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On the Reaction of Lupulones, Hops β-Acids, with 1-Hydroxyethyl Radical

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Supporting Information

ABSTRACT: Lupulones, hops β-acids, are one of the main constituents of the hops resin and have an important contribution to the overall bacteriostatic activity of hops during beer brewing. The use of lupulones as natural alternatives to antibiotics is increasing in the food industry and also in bioethanol production. However, lupulones are easy oxidizable and have been shown to be very reactive toward 1-hydroxyethyl radical with apparent bimolecular rate constants close to diffusion control $k = 2.9 \times 10^8$ and 2.6×10^8 L mol^{-1} s^{-1} at 25.0 ± 0.2 °C in ethanol−water solution (10% of ethanol (v/v)) as probed by EPR and ESI-IT-MS/MS spin-trapping competitive kinetics, respectively. The free energy change for an electron-transfer mechanism is $\Delta G° = 106$ kJ/mol as calculated from the oxidation peak potential experimentally determined for lupulones (1.1 V vs NHE) by cyclic voltammetry and the reported reduction potential for 1-hydroxyethyl radical. The major reaction products identified by LC-ESI-IT-MS/MS and ultrahigh-resolution accurate mass spectrometry (orbitrap FT-MS) are hydroxylated lupulone derivatives and 1-hydroxyethyl radical adducts. The lack of pH dependence for the reaction rate constant, the calculated free energy change for electron transfer, and the main reaction products strongly suggest the prenyl side chains at the hops β-acids as the reaction centers rather than the β,β′-triketone moiety.

KEYWORDS: *lupulones, β-acids, beer, 1-hydroxyethyl radical, kinetics*

INTRODUCTION

The use of hops (*Humulus lupulus*) in beer brewing is mandatory, resulting in the unique sensorial properties of beer such as a typical bitter taste, particular aroma, and foam stability.[1−3] It is well-known that the advantage of adding hops during beer brewing as a natural antiseptic and flavoring agent is due to the secondary metabolites found in the lupulin glands of the female hop cones.[2,4] The secondary metabolites found in the lupulin powder are classified as resinous bitter acids, volatile oil, and polyphenols.[3,4] The classes of secondary metabolites responsible for the main antiseptic, bitter taste and foam properties of beer are the hop bitter acids, which consist of two related series, the β-acids or lupulones (Figure 1, **1a−c**) and the α-acids or humulones (**2a−c**), identified according to the acyl side chain in three analogues as co- (**1a** and **2a**), (n)- (**1b** and **2b**), and ad- (**1c** and **2c**) lupulone or humulone, respectively.[3] It is well-known that humulones undergo isomerization during wort boiling, giving rise to a mixture of six isomers, the so-called *trans*-isohumulones (**3a−c**) and *cis*-isohumulones (**4a−c**). On the other hand, lupulones are not isomerized due to the lack of tertiary alcohol function at C-6, thus making the β-acids less acidic and water-soluble than the respective isomerized α-acids. Therefore, lupulones are essential to control the growth of *Lactobacillus* spp. during fermentation[3] and known to be partially transformed by oxidation and proton-catalyzed cyclization to more soluble and sensory active compounds during wort boiling.[1]

The antiseptic action of hop bitter acids has been investigated for many years, and this property is due to their pronounced bacteriostatic activity.[2,4−9] In this context, hop bitter acids act as ionophores against Gram-positive bacteria,

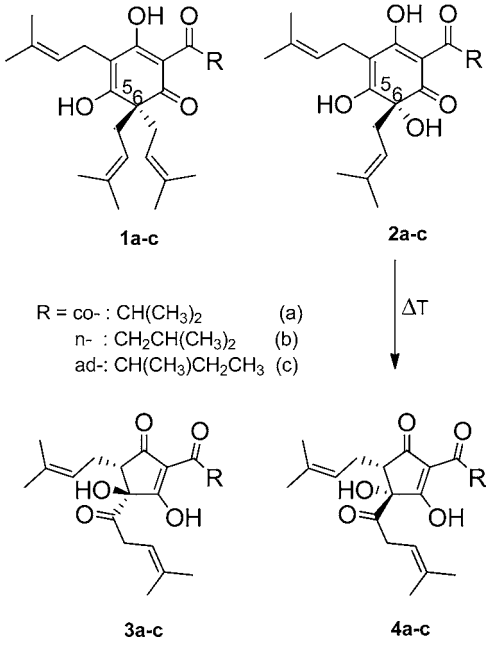

Figure 1. Chemical structures of lupulones (**1a−c**), humulones (**2a−c**), *trans*-isohumulones (**3a−c**), and *cis*-isohumulones (**4a−c**).

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such as *Lactobacillus* spp., *Streptococcus* spp., *Staphylococcus* spp., *Micrococcus* spp., and *Bacillus* spp., inhibiting or reducing their growth.[4,10] In beer, hop acids inhibit the growth of *Lactobacillus* during wort boiling and postboiling operations without affecting the yeast performance, leading to a sterile beer.[4] This bacteriostatic activity of hop bitter acids has been assigned to hydrophobic interactions of prenyl groups, present in α- and β-acid structures, with the bacterial cell walls.[6,8] Lupulones have been shown to be more active than humulones and isohumulones because β-acids have three prenyl side chains rather than two in the α- and iso-α-acids.[5,11,12]

Several technological applications have recently been developed to explore the bacteriostatic activity of lupulones, for example, the use of β-acids in the control of bacterial activity during extraction of sugar beet and in industrial ethanol production, replacing conventional antibiotics such as penicillin.[2,4,13] However, lupulones are very sensitive to oxidative decomposition,[2,4] resulting in loss of their antimicrobial activity and giving rise to oxidation reaction products that may exhibit unpleasant organoleptic characteristics.[14−17] Aiming to provide a better understanding of the mechanism behind oxidative decomposition of lupulones, herein, we report the reactivity of lupulones (**2a−c**) toward 1-hydroxyethyl radical, the major radical species formed in the brewing process, as probed by spin-trapping EPR and ESI-IT-MS/MS using a competitive kinetic approach.

MATERIALS AND METHODS

Chemicals and Materials. Ethanol, hydrogen chloride, and dichloromethane were from J. T. Baker (Xalostoc, Mexico). Ferric chloride ($FeCl_2{\cdot}4H_2O$) and hydrogen peroxide 30% were from Merck (Darmstadt, Germany). Acetonitrile and ethyl acetate were from Tedia (Fairfield, OH, USA). Formic acid, catalase from bovine liver, α-(4-pyridyl-1-oxide)-*N*-*tert*-butylnitrone (4-POBN), tetrabutylammonium perchlorate, and ferrocene were from Sigma-Aldrich (Steinheim, Germany). Lupulones (β-acids) were from Hopsteiner (Mainburg, Germany). Water was purified (18 MΩ cm) by means of a Milli-Q purification system (Millipore, Bedford, MA, USA). Argon (purity grade 99.999%) and nitrogen (purity grade 99.999%) were from White Martins (Sertãozinho, São Paulo, Brazil). Sodium sulfate (Na_2SO_4) was from Chemis (São Paulo, São Paulo, Brazil) and ammonium chloride (NH_4Cl) from Synth (Diadema, São Paulo, Brazil). All solvents used were of HPLC grade, and chemicals of analytical grade were used without further purification.

Formation of 1-Hydroxyethyl Radicals and Competitive Kinetics Studies Using 4-POBN as Spin-Trap. The formation of 1-hydroxyethyl radicals and the competitive kinetics approach by spin-trapping 1-hydroxyethyl radical with 4-POBN were conducted at 25.0 ± 0.2 °C following the procedure reported by de Almeida and co-workers.[18] Reactions were initiated by adding 80 μL of H_2O_2 (58.0×10^{-3} mol/L) in an argon-saturated solution containing 60 μL of $FeCl_2{\cdot}4H_2O$ (2.0×10^{-3} mol/L), 1 mL of 4-POBN (3.2×10^{-3} mol/L in aqueous solution containing 12% (v/v) of ethanol), and different concentrations of lupulones with concentrations ranging from 7.2×10^{-5} to 1.6×10^{-4} mol/L. The reaction was completed within 1 min by adding 100 μL of catalase (47.4 mg/mL).[19] Thus, the competitive kinetics were conducted through monitoring the content of the spin adduct 1-hydroxyethyl/4-POBN radical by EPR spectroscopy and/or ESI-IT-MS/MS.

Electron Paramagnetic Resonance Spectroscopy. Analysis of the spin adduct radical was carried out in a Bruker EMX Plus spectrometer (Rheinstetten, Germany) operating at X-band, frequency of 9.76 GHz, microwave power of 1 mW, and modulation amplitude of 1 G using a rectangular TE 102 cavity and quartz capillary (i.d. = 0.75 mm) as sample cell (Wilmad Glass, Buena, NJ, USA).

Direct Injection ESI-IT-MS/MS. Identification and quantitation of the 1-hydroxyethyl/4-POBN spin adduct radical (m/z 240) was carried out in a direct batch injection in the mass spectrometer supported by isocratic HPLC in which the mobile phase consisted of a mixture of water and formic acid (99.9:0.1 v/v) with a sample loop of 20 μL. The electrospray mass spectra were obtained using a mass spectrometer with an ion-trap analyzer from Bruker Daltonics, model Esquire 4000 (Bremen, Germany) operating in the positive ion mode. Samples were directly infused into the HPLC system with a flow rate of 100 μL/min. General spectrometer conditions were as follows: N_2 drying gas temperature, 250 °C; N_2 drying gas flow, 9 L/min; nebulizer pressure, 30 psi; capillary voltage, 3000 V.

Identification of Oxidation Products of Lupulones in the Presence of 1-Hydroxyethyl Radicals. The degradation products of lupulones were analyzed through LC-ESI-IT-MS/MS or by flow injection ultrahigh-resolution accurate mass spectrometry (orbitrap ESI-FT-MS) from the reaction mixture by the addition of 100 μL of H_2O_2 (58.0×10^{-3} mol/L) in an alcoholic solution containing lupulones (2.4×10^{-4} mol/L, in ethanol or d_6-ethanol) and 80 μL of $FeCl_2{\cdot}4H_2O$ (2.0×10^{-3} mol/L). The reaction was incubated for 1 min at 25.0 ± 0.2 °C, and the final composition was analyzed by LC-ESI-IT-MS/MS and direct infusion orbitrap ESI-FT-MS.

LC-ESI-IT-MS/MS. The LC-ESI-IT-MS/MS analysis was performed using a Shimadzu Prominence HPLC equipped with two LC-20AD solvent delivery units, an online Shimadzu degasser model DGU20A3, and a CBM-20A Shimadzu Prominence communications bus module. Samples of 20 μL were injected using a manual injection valve, Rheodyne model 8125, and separation was achieved using a 150 mm × 2.1 mm i.d., 5 μm, Zorbax Extended C18 (Agilent Technologies, Santa Clara, CA, USA). Mobile phases, at a flow rate of 0.3 mL/min, were A (water/formic acid, 99.9:0.1 v/v) and B (acetonitrile/formic acid, 99.9:0.1 v/v) using the following linear eluting gradient: 0−5 min, 0−30% B in A; 5−15 min, 30−70% B in A; 15−50 min, 70−90% B in A; 50−55 min, 90− 90% B in A; 55−60 min, 90−0% B in A. The mass spectra were collected in the positive ion mode for the identification of the target compounds using a Bruker Daltonics ion trap mass spectrometer model Esquire 4000 equipped with an electrospray interface. General conditions were as follows: N_2 drying gas temperature, 350 °C; N_2 drying gas flow, 9 L/min; nebulizer pressure, 30 psi; capillary voltage, 3000 V.

Direct Infusion Ultrahigh-Resolution and Accurate Mass Spectrometry (Orbitrap ESI-FT-MS). Flow injection analysis of reaction samples (5 μL/min) was carried out with an LTQ Orbitrap Velos FT-MS instrument (Thermo Fischer Scientific, Bremen, Germany) equipped with an electrospray source (HESI-II) operating in full scan positive ionization mode.

Cyclic Voltammetry of Lupulones. Cyclic voltammetry of lupulones was carried out with an Autolab GPES Eco Chemie BV potentiostat/galvanostat (Metrohm, Utrecht, The Netherlands) using a boron-doped diamond electrode (8000 ppm) (working electrode) and a platinum wire (auxiliary electrode). The ferrocene/ferrocenium couple was used as internal standard, and the measured potential was reported against the normal hydrogen electrode (NHE) assuming the potential for the Fe^+/Fe couple ($E°$) versus NHE to be equal to +630 mV in acetonitrile.[20] The supporting electrolyte (0.2 mol/L of tetrabutylammonium perchlorate in acetonitrile) and the lupulones (1.0×10^{-3} mol/L) were purged with high-purity nitrogen for 15 min, and the measurements were conducted be varying the scan rate from 10 to 200 mV/s at 25 ± 0.2 °C.

RESULTS AND DISCUSSION

Apparent Second-Order Rate Constants. Following the procedure previously reported in the literature,[18] the apparent second-order rate constants for the reaction between lupulones and 1-hydroxyethyl radicals were obtained using a competitive kinetics approach.[21,22] In this context, the signal intensity of the 1-hydroxyethyl/POBN radical spin adduct and the inhibition of the spin adduct formation in the presence of analytical concentrations of lupulones were monitored by ESI-IT-MS/MS or EPR. Figure 2 illustrates the decrease in spin adduct formation in the presence of increasing concentrations of

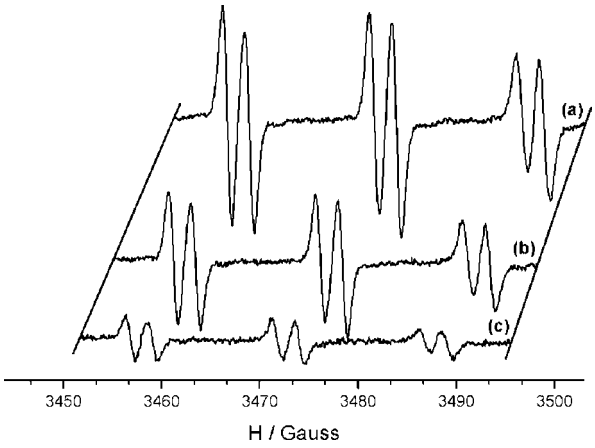

Figure 2. Experimental EPR spectra of the spin adduct radical derived from the 1-hydroxyethyl radical trapped by 4-POBN. Reactions were carried out in argon-saturated aqueous solution containing 10% of ethanol (v/v) at 25.0 ± 0.2 °C. The EPR spectra were obtained in the presence of different concentrations of lupulones: (a) 7.4×10^{-5} mol/L, (b) 1.1×10^{-4} mol/L, and (c) 1.5×10^{-4} mol/L.

lupulones as probed by EPR spectroscopy. The EPR spectrum is characterized by a triplet of doublets with a nitrogen hyperfine coupling constant of 15.6 G and a hydrogen super hyperfine coupling constant of 2.6 G, in agreement with the values expected for the spin adduct radical formed from the reaction of 1-hydroxyethyl radical and 4-POBN.[14] Thus, plotting $(F/1 - F) \times k_2 \times [\text{4-POBN}]$ against the respective added concentrations of lupulones, as shown in Figure 3, allows

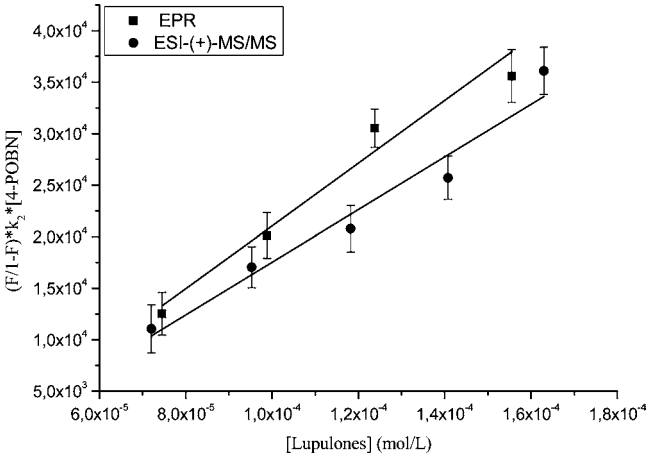

Figure 3. Plot of $(F/1 - F) \times k_2 \times [\text{4-POBN}]$ versus the concentration of lupulones as obtained by spin-trapping EPR or ESI-IT-MS/MS competitive kinetics.

for calculation of the apparent second-order rate constants (k_2) from the slope of the linear dependence as established by eq 1

$$((F/(1 - F)) \times [\text{4-POBN}] \times 3.1 \times 10^7 = k_2 \times [\text{lupulones}] \quad (1)$$

where F denotes the percentage of inhibition for the formation of the spin adduct, 3.1×10^7 is the second-order rate constant (L $\text{mol}^{-1}\text{s}^{-1}$) for the reaction of the spin trap with 1-hydroxyethyl radical, and [4-POBN] and [lupulones] account for the concentrations of the spin trap and lupulones, respectively.[23]

The apparent second-order rate constants for the reaction of the 1-hydroxyethyl radical with lupulones obtained from the linear regression of the plot in Figure 3 were 2.9×10^8 and 2.6×10^8 L mol^{-1} s^{-1} as probed by EPR and ESI-IT-MS/MS, respectively. As observed, the apparent second-order rate constants obtained by EPR or ESI-IT-MS/MS are similar ($p = 0.05$), the EPR detection being more sensitive and accurate as expected, nevertheless demonstrating the feasibility of both detection methods to afford the apparent rate constant by a combined spin-trapping competitive kinetics approach. The observed apparent rate constant for the reaction of lupulones with 1-hydroxyethyl radical expresses the high reactivity and sensitivity of lupulones toward oxidation by the 1-hydroxyethyl radical, being as reactive as *cis*-isohumulones and 33 times less reactive than *trans*-isohumulones.[18] In addition, the apparent second-order rate constant for the reaction of undissociated lupulones ($pK_a = 6.1$; $k = 2.5 \times 10^8$ L mol^{-1} s^{-1}) with the 1-hydroxyethyl by ESI-IT-MS/MS was determined. The experimental apparent rate constant obtained for the lupulones in their undissociated form was comparable to the apparent rate constant observed for dissociated lupulones (data not shown). Therefore, it strongly suggests that the mechanism for oxidative degradation of lupulones by the 1-hydroxyethyl radical involves hydrogen atom transfer (HAT) at the prenyl side chains rather than being governed by electron transfer (ET) from the cyclohexane-1,3,5-trione, β,β'-triketone group, leading to stabilized allylic radicals that eventually are trapped by other radical species or residual molecular oxygen.

Electrochemical Studies of Lupulones. To the best of our knowledge, the oxidation potential of lupulones has hitherto not been reported, although being an intriguing target for oxidation, with a number of Π and n electrons available within a conjugated system.

It is well-known that hop bitter acids are susceptible to degradation by means of oxidative processes. In this context, the oxidative degradation of these compounds by one-electron oxidation from the β,β'-triketone moiety is one possible way.[18,24−26] On the other hand, the electrochemical behavior of lupulones has not been reported. Thus, to determine the oxidation potential of lupulones, cyclic voltammetric experiments were conducted, at various scan rates, as presented in Figure 4. The oxidation potential observed was +1.10 V versus

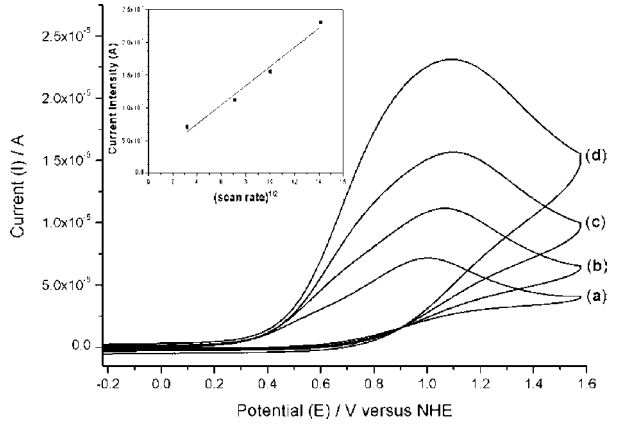

Figure 4. Cyclic voltammograms of lupulones (1.0×10^{-3} mol/L); supporting electrolyte tetrabutylammonium perchlorate (0.2 mol/L) in acetonitrile, at 25 °C and different scan rates: (a) 10 mV/s; (b) 50 mV/s; (c) 100 mV/s; and (d) 200 mV/s.

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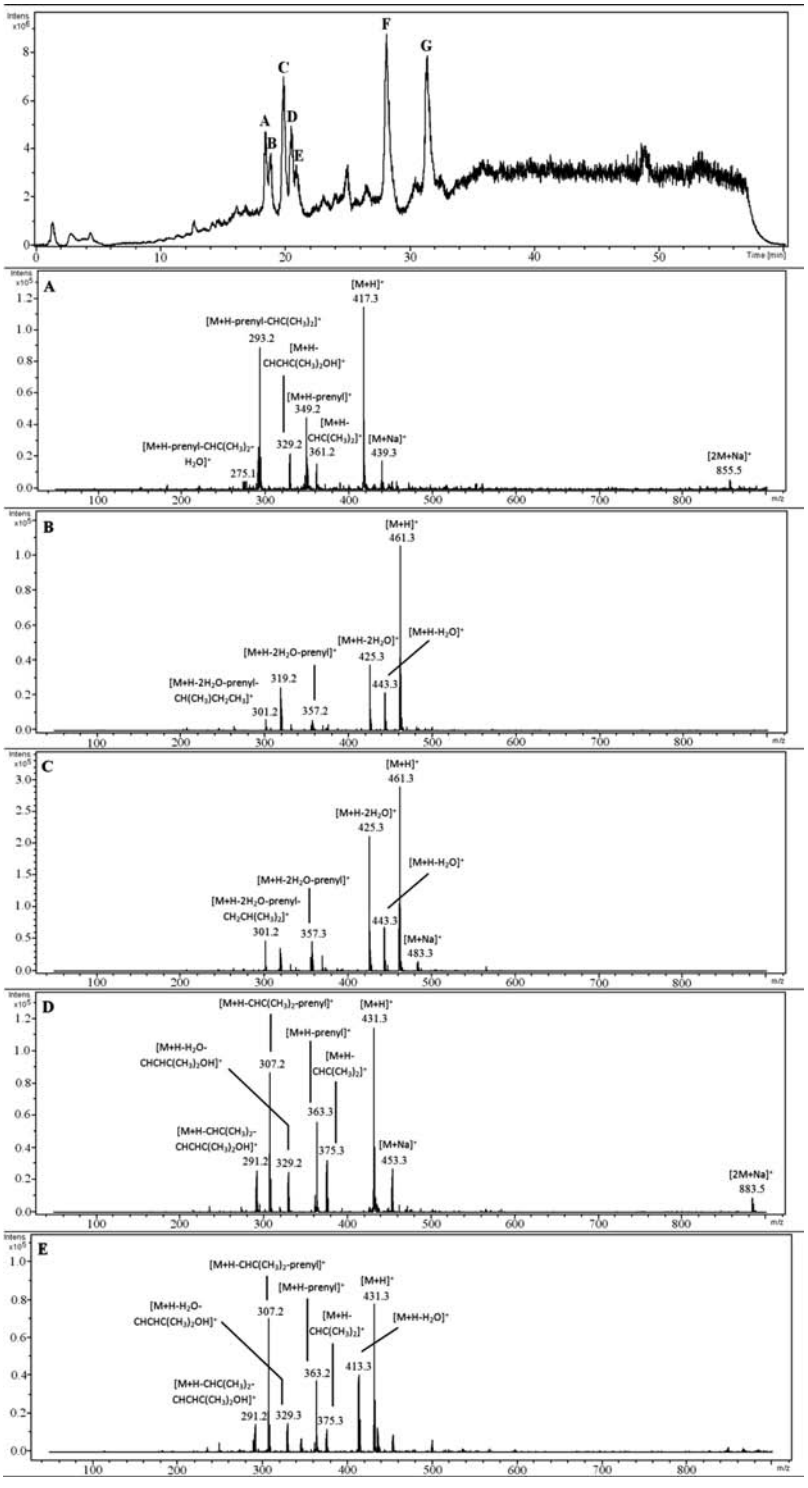

Figure 5. LC-ESI-IT-MS/MS chromatogram of the final composition obtained from the reaction between lupulones and 1-hydroxyethyl radicals, after 1 min, in aqueous ethanol solution 12% v/v at 25 °C, operating in the positive ion mode using an electrospray ion source; mass spectra of chromatographic peaks A, B, C, D, and E.

NHE (at a scan rate of 100 mV/s) as an irreversible process. In addition, a diffusion-controlled electrochemical process was verified due to the linear dependence of the anodic peak current with the square root of the scan rate.

Although the oxidation potential of lupulones is higher than the reduction potential of the 1-hydroxyethyl radical (E = 0.98 V versus NHE),[27] the reaction mechanism is probably not governed by electron transfer ($\Delta G°$ = 106 kJ/mol). Indeed, this information corroborates the finding that HAT from the prenyl side chains occurs rather than electron transfer.

Identification of the Oxidation Products of the Lupulones. The oxidation products arising from the reactions of the lupulones toward the 1-hydroxyethyl radical were determined by analysis of a reaction mixture by LC-ESI-IT-MS/MS. The chromatogram of the reaction mixture, containing ferric ions, hydrogen peroxide, and lupulones

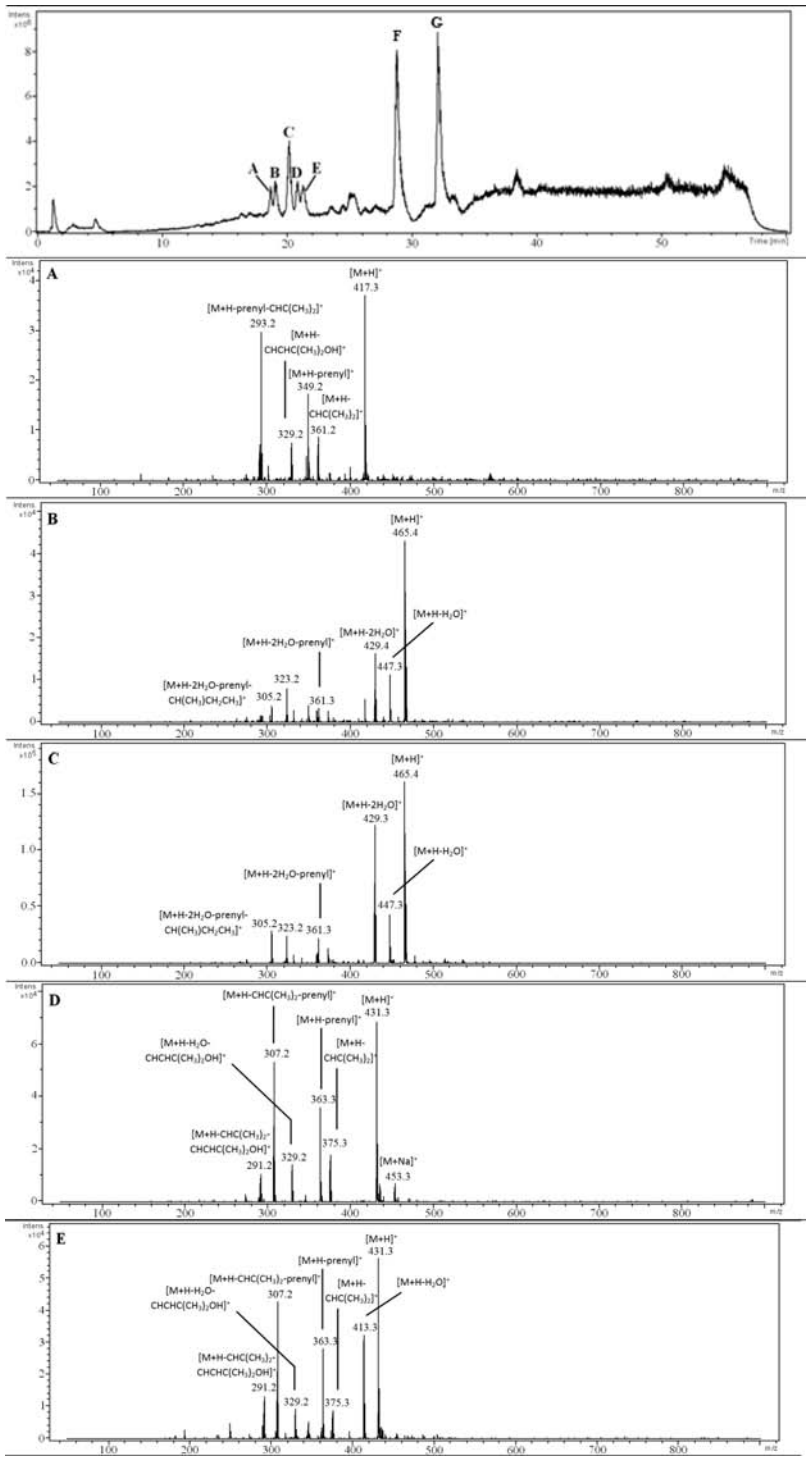

Figure 6. LC-ESI-IT-MS/MS chromatogram of the final composition obtained from the reaction between lupulones and 1-hydroxyethyl radicals, after 1 min, in aqueous ethanol-d_6 solution 12% v/v at 25 °C, operating in the positive ion mode using an electrospray ion source; mass spectra of chromatographic peaks A, B, C, D, and E.

dissolved in ethanol, is shown in Figure 5. The chromatogram of the oxidation products showed mainly seven peaks, of which the first five peaks are reaction products, whereas the last two peaks are the initial lupulones (peak F, colupulone; peak G, n- and ad-lupulone).

Among all recorded LC peaks, the MS spectrum of peak A revealed the presence of the $[M + H]^+$ quasi-molecular ion at m/z 417 with t_r = 18.7 min, whereas the same quasi-molecular ion value (m/z 431) was observed for peaks D and E, with t_r = 20.8 and 21.3 min, respectively. Recent studies described in the literature reported compounds derived from isohumulones containing a hydroxyl group in the side chain.[1,16,18] These compounds are formed by oxidation in the presence of radical species, mainly 1-hydroxyethyl radicals, which abstract an allylic hydrogen atom of the unsaturated side chain of the isohumulones. The observed quasi-molecular ion at m/z 417 (peak A) suggests addition of a hydroxyl group to colupulone, whereas the quasi-molecular ions at m/z 431 (peaks D and E)

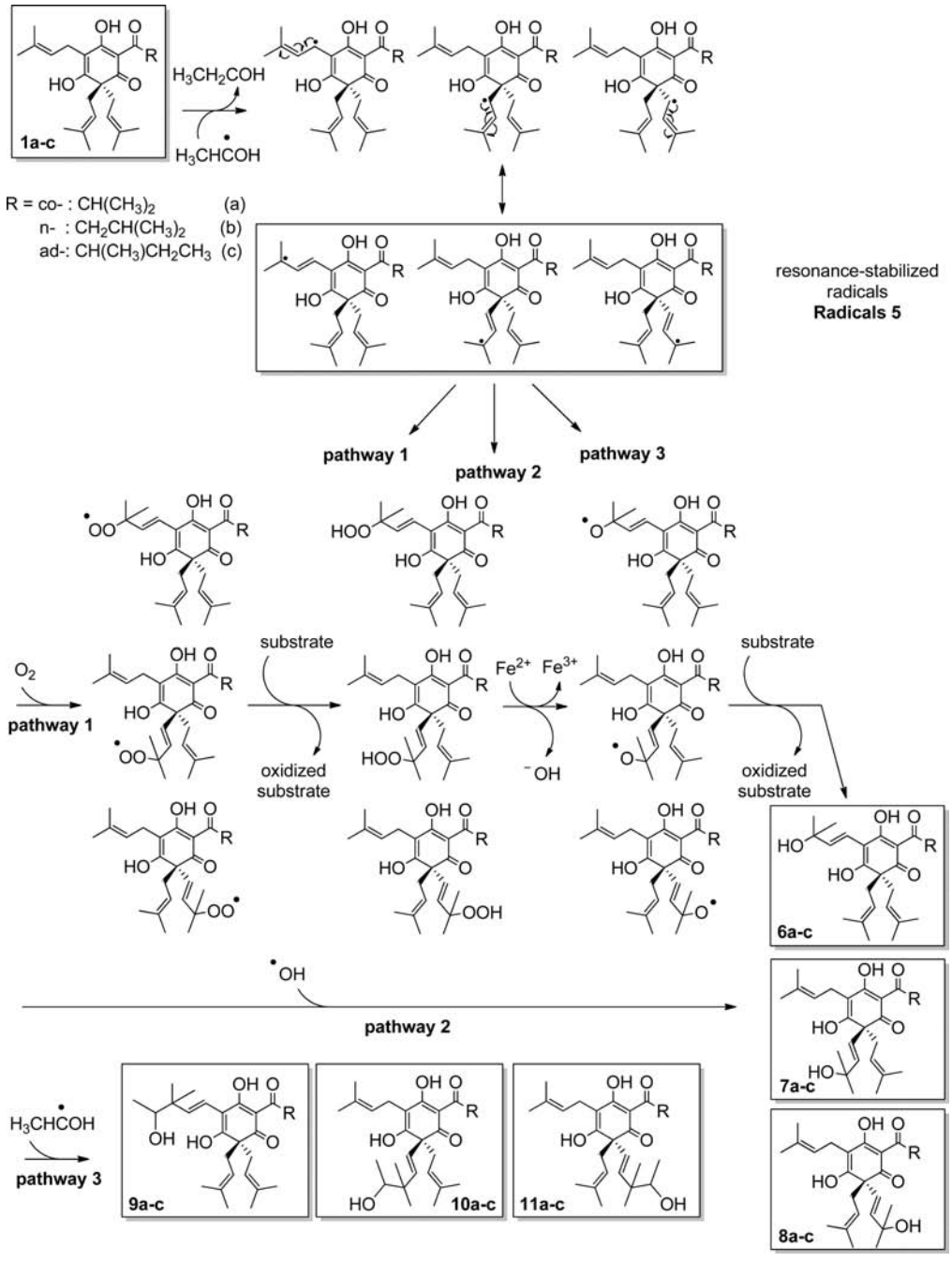

Figure 7. Proposed reaction pathways for the reaction between 1-hydroxyethyl radicals and lupulones for the formation of hydroxylated derivatives (**6a−c**, **7a−c**, **8a−c**) and allylic alcohol derivatives (**9a−c**, **10a−c**, **11a−c**) of lupulones.

indicate addition of a hydroxyl group in the n- and ad-lupulones.

On the other hand, peaks B and C showed the same quasi-molecular ion at m/z 461 (t_r = 19.1 and 20.1 min, respectively), which suggests formation of compounds arising from covalent binding of a 1-hydroxyethyl radical to the homologous ad- and n-lupulones. To confirm this supposition, the reaction was performed in deuterated media (d_6-ethanol), and then the mixture was analyzed by LC-ESI-IT-MS/MS (Figure 6). The chromatographic profile of the oxidation products was identical for both media as well for the mass spectra of peaks A, D, and E. The quasi-molecular ions of peaks B and C, at m/z 465, presented the addition of 4 mass units relative to the quasi-molecular ions from the same peaks in nondeutered medium. Indeed, these data corroborate the fact that the peaks corresponding to m/z 461 (Figure 5) and m/z 465 (Figure 6) refer to 1-hydroxyethyl radical addition products to ad- and n-lupulones.

According to de Almeida et al.[18] the bond dissociation enthalpies (BDE) of allylic hydrogen atoms in the isohumulone alkenyl side chains are similar, suggesting that hydrogen atom abstraction may occur at both side chains. Thus, in our case, three possible radical chemical structures arising for each lupulone analogue are expected, because all three prenyl side chains are susceptible to hydrogen atom abstraction and are likely to show similar values of BDE for the allylic hydrogens. Therefore, the quasi-molecular ions at m/z 417 refer to oxidation products that contain a hydroxyl function in one of

the prenyl side chains of colupulone (**6a**, **7a**, **8a**) (Figure 7), and the quasi-molecular ions m/z 431 refer to the products formed from the addition of a hydroxyl function in the prenyl side chains of the homologous n- and ad-lupulones (**6b,c**, **7b,c**, **8b,c**). The quasi-molecular ions at m/z 461 are a result of the addition of 1-hydroxyethyl radical in the side chains of n- and ad-lupulones (**9b,c**, **10b,c**, **11b,c**). However, it was not possible to detect by HPLC-ESI-IT-MS compounds derived from colupulones that contain a 1-hydroxyethyl radical attached to the prenyl side chains (**9a**, **10a**, **11a**). To circumvent this fact and to obtain unequivocal identification of reaction products, ultrahigh-resolution and accurate mass spectrometry in the positive ion mode were performed in a hybrid linear ion trap-orbitrap Fourier transform mass spectrometer (see the Supporting Information). Reaction samples were directly infused into the ESI source (5 μL/min), allowing the qualitative identification of compounds **6a**−**c**, **7a**−**c**, **8a**−**c**, **9a**−**c**, **10a**−**c**, and **11a**−**c**. Compounds **6a**−**c**, **7a**−**c**, and **8a**−**c** were identified by the presence of the pseudomolecular ions at m/z 417.26317 (**6a**, **7a**, and **8a**/calculated for $[C_{25}H_{36}O_5 + H]^+$ = 417.26355, error of −0.9 ppm) and m/z 431.27886 (**6b,c**, **7b,c**, and **8b,c**/calculated for $[C_{26}H_{38}O_5 + H]^+$ = 431.27920, error of −0.8 ppm). Compounds **9a**−**c**, **10a**−**c**, and **11a**−**c** were identified by the presence of pseudomolecular ions at m/z 445.29446 (**9a**, **10a**, and **11a**/calculated for $[C_{27}H_{40}O_5 + H]^+$ = 445.29485, error of −0.9 ppm) and m/z 459.310212 and 461.32579 (**9b,c**, **10b,c**, and **11b,c**/calculated for $[C_{28}H_{42}O_5 + H]^+$ = 459.3105, error of −0.8 ppm and $[C_{28}H_{44}O_5 + H]^+$ = 461.32615, error of −0.8 ppm, respectively).

Thus, there are three possible reaction mechanisms as shown in Figure 7, in which pathways 1 and 2 were previously reported in the literature[18] for the reaction between 1-hydroxyethyl radicals and isohumulones, which could be used to describe the formation of the identified hydroxylated derivatives. All reaction mechanisms are initiated by hydrogen atom abstraction from the prenyl side chains of lupulones by a 1-hydroxyethyl radical. The primary formed radicals are resonance-stabilized (radicals **5**) (Figure 7), and then the reaction may follow three different pathways.

The first pathway considers that residual molecular oxygen reacts with the resonance-stabilized radicals (radicals **5**) (Figure 7), giving rise to peroxyl radicals, which abstract hydrogen atoms from substrates, yielding hydroperoxides. The formed hydroperoxides are cleaved by the presence of high amounts of Fe(II) ions producing the hydroxylated derivatives of lupulones (**6a**−**c**, **7a**−**c**, **8a**−**c**) through hydrogen abstraction from the initial lupulones. In pathway 2, the hydroxylated derivatives of lupulones (**6a**−**c**, **7a**−**c**, **8a**−**c**) are formed from direct trapping of radicals **5** (Figure 7) by hydroxyl radicals, which arise from the excess of hydrogen peroxide and Fe(II) ions in the reaction medium. Finally, the third pathway considers that radicals **5** are trapped by 1-hydroxyethyl radicals, giving rise to 1-hydroxyethyl radical addition at the unsaturated side chains of lupulones (**9a**−**c**, **10a**−**c**, **11a**−**c**) as probed by using isotopically labeled ethanol (d_6-ethanol). The oxidation products herein described have not been previously reported, and they share the property of moving the olefinic double bond to an allylic position in the prenyl side chains of lupulones to stabilize the formed radical, which may further react with molecular oxygen or a hydroxyl radical or a 1-hydroxyethyl radical.

In conclusion, the lupulones have been shown to be very reactive toward 1-hydroxyethyl radicals with apparent rate constants close to the diffusion control as probed by EPR and ESI-IT-MS/MS spin trapping using a competitive kinetic approach. The prenyl side chains of the lupulones could be assigned as the reactive sites, because allylic hydrogen abstraction gives rise to resonance-stabilized radicals (radicals **5**) (Figure 7) that subsequently react with molecular oxygen or a hydroxyl radical or a 1-hydroxyethyl radical, yielding the identified oxidation products. The results of the present investigation may contribute to a better knowledge of the mechanism of decomposition of lupulones during beer brewing processes and aging and may provide a better understanding of the mechanism behind the decrease of antiseptic activity of the added lupulones during bioethanol production yield to occasional bacterial secondary fermentation and decreasing the overall ethanol yield.

■ ASSOCIATED CONTENT

Ⓢ Supporting Information

Ultrahigh-resolution mass spectrum (m/z 400−480) of the reaction sample acquired by direct sample infusion (5 μL/min) in the positive ion mode of electrospray (Figure S1) and Figures S2−S6 showing the enlargement of relevant spectrum regions. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

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