson, B.; Chen, W.; Wong, M. W.; Gonzalez, C.; Pople, J. A.; *Gaussian 03*, Gaussian, Inc., Wallingford, CT, USA, 2004.
33. Hohenberg, P.; Kohn, W.; *Phys. Rev. B* **1964**, *136*, B864.
34. Kohn, W.; Sham, L. J.; *Phys. Rev. A* **1965**, *140*, A1133.
35. Parr, R. G.; Yang, W.; *Density-Functional Theory of Atoms and Molecules*; Oxford University Press: Oxford, UK, 1989.
36. Becke, A. D.; *J. Chem. Phys.* **1993**, *98*, 5648.
37. Lee, C.; Yang, W.; Parr, R. G.; *Phys. Rev. B* **1988**, *37*, 785.
38. Rassolov, V.; Ratner, M. A.; Pople, J. A.; Redfern, P. C.; Curtiss, L. A.; *J. Comput. Chem.* **2001**, *22*, 976.
39. Tomasi, J.; Mennucci, B.; Cammi, R.; *Chem. Rev.* **2005**, *105*, 2999.
40. Pascual-Ahuir, J. L.; Silla, E.; Tuñón, I.; *J. Comput. Chem.* **1994**, *15*, 1127.

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