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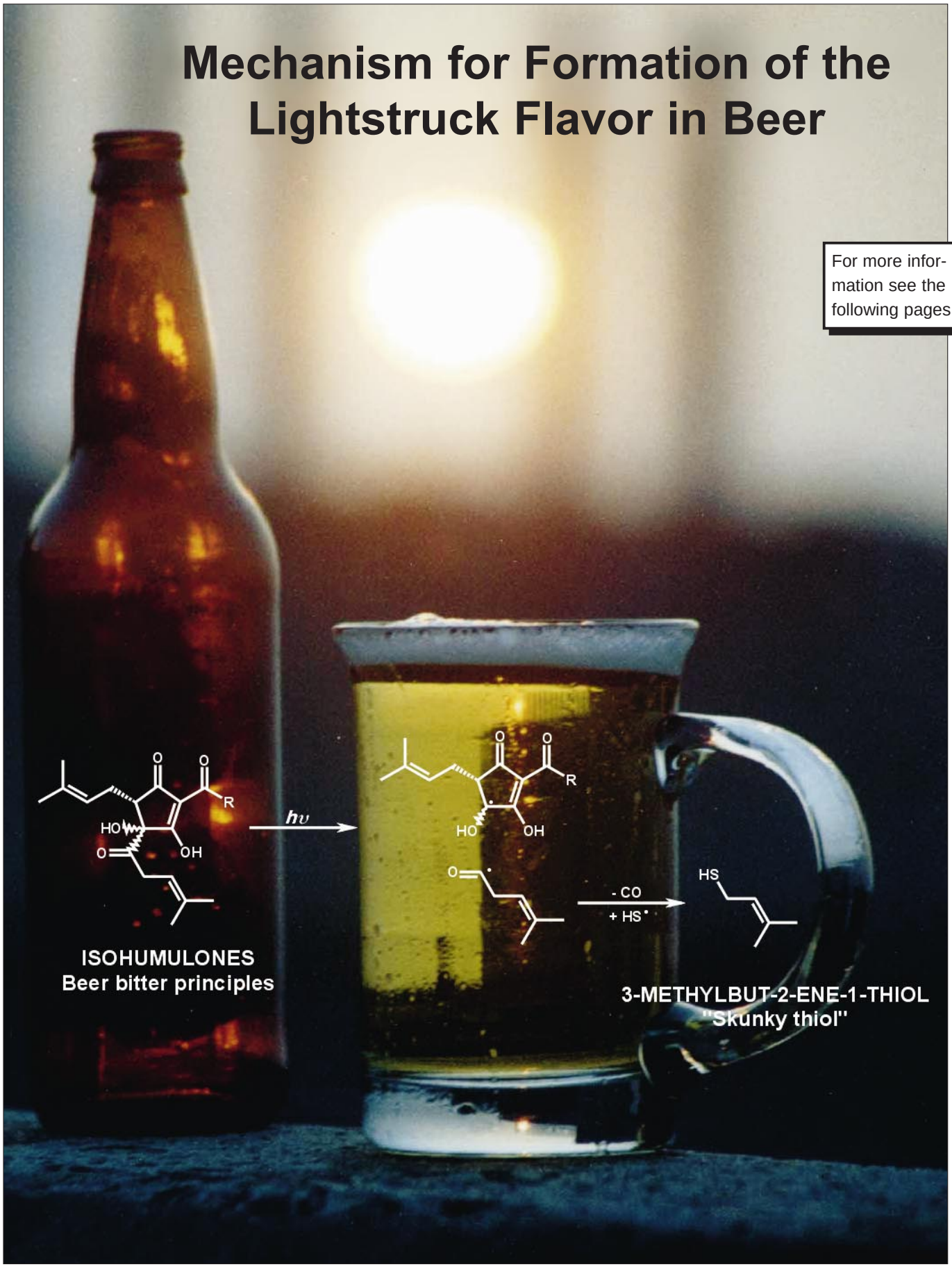

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Mechanism for Formation of the Lightstruck Flavor in Beer Revealed by Time-Resolved Electron Paramagnetic Resonance

Colin S. Burns,[a] Arne Heyerick,[b] Denis De Keukeleire,*[b] and Malcolm D. E. Forbes*[a]

Abstract: Time-resolved electron paramagnetic resonance (TREPR) data collected during the photodegradation of iso-α-acids (isohumulones), the principal bittering agents from hops in beer, are presented and discussed, and, from the data, the photophysics leading to free-radical production as the primary step in the photodecomposition of iso-α-acids towards the development of "skunky" beer are explained. During laser flash photolysis of iso-α-acids at 308 nm in toluene/methylcyclohexane (1:1), TREPR spectra exhibit net emissive signals that are strongly spin polarized by the triplet mechanism of chemically induced electron spin polarization. From two potential photochemically active sites, the TREPR data show that although the first site, an enolized β-triketone, is the primary light-absorbing chromophore, an uphill intramolecular triplet energy transfer process leads to Norrish type I α-cleavage at a second site, an α-hydroxycarbonyl. The energy transfer mechanism is supported by additional TREPR experiments with chemically modified hop compounds. Structural parameters (hyperfine coupling constants, *g* factors, line widths) for the observed free radicals, obtained from computer simulations, are presented and discussed.

Keywords: beer · EPR spectroscopy · isohumulones · photolysis

Introduction

Beer is a complex mixture consisting mainly of water and ethanol, with a fraction of about 0.5% solids that contains over 200 different substances. These constituents are derived from various raw materials, principally barley malt, water, hops, and yeast. In an early stage of the brewing process, a solution of carbohydrates, called wort, is produced by enzymatic degradation of starch provided by barley malt and, in some cases, cereal grains or rice. The boiling of the wort, with hops added as flavoring agents, produces a liquid called hopped wort in which fermentation takes place after addition of yeast. The compounds shown in Scheme 1, which are contained in the powdery lupulin glands of female hop cones, are called the α-acids, and are generally present as a mixture of humulone (**1a**), cohumulone (**1b**), and adhumulone (**1c**). During the boiling of the wort, these compounds undergo thermal isomerization to produce both *trans*- and *cis*-iso-α-acids (shown as structures **2** and **3**, respectively). As there are three main α-acids in hops, differing in the side chain structure, six iso-α-acids occur in beer as epimeric mixtures of isohumulones (**2a**, **3a**), isocohumulones (**2b**, **3b**), and isoadhumulones (**2c**, **3c**), respectively.

The typically bitter beer taste is due to the presence of the iso-α-acids in concentrations varying between 15 and 100 ppm (normally $\sim 25\ mg\,L^{-1}$). Additionally, these acids contribute to the bacteriostatic properties of beer and they function as pivotal elements in the formation of a stable foam head on beer.[1] Problematic to the iso-α-acids is their pronounced light sensitivity. Exposure of beer to light causes the development of an offensive taste and a "skunky" odor termed the "lightstruck flavor". This phenomenon has been reported in the literature as early as 1875,[2] but until now the detailed mechanism has not been unraveled. As the lightstruck flavor is not observed in unhopped beers, hop-derived compounds play a paramount role in this process. As will be demonstrated in detail below, there is mounting evidence that photolysis of the iso-α-acids is particularly important, and, therefore, we have undertaken a study of their direct photolyses. In this report we present time-resolved electron paramagnetic resonance (TREPR) data that give significant details about the primary mechanism of this photochemical reaction. While steady-state EPR methods have been used to study oxidative

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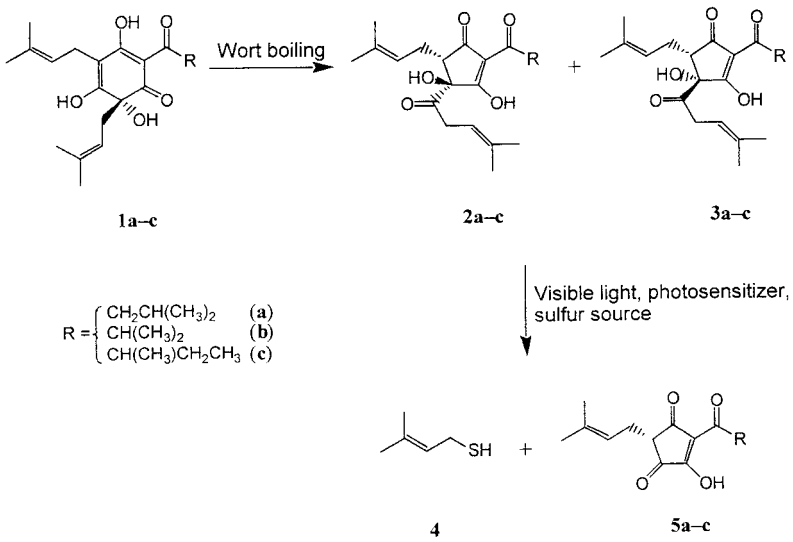


Scheme 1.

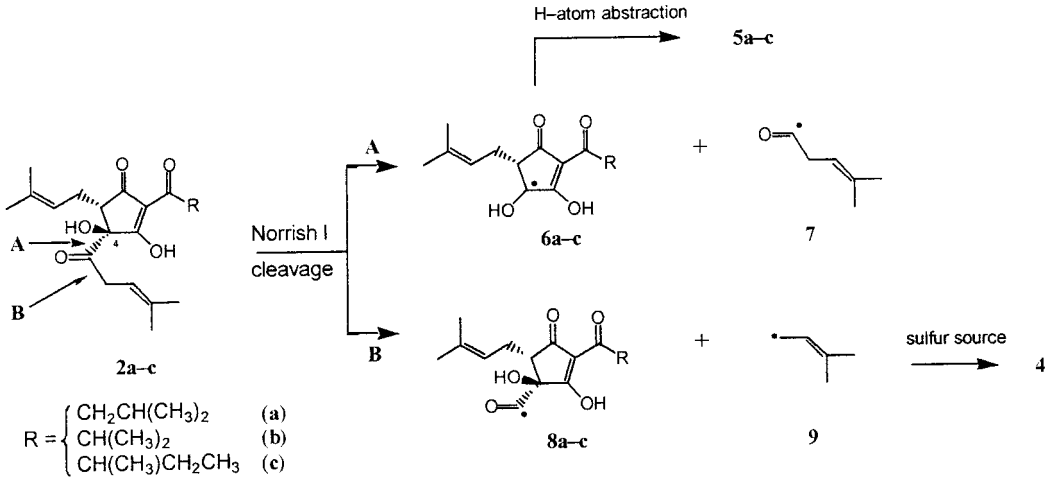
stress in beer,[3, 4] this paper represents the first use of any direct time-resolved spectroscopic technique for the study of the photodegradation of hop-derived beer bittering agents.

Model reactions carried out by Kuroiwa et al. have shown that photolysis with visible light (up to 500 nm) of solutions containing a mixture of riboflavin, isohumulones, ascorbic acid, and sulfur-containing amino acids produces 3-methyl-but-2-ene-1-thiol (**4** in Scheme 1), the compound believed to be largely responsible for the offending flavor and odor.[5] The flavor threshold of this thiol is so low that concentrations of even a few parts-per-trillion (ppt; $ng\,L^{-1}$) can make beer unpalatable; therefore, even very small photochemical conversion rates of iso-α-acids can produce this effect. Gunst and Verzele have confirmed that the thiol is present in light-exposed beer by GC-analysis of the beer headspace.[6] Although the iso-α-acids do not absorb in the visible region, the thiol can be formed by exposure to visible light, most probably due to sensitization by compounds such as riboflavin (a few hundreds of $\mu g\,L^{-1}$ in beer);[7] this reaction is still under further investigation.

Photolysis of *trans*-isohumulone (**2a**) in methanol at 300 nm has been reported to give dehydrohumulinic acid (**5a** in Scheme 1) as a major reaction product.[8] Formation of **5a** apparently results from a mechanism involving light-induced α-cleavage of the carbonyl group in the 4-methyl-pent-3-enoyl group at carbon 4. Such Norrish type I photoreactions usually originate from the $n-\pi^*$ state of an excited ketone.[9] Loss of the unsaturated side chain from this site would then furnish radical precursors to **4**.

The cleavage reaction for *trans*-iso-α-acids (**2a–c**) is detailed in Scheme 2. The ketone triplet state can cleave on either side of the carbonyl, indicated as pathways A and B. In either case, acyl radicals (**7** or **8a–c**) and other radicals stabilized by electron delocalization emerge (**6a–c** or **9**). Pathway A leads to a ketyl radical, while pathway B leads to an allylic radical. There is evidence that C–C bond cleavage of α-hydroxy carbonyls can be faster than that for alkyl-substituted aliphatic structures;[10] this leads us to favor pathway A. Photolysis products **5a–c** from Scheme 1 result from abstraction of a hydrogen atom from radicals **6a–c**. These products are also produced by the same reaction after the expected rapid decarbonylation of radicals **8a–c**. The compound responsible for "skunky" beer, **4**, should evidently be produced from trapping of the acyl radical **7** (pathway A, after decarbonylation on the sub-microsecond timescale) or directly from the allyl radical **9** (pathway B). The trapping agent can be any sulfur-containing species in the beverage, for example, sulfur-rich proteins derived from barley malt or low-molecular-weight sulfur-containing compounds, including cysteine.

On dissolving **4** in water, an obnoxious taste is apparent already at concentrations below 1 $ng\,L^{-1}$. In beer, the organoleptic activity is somewhat weaker, since the beer matrix contains a number of constituents able to mask the offending



Scheme 2.

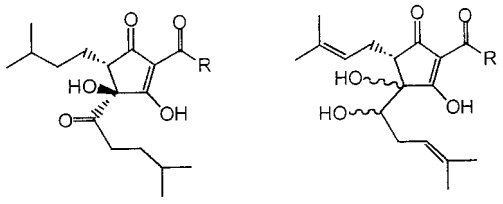
flavor. Values quoted in the literature vary: 1.25 – 2.5 ng L^{-1},[11] 4.4 – 35 ng L^{-1},[12] and 0.46 – 338 ng L^{-1},[13] have been reported, depending on the beer type and the sensitivity of each individual person. In view of these very low threshold values, compound **4** is one of the most powerful taste- and flavor-active compounds known.

The goal of the present study is to examine the primary photochemical events by using time-resolved (continuous wave) electron paramagnetic resonance (TREPR) spectroscopy[14] and computer simulations of the spectra to identify the free radicals produced. The TREPR experiment has superior time response (~60 ns) over steady-state EPR methods yet retains the high structural resolution needed to measure the hyperfine interactions used for assignment of the signal carriers. Not only have we accomplished this original goal, but, due to the presence of chemically induced electron spin polarization (CIDEP)[15] in the TREPR signals, we have also obtained important new insight into the photophysics of the excited states leading to the observed radicals.

Experimental Section

The iso-α-acids are commercially available as an aqueous solution (ca. 30% w/v) of the potassium salts of *trans*-iso-α-acids (**2a – c**; Scheme 1; **2a**: 12%, **2b**: 7%, **2c**: 3%) and *cis*-iso-α-acids (**3a – c**; **3a**: 42%, **3b**: 24%, **3c**: 12%) (Wigan Co., Eardiston, Near Tenbury Wells, Worcestershire, England).[16, 17] The iso-α-acids were freed from the salts by ten-fold dilution, acidification to pH 1, extraction with isooctane, and removal of the solvent. The *trans*-iso-α-acids (**2a – c**) were accessed, using literature methods,[18] by selective precipitation with dicyclohexylamine (DCHA) in ethyl acetate, recovery of the DCHA-salts, re-dissolution in warm ethyl acetate, addition of acidified water (pH 1), phase separation, and evaporation of the organic solvent. These compounds were determined to be >99% pure by HPLC.

The TREPR experiment can distinguish between radicals of different structure, and, through the observation of CIDEP, the experiment also allows characterization of the excited state precursors. To take advantage of these possibilities, two chemically modified sets of iso-α-acids were also studied. These are the *trans*-tetrahydroiso-α-acids (**10a – c**) and dihydroiso-α-acids (**11a – c**). The tetrahydroiso-α-acids are commercially available as



10a–c **11a–c**

$$R = \begin{cases} CH_2CH(CH_3)_2 & \textbf{(a)} \\ CH(CH_3)_2 & \textbf{(b)} \\ CH(CH_3)CH_2CH_3 & \textbf{(c)} \end{cases}$$

an aqueous solution (ca. 10% w/v) of their potassium salts (Wigan Co., Eardiston, Near Tenbury Wells, Worcestershire, England).[17, 19] *Trans*-tetrahydroisohumulone (**10a**; 44%), *trans*-tetrahydroisocohumulone (**10b**; 42%), and *trans*-tetrahydroisoadhumulone (**10c**; 14%) were separated from the *cis*-epimers according to a procedure described above for the isolation of the *trans*-iso-α-acids. Dihydroiso-α-acids (**11a – c**; five major constituents, diastereomers not assigned individually) were a gift from Kalsec Corp. (Kalamazoo, Michigan, USA).[17, 20] These compounds were determined to be >97% pure by HPLC.

Our time-resolved electron paramagnetic resonance apparatus has been described in detail elsewhere.[21] Briefly, it operated as follows: samples ranging in concentration from 10^{-2} to 10^{-3} M were allowed to flow through a quartz flat cell (0.4 mm path length) positioned in the center of a TE_{103} rectangular optical transmission cavity of a JEOL RE-1X spectrometer. The samples were irradiated with 308 nm light from a Lambda Physik LPX100i excimer laser operating at 308 nm (~20 mJ per pulse hitting the sample). The concentrations of radicals produced by each laser flash was below 10^{-5} M. The TREPR spectrum was collected at a fixed delay time after the laser flash in the absence of field modulation. Spectra were recorded as the analogue output of a two-gate boxcar integrator, while the external magnetic field was swept. All solvents were HPLC grade and were used as received from Fisher Scientific and Aldrich.

Results and Discussion

Figure 1A shows the X-band (9.5 GHz) TREPR spectrum obtained at a delay time of 0.3 μs after photolysis at 308 nm of a solution of *trans*-iso-α-acids (**2a – c**) in toluene/methylcy-

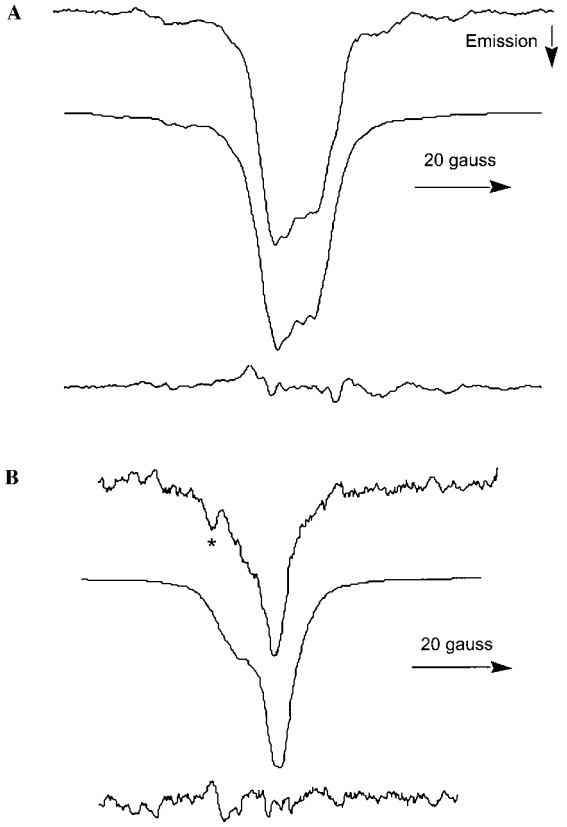


Figure 1. A) Top: Experimental TREPR spectrum recorded during 308 nm photolysis of a solution of *trans*-iso-α-acids (**2a – c**) in toluene/methylcyclohexane (1:1). In this and all subsequent spectra transitions below the baseline are in emission and those above the baseline exhibit enhanced absorption, in line with the direct detection method employed. Bottom: Simulation using the magnetic parameters listed in Table 1 for radicals **6a** and **9** (Scheme 2). B) Top: Experimental TREPR spectrum recorded during 308 nm photolysis of a solution of *trans*-tetrahydroiso-α-acids (**10a – c**) in methylcyclohexane. See text for an explanation of the marked * transitions. Bottom: simulated spectrum using magnetic parameters listed in Table 1 for radicals **6a′** and **7′**. Both experimental spectra were obtained at room temperature at a delay time of 0.3 μs after the laser flash. Residuals (simulation subtracted from spectrum) are shown at the bottom of each data set. See text for details.

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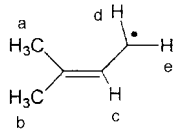
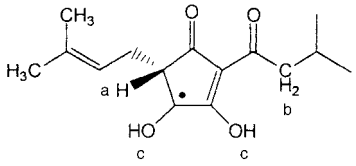
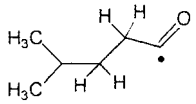
Table 1. Magnetic parameters used for simulations in Figure 1.

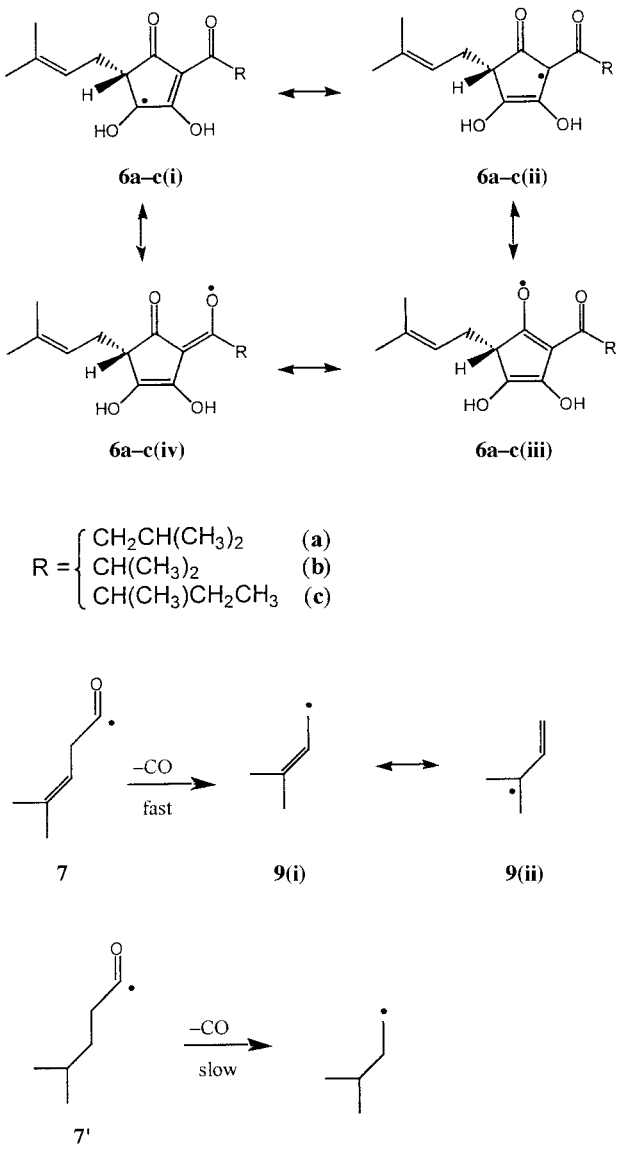
Radical[a]	Structure	Hyperfine coupling constants [Gauss]	*g* factor	Line width [Gauss]	Ref.
9		11.0 ($3H_a$) 14.0 ($3H_b$) 4.0 ($1H_c$) 13.8 ($1H_d$) 13.1 ($1H_e$)	2.0026	2.2	23
6a (**6a′**)		8.4 ($1H_a$) 2.2 ($2H_b$) 2.9 ($2H_c$)	2.0026	4.1	this work
7′		–	2.0008	4.0	this work

[a] Radicals **6a′** and **7′** arise from photolysis of **10a**. Radical **6a′** (not shown) is identical to radical **6a** except that the double bond in the 3-methylbut-2-enyl side chain is not present. Radical **6a′** has the same hyperfine coupling constants, *g* factor, and line width as **6a**.

clohexane (1:1 ratio). Immediately below the experimental spectrum is a computer simulation calculated by using the parameters listed in Table 1, and below this is the residual (spectrum minus simulation), to be discussed below. The fact that a strong TREPR signal is detected indicates that free radicals are indeed produced when UV light strikes iso-α-acids. The strong intensity in the center of the spectrum and weak signals on the edges indicate the presence of at least two different radical species with significant electron–nuclear hyperfine interactions. Acyl radicals are not expected to have large hyperfine coupling constants, and, since decarbonylation in both cases leads to free radicals stabilized by significant resonance delocalization, it is likely that we are observing radicals that have lost CO regardless of the cleavage pathway (A or B).

Photolysis of *trans*-tetrahydroiso-α-acids (**10a–c**) yields the TREPR signal shown in Figure 1B, which again has a simulation shown immediately below it and a residual at the bottom. This simple modification of *trans*-iso-α-acids by hydrogenation of the unsaturated side chains significantly alters the shape of the EPR signal, indicating that at least one of the radical centers originating from photolysis of *trans*-iso-α-acids is located in close proximity to an alkene functionality. It is noteworthy that the signal from this hydrogenated sample has narrowed considerably, which is to be expected if, after cleavage, decarbonylation to the allyl radical is no longer taking place on the μs timescale. These results are consistent with literature data on the decarbonylation rates of acyl radicals leading to saturated and unsaturated carbon centered radicals.[22] Photolysis of a solution of dihydroiso-α-acids (**11a–c**) at 308 nm does not lead to observable TREPR signals; this significant result will be discussed in more detail below.

The structures of the possible free radicals formed by this photochemistry, along with the most probable resonance contributors to them, are shown in Scheme 3. Acyl radical **7** should decarbonylate quickly (within approximately 100 ns) to dimethylallyl radical **9**. Resonance form **9(ii)** allows spin



Scheme 3.

density on, and, therefore, hyperfine interaction with the terminal methyl protons, and our simulation uses values very close to the literature hyperfine coupling constants[23] for the dimethyl allyl radical, listed in Table 1 (three of the couplings are exactly the same as in ref. [23], and the other two are within 10% of their literature values). Radicals **6a–c** each have four resonance contributors, although it is unlikely that they contribute equally to their overall stability. For example, resonance structures **6a–c(iv)** contain an *exo*-double bond and an electron-deficient oxygen. Structures **6a–c(iii)**, as cyclopentadiene derivatives with an electron-deficient oxygen, are also probably of minor importance. Still, contributors **6a–c(i–iv)** taken together provide significant stabilization of the radical center and, because they all pull electron density away from the point of cleavage, hyperconjugation to the lone H atom on the ring is probably not as large as it would be for a radical without these substituents.

The only radical close in structure to **6a–c**, for which literature hyperfine couplings are known, is the hydroxycyclopentenyl radical,[24] which has a coupling constant of 20 Gauss for an H atom in a similar position to the lone H atom on the ring system of **6**. Our simulation of radical **6a** gives a coupling constant of only 8.4 Gauss for the lone ring proton. This is consistent with the expected lower spin density in our system due to delocalization of the unpaired spin away from the other side of the five-membered ring. The hydroxyl coupling constants are also close to those reported in the literature for similar radicals.[25] Presumably, they are resolved here due to the use of non-aqueous solvents in our experiments; in aqueous solution, hydrogen bonding and/or exchange processes broaden them significantly.

It is perhaps somewhat surprising to require hyperfine interaction on the alkyl group on the other side of the triketone from the ring (position b for **6a** in Table 1) in order to fit the spectrum in Figure 1A. However, with delocalized radicals such as **6a–c**, there is certainly the possibility of hyperconjugation to the π system leading to these small couplings. Although there are two such couplings for radical **6a** and only one for both **6b** and **6c**, we obtain a best fit with two. This is consistent with our samples having a considerably higher mole fraction of precursors **2a** and **3a**. The possible existence of a superposition of radicals with one or two couplings at this position is most likely responsible for the small discrepancies in the overall line shape of the simulation of Figure 1A, especially at the perimeter of the transitions from radicals **6a–c**. We dismiss the possibility of hyperfine interaction with the methylene hydrogens of the 3-methylbut-2-enyl substituent side chain, as the carbon atom bearing them is not directly bonded to the delocalized electrons; hyperconjugation would be very weak in this case.

The relative intensities of the two radicals in Figure 1A appear to be quite different, but this is easily understood. The dimethylallyl radical **9** has its intensity distributed over a very large number of hyperfine lines (128) that cover more than 100 Gauss. Therefore, even its most intense lines are diluted over much of the sweep width, and, in fact, the least intense lines on the perimeter are undetectable and left off the total sweep in the figure. Radicals **6a–c** on the other hand have EPR signals distributed over just 18 closely spaced lines, so this signal appears to be much more intense. In fact, the integrated intensity of each radical type is nearly the same. Small differences in the electron spin lattice relaxation time of the radicals or the decarbonylation rates of **8a–c** and **7** may be responsible for the integrations being not precisely equal. In regard to the simulation of Figure 1A, it should also be noted that a small amount of multiplet spin polarization (emission/absorption or E/A) has been added to this spectrum for best fit. This contribution is from the radical pair mechanism (RPM)[26] of CIDEP, and we note that it is of the correct phase for a triplet precursor to the radical pair.

If we are indeed observing in Figure 1B the saturated analogue of acyl radical **7**, that is, the 4-methylpentanoyl radical (labeled as radical **7′** in Table 1), then we can conclude that decarbonylation is very slow because the resulting species is a much less stable primary 3-methylbutyl radical. The changes expected in the TREPR spectrum upon hydrogenation of this double bond should be 1) the disappearance of the hyperfine structure due to radical **9** and 2) the appearance of a broad line with a different *g* factor and no resolvable hyperfine interaction for radical **7′**. The simulation immediately below Figure 1B supports this conclusively.

There is an additional emissive transition seen on the low-field side of the spectrum in Figure 1B that is possibly accompanied by an additional absorptive peak on the high-field side. This transition, marked with a * in Figure 1B, is due to an unknown radical that could not be simulated by using reasonable parameters for any of the radical structures shown in Scheme 3 above. This signal may arise from a competing photochemical reaction, for example, H-atom abstraction from the solvent, from decarbonylation of one of the acyl radicals, or from secondary photochemical processes. The fact that this signal appears to be more strongly RPM spin polarized (E/A) indicates that it does not result from a primary photochemical process. It is more likely to have been generated at a later delay time, after the strong net emissive polarization from the parent ketone triplet state has relaxed.

Some comments should be made about the residual signals (spectra minus their simulations) shown in Figure 1. In Figure 1B the residual contains no major peak above the noise level of the spectrum apart from the one marked with an asterisk, which as discussed above is most likely from an independent and later photochemical process. The residual for Figure 1A appears to show more structure, but it should be pointed out that the total area of the residual is less than 3% of the total area of the spectrum, which, by most standards for spectral simulation, represents an excellent fit. The major discrepancies are at the wings of the signal from radical **6a**, and this is not surprising as it is where the two signal carriers meet. Still, the error is small and may be due to small line shape errors rather than coupling constant errors. Another minor discrepancy is the lack of a good fit on the high-field side, but this is where the ratio of the two polarization mechanisms (RPM and TM) comes into play and this can be notoriously difficult to fit because the radicals may have different chemical lifetimes and spin-lattice relaxation times, leading to intensity distortions in this region of the spectrum. Overall, the simulation is satisfactory, especially with regard to the resolved hyperfine structure of radical **6a**.

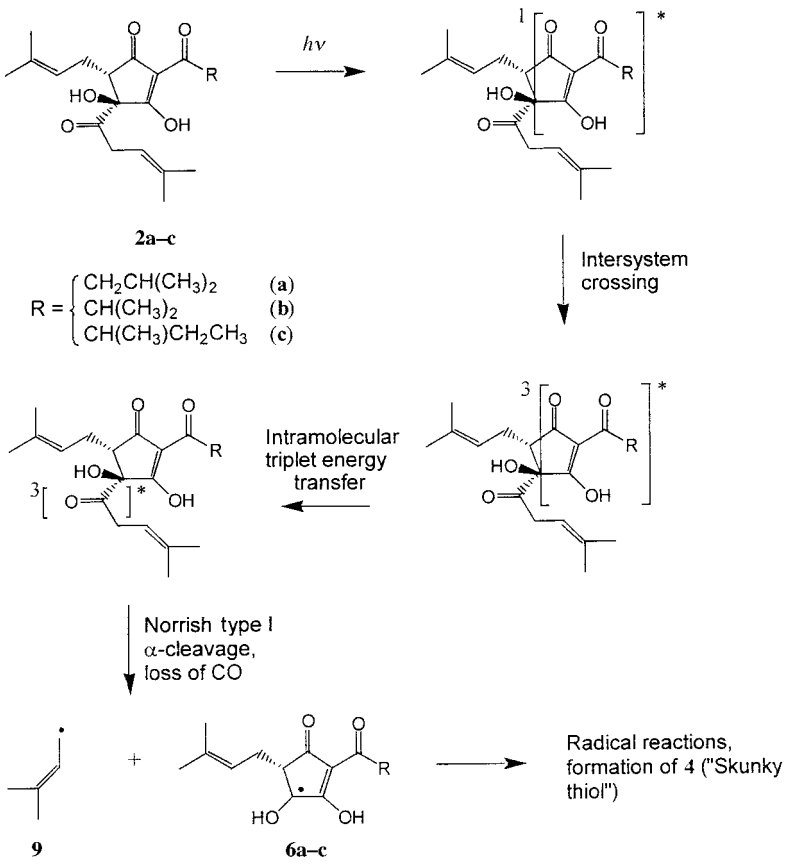
0947-6539/01/0721-4558 $ 17.50+.50/0 *Chem. Eur. J.* **2001**, *7*, No. 21

From the TREPR results and simulations, in conjunction with the product analyses outlined above, it can be concluded that the radicals result from Norrish type I α-cleavage of the isolated α-hydroxy ketone shown in Scheme 2. This is further supported by the fact that the dihydroiso-α-acids (**11a–c**) do not produce a TREPR signal upon 308 nm laser photolysis. This observation provides the first direct spectroscopic evidence for the resistance of dihydroiso-α-acids to photolysis and is the most conclusive proof to date that the photochemistry leading to the lightstruck flavor in beer originates at this site.

The net emission exhibited in both spectra in Figure 1 is generated by the triplet mechanism (TM) of CIDEP.[27] Briefly, this is caused by unequal populating of the three triplet spin levels during the intersystem crossing process from the first excited singlet state. The origin of the polarization and its phase (net E rather than net A) are topics worthy of further discussion, as consideration of these issues allows further mechanistic information to be obtained regarding the photophysics of the precursor molecules. In this regard, we note that the phase of the TM is dependent on the sign of the zero-field splitting parameter D of the parent ketone. In saturated ketones, the TM is usually weak and absorptive, while ketones conjugated to unsaturated bonds exhibit stronger TM polarization that is emissive. This reversal of TM phase is supported by reports of the sign of D for the triplet states of carbonyl compounds such as cyclohexanone[28] versus cyclohexenone,[29] which are opposite.

Because of the emissive polarization in the spectra, we propose the photophysical mechanism shown in Scheme 4 for the *trans*-iso-α-acids (**2a–c**). The first step involves absorption of UV radiation by the β-triketone portion of the molecule (indicated by brackets). This is easily concluded from the UV absorption spectrum of the compounds, to be discussed below. After intersystem crossing (ISC) to the triplet state, triplet energy transfer occurs to the isolated α-hydroxyketone, which in its excited state ultimately cleaves, and radical chemistry ensues. There is good evidence supporting the assumption that spin polarization transfer accompanies triplet energy transfer;[30, 31] therefore, we expect the isolated monocarbonyl triplet to show emission from the original absorbing chromophore rather than the enhanced absorption from the TM expected if the monocarbonyl were being directly excited.

The β-triketone moiety has a strong electronic absorption at 308 nm in all of the types of iso-α-acids under consideration, that is, *trans/cis*-iso-α-acids (**2** + **3**), *trans*-tetrahydroiso-α-acids (**10a–c**), and dihydroiso-α-acids (**11a–c**). Reduction of the monoketone, as in dihydroiso-α-acids, does not alter the absorption spectrum significantly, as the weak n–π^* transition of the monoketone must be hidden underneath the long-wavelength edge of the strong absorption band of the β-triketone. As an example, the ε value for dihydroiso-α-acids (**11a–c**) at 308 nm in methanol is 1750 L cm mol^{-1}, while an ε value of 15 L cm mol^{-1} is reported for the n–π^* transition of a model compound, 3-hydroxy-3-methylbutan-2-one, also at 308 nm in methanol. These facts corroborate our suggestion that the β-triketone moiety is the primary light-absorbing chromophore in the iso-α-acids at 308 nm.



Scheme 4.

To provide additional support for this mechanism, the triplet spectrum of a mixture of frozen dihydroiso-α-acids (**11a–c**) in methylcyclohexane was generated and observed by TREPR (Figure 2A). This experiment was carried out in order to characterize the β-triketone triplet in the absence of the energy transfer process. The strongly emissive half-field transitions in Figure 2A, indicated by an asterisk, are a strong indicator that the net emissive polarization in Figure 1A originates from the β-triketone chromophore.[32] A rough estimate of the dipolar interaction D in the triplet can be made by measurement of the separation (in Gauss) between the outermost $\Delta m = 1$ transitions in Figure 2A, indicated by dashed vertical lines. This gives $D \sim 1100 \pm 100$ Gauss or approximately 0.1 cm^{-1}, which is very consistent with a delocalized triplet such as this triketone.

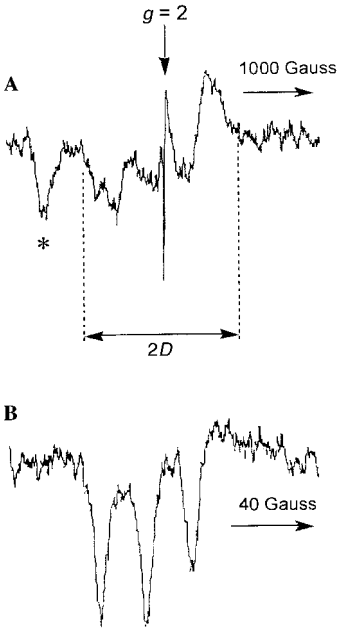


Figure 2. A) TREPR spectrum of the triplet state of the dihydroiso-α-acids (**11a–c**) frozen in methylcyclohexane. The data were obtained at a temperature of 100 K at a delay time of 1.0 μs after the 308 nm laser flash. The emissive $\Delta m = 2$ transitions (half-field lines) are marked by *, and dotted lines indicate the peripheral $\Delta m = 1$ transitions used to evaluate the zero-field splitting parameter D for the triplet. B) TREPR spectrum obtained after photoexcitation of a solution containing a mixture of TEMPO and dihydroiso-α-acids (**11a–c**) in methycyclohexane at room temperature taken 1.0 μs after the laser flash.

The mechanism is further supported by experiments involving photolysis of a solution containing a mixture of dihydroiso-α-acids (**11a–c**) and 2,2,6,6-tetramethylpiperidine-1-oxyl (TEMPO), a stable free radical. In the TREPR spectrum obtained from this sample an emissively polarized three-line spectrum of TEMPO is observed (Figure 2B). No TREPR spectrum is obtained with either dihydroiso-α-acids alone or TEMPO alone. The emissive polarization of the TEMPO free radical observed in this case is a result of the radical triplet-pair mechanism[33] of CIDEP, which results from the diffusive encounter and magnetic interaction between an excited triplet molecule, in this case that of the dihydroiso-α-acids, and a doublet-state free radical. The net emission observed here must arise from the polarized triplet state of the dihydroiso-α-acids, as the nitroxide does not absorb the light nor does it have any spin polarization it can acquire on its own. Both spectra in Figure 2 allow us to conclude that the emissive polarization observed in the TREPR spectra in Figure 1 is originally generated in the intersystem crossing process from S_1 to T_1 of the β-triketone, the primary light-absorbing chromophore in the iso-α-acids.

The intramolecular triplet energy transfer process from the β-triketone to the isolated α-hydroxyketone is noteworthy, as it is expected that it should be uphill in energy by $\sim$25 kJ mol^{-1}.[34] Even so, the radicals are formed from the α-hydroxyketone within a few hundred nanoseconds (the TREPR signals from **6a–c**, for example, show maximum intensity at approximately 400 ns after the laser flash). For two chromophores diffusing in free solution, collisional triplet energy transfer is relatively slow ($\sim 10^5$ s^{-1}) for this large an energy difference between the states.[34] However, when the two chromophores are connected as in this case, the triplet energy transfer rate can be much faster. Johnson et al. measured triplet energy transfer from naphthalene to biphenyl at greater than diffusion-controlled rates (7.7×10^9 s^{-1}) when the two chromophores were connected to an alkane spacer and separated by only two C–C σ bonds.[35] Although that result was for a downhill reaction ($\sim$17 kJ mol^{-1}), it demonstrated that covalently linking the triplet donor and acceptor can increase the intrinsic triplet energy transfer rate. It is possible that the two chromophores are in fact very strongly coupled in our system. Because the hydroxyl group and the acyl side chain at C-4 are oriented above and below the five-membered ring that contains the β-triketone, the two chromophores are in a fair geometry for interaction directly through space, and through-bond coupling is also expected to be very strong. Therefore, it is not unreasonable to observe this uphill energy transfer process taking place on the submicrosecond timescale. Of course, this means that the cleavage reaction at the site producing radicals must be fast ($<$20 ns) in order to compete with back transfer of the triplet energy. Based on our comments above regarding the faster cleavage of α-hydroxyketone excited states, this seems feasible.

In order for the emissive TM polarization to be preserved during the energy transfer process, the transfer rate must also take place within the electron spin-lattice relaxation times of both of the triplets involved in the energy transfer process. This suggests that either these relaxation times are quite long or that the intersystem crossing rate from the initial singlet excited state is fairly slow. The spin lattice relaxation rate, T_1, of the triplet electron spins depends on the tumbling rate of the triplet in solution. In fact, the magnitude of the TM polarization also depends on this tumbling rate. With so many alkyl appendages, tumbling will be slower than for less substituted and more symmetric triplet states, which have electron T_1 values on the order of 1–10 ns. Therefore, a longer triplet electron spin-lattice relaxation rate than this for the triplet states of **2a–c** and **3a–c** is reasonable.

Conclusion

We have demonstrated that the primary photophysics involved in the lightstruck flavor of beer are absorption and formation of the triplet state of the delocalized β-triketo chromophore present in the iso-α-acids, followed by intramolecular energy transfer to the localized α-hydroxyketone moiety. Subsequent photochemistry from this ketone leads to the formation of free radicals through a Norrish type I α-cleavage. The events can be followed by TREPR because of strong emissive triplet-mechanism CIDEP, which is produced initially and then propagated throughout the various photophysical and photochemical pathways. Simulation of the TREPR spectra provides unambiguous assignment of the signal carriers to the radicals proposed in the mechanism. Future work will include attempts to obtain kinetic studies on

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the intersystem crossing, spin relaxation, and decarbonylation processes involved in this photochemistry.

Apart from storing beer in light-proof containers, such as dark glass or cans, or immediate consumption, the photosensitivity can be circumvented by reduction of the iso-α-acids so that the deleterious photochemical process is prohibited. This has been conclusively shown in the present work for the dihydroiso-α-acids in which the photoreactive α-hydroxyketone group is reduced to a photoinactive 1,2-diol entity, resulting in complete light resistance. Conversely, the tetrahydroiso-α-acids are as photoreactive as the iso-α-acids; however, 3-methylbut-2-ene-1-thiol ("skunky" thiol) cannot be formed from these compounds subsequent to photocleavage. As a consequence, the lightstruck flavor derived from tetrahydroiso-α-acids must be distinctly different from the "natural" lightstruck flavor, perhaps having less obnoxious organoleptic features.

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