

## Photooxidative Degradation of Beer Bittering Principles: A Key Step on the Route to Lightstruck Flavor Formation in Beer

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Isohumulones, dihydroisohumulones, tetrahydroisohumulones, and humulinones, important hop-derived bittering compounds in beer, were shown to give rise to reactive triacylmethyl radicals on interaction with triplet-excited riboflavin after spin trapping by 5,5-dimethyl-1-pyrroline *N*-oxide or 2-methyl-2-nitrosopropane, followed by electron paramagnetic resonance spectroscopy combined with spectral simulation. Electron abstraction from the ionized  $\beta$ -tricarbonyl chromophore, which is common to all five-membered ring hop derivatives, is the initial event on photoinduced degradation. Radicaloid decomposition of isohumulones leads to precursors for 3-methylbut-2-ene-1-thiol, the lightstruck constituent in beer. Interaction of reduced derivatives of isohumulones with triplet-excited riboflavin furnished radical precursors of volatile aldehydes, which may lead to the development of unpleasant stale or cardboard flavors.

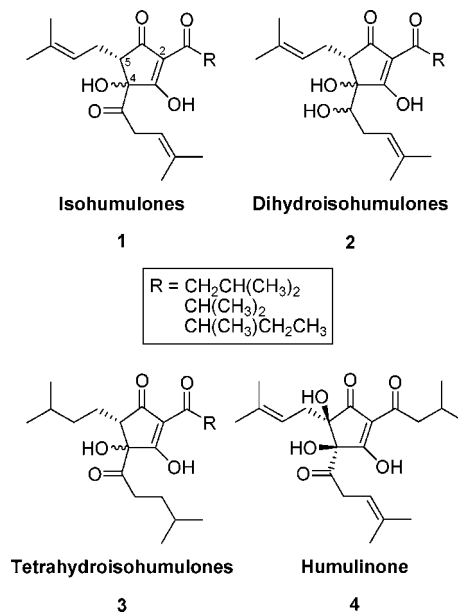
**KEYWORDS:** Isohumulones; lightstruck flavor; EPR spectroscopy; photooxidation; beer

### INTRODUCTION

The quality of beers decreases gradually, continuously, and irreversibly on storage as a result of the reactivity of constituents in the complex beer matrix. Thus, significant flavor and taste alterations affect the beer organoleptic features adversely with respect to the fresh brew (1). Indeed, thermal energy may lead to increased reactivity when the temperature is controlled improperly, while the presence of suitable redox couples could provoke oxidation processes, even in the absence of oxygen (2). Although the influence of light can be minimized by storage in opaque kegs or dark-colored bottles, still, light-induced changes occur rapidly during beer consumption. On exposure to light, a particular off-flavor, known as lightstruck flavor (LSF), is perceived. The occurrence of this phenomenon, reported as early as in 1875 (3), has later been attributed to the formation of 3-methylbut-2-ene-1-thiol (MBT) (4), which produces a foxy, skunky odor that is readily detected in concentrations of ca. 4 ng L<sup>-1</sup> (5).

As unhopped beers do not develop LSF, intervention of hop-derived substances seems essential (6). In particular, a pivotal role in the development of LSF is imputed to isohumulones. These bittering agents, present in concentrations between 10 and 35 mg L<sup>-1</sup> in lager beers, consist of a mixture of *cis*- and *trans*-isohumulones [usual ratio, 7:3; the descriptors *cis* and *trans*

**Scheme 1.** Structures of Isohumulones (1), Dihydroisohumulones (2), Tetrahydroisohumulones (3), and Humulinone (4)



refer to the relative configuration of the tertiary hydroxyl at C<sup>4</sup> and the prenyl substituent at C<sup>5</sup> (**Scheme 1**), which are formed by thermal isomerization of humulones ( $\alpha$ -acids) that are present in the powdery lupulin glands of the female cones of hops (*Humulus lupulus* L.). Isohumulones are prone to light-induced

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decomposition on direct irradiation with UV-B light (280–320 nm) (7). Time-resolved electron paramagnetic resonance (EPR) spectroscopy revealed the formation of radicals after the enolized  $\beta$ -tricarboxyl chromophore of the isohumulones was excited by a 308 nm laser flash (8). Triplet energy from this moiety is transferred to the  $\alpha$ -hydroxyketone group resulting in a Norrish type I  $\alpha$ -cleavage. Fast decarbonylation of the 4-methylpent-3-enoyl radical thus formed produces a 3-methylbut-2-enyl radical, which, if trapped by a suitable sulfur source, eventually leads to MBT. These findings were nicely corroborated by a detailed product analysis after photolysis of *trans*-isohumulones and derivatives at 300 nm (9).

Kuroiwa et al. found that LSF was mainly evident on irradiation with wavelengths from 350 nm up to 500 nm (10), although isohumulones are transparent in this region (11). They suggested the interference of riboflavin (RF), a natural photosensitizer present in beer (12). As RF exhibits two absorption maxima at 375 and 445 nm, irradiation in this particular wavelength region furnishes triplet-excited RF ( $^3\text{RF}^*$ ) (13). A mechanism involving energy transfer from  $^3\text{RF}^*$  was proposed, but because the triplet energy of isohumulones is ca. 90 kJ mol<sup>-1</sup> higher than that of RF, this process is thermodynamically unfeasible (14). Furthermore, it has been reported that reactive oxygen species produced via photosensitization attack flavor active compounds (15); however, it is most likely that other pathways are responsible for the formation of LSF. Indeed, laser flash photolysis experiments indicated that an electron is released from isohumulones on interaction with  $^3\text{RF}^*$  (16), possibly forming radical precursors for MBT.

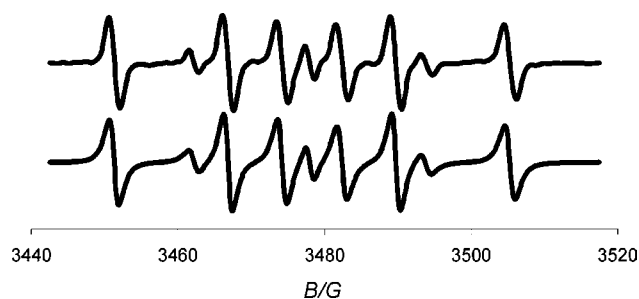
The purpose of this study was to identify radicals formed on interaction between excited RF and five-membered ring hop derivatives and to elaborate the reaction mechanism for the formation of the LSF in beer on visible light exposure. Therefore, spin traps were added to solutions containing RF and isohumulones and spin adducts formed on irradiation were analyzed by continuous wave EPR spectroscopy. Reaction mechanisms of reduced derivatives, showing only minor structural changes with respect to isohumulones, were investigated as well, i.e., dihydroisohumulones (also known as  $\rho$ -isohumulones) and tetrahydro-isohumulones. Humulinone, an oxidized derivative of isohumulone, was also included in this study.

## MATERIALS AND METHODS

**Chemicals.** Isohumulones (**1**), consisting of a mixture of *trans*-isohumulones and *cis*-isohumulones, as well as dihydroisohumulones (**2**) and tetrahydroisohumulones (**3**) are commercially available as aqueous solutions of the corresponding potassium salts (ca. 30% w/v, ca. 35% w/v, and ca. 10% w/w, respectively) (a kind gift by Botanix, Eardiston, Near Tenbury Wells, Worcestershire, England).

The sodium salt of humulinone (**4**) was prepared according to a literature procedure (17) by dissolving humulone (2.5 g) in a biphasic system of diethyl ether (150 mL) and a saturated sodium hydrogen carbonate solution (150 mL). After a catalytic amount of cumenylhydroperoxide was added, the reaction mixture was left for 2 days under ambient light conditions. The sodium salt of humulinone separated at the interface between the two liquid phases as white crystals (1.46 g, 56%).

RF, 5,5-dimethyl-1-pyrroline *N*-oxide (DMPO), and 2-methyl-2-nitrosopropane (MNP) were obtained from Aldrich (Bornem, Belgium). Solutions were prepared by dissolving isohumulones or derivatives (10 mM), the spin trap (10 mM), and RF (1 mM) in acetonitrile/water (v/v 1:1) or aqueous citrate buffer (pH 4.6)/acetonitrile (v/v 1:1). All solutions were made using Milli-Q (Millipore, Bedford, MA) double distilled water ( $R = 18 \text{ m}\Omega/\text{cm}^2$ ).



**Figure 1.** Experimental (upper trace) and simulated (lower trace) spin patterns of radicals derived from dihydroisohumulones (**2**) after photo-oxidation by  $^3\text{RF}^*$  and subsequent spin trapping by DMPO under a nitrogen atmosphere (for conditions, see Materials and Methods).

**Irradiation and EPR Spectroscopy.** A Bruker ECS 106 spectrometer (Bruker, Karlsruhe, Germany) was used for EPR spectroscopy. The settings were applied as follows: center field, 3480 G; sweep width, 80 G; microwave power, 10 mW; modulation frequency, 100 kHz; modulation amplitude, 1.0 G; conversion time, 82 ms; and sweep time, 84 s.

Samples were degassed with nitrogen or saturated with oxygen before being transferred into a flat quartz cell inside the EPR cavity by a peristaltic pump. Irradiation experiments were carried out inside the EPR cavity using a 60 W incandescent lamp. Simulation and fitting of EPR spectra to calculate hyperfine coupling constants were performed by the PEST WinSIM program (18).

## RESULTS

Although photochemical reactions of isohumulones (**1**) and derivatives (**Scheme 1**) on direct UV-B irradiation have been studied in detail (8, 9), decomposition pathways on illumination with visible light under sensitized conditions remain elusive. All five-membered ring hop derivatives share an enolized  $\beta$ -tricarboxyl moiety with a maximum absorption around 250–255 nm (shoulder around 270–280 nm) and molar absorptivities between 16000 and 19000 L mol<sup>-1</sup> cm<sup>-1</sup> (11). Hence, these compounds are transparent in the visible part of the electromagnetic spectrum.

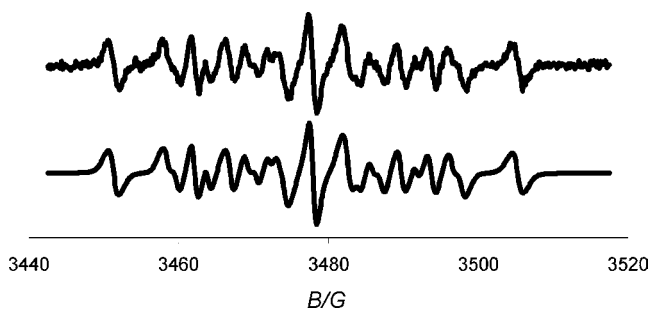
As was advanced previously (2), it was not necessary to isolate the individual constituents of the isohumulones or derivatives. Indeed, the nature of the R group (a hydrocarbon residue, representing isobutyl, isopropyl, or secondary butyl) in the side chain at C(2) does not significantly affect the reactivities of functionalities present elsewhere. Likewise, the stereochemistry at C(4) showed no significant influence on the reaction kinetics regarding interaction with  $^3\text{RF}^*$  and is, therefore, not relevant (15). Isohumulones have pK<sub>a</sub> values around 3 and, as the pH of lager beers is generally between 4.2 and 4.4, they occur predominantly in their anionic forms (19). Thus, five-membered ring hop derivatives were investigated as salts.

Detectable steady state radical concentrations could not be reached, when a solution of dihydroisohumulones (**2**) and RF was irradiated with visible light inside the EPR cavity. Therefore, spin traps were added and spin adducts with radicals formed on irradiation of isohumulones and derivatives in the presence of RF were detected. DMPO and MNP were used as trapping agents, whose reactivities under the irradiation conditions were examined also.

**Spin Trapping with DMPO.** Visible light exposure of a nitrogen-saturated solution (acetonitrile/water, v/v 1:1), containing a 10-fold molar excess of dihydroisohumulones (**2**) and DMPO with respect to RF, led to detection of two spin adducts (**Figure 1**). The main species was identified as a triplet of

**Table 1.** Relevant Data from Simulations of the EPR Spectra, Recorded at Varying Irradiation Times, of a Nitrogen-Degassed Solution of Dihydroisohumulones (**2**), RF, and a Spin Trap (DMPO and MNP, Respectively)<sup>a</sup>

time (min)		DMPO			MNP		
2	RA (%) <sup>a</sup>	82	18	70	20	10	
	a <sub>N</sub> (G)	15.5	15.8	16.5	15.0	16.5	
	a <sub>H</sub> (G)	22.9				4.6	
5	RA (%)	84	16	51	44	5	
	a <sub>N</sub> (G)	15.5	15.8	16.5	15.0	16.4	
	a <sub>H</sub> (G)	22.9				4.7	
10	RA (%)	86	14	63	32	5	
	a <sub>N</sub> (G)	15.5	15.8	16.5	15.1	16.4	
	a <sub>H</sub> (G)	22.9				4.7	
20	RA (%)	85	15	64	31	5	
	a <sub>N</sub> (G)	15.5	15.8	16.5	15.2	16.4	
	a <sub>H</sub> (G)	22.9				4.7	
30	RA (%)	86	14	57	26	17	
	a <sub>N</sub> (G)	15.5	15.8	16.4	15.2	16.3	
	a <sub>H</sub> (G)	22.9				4.6	

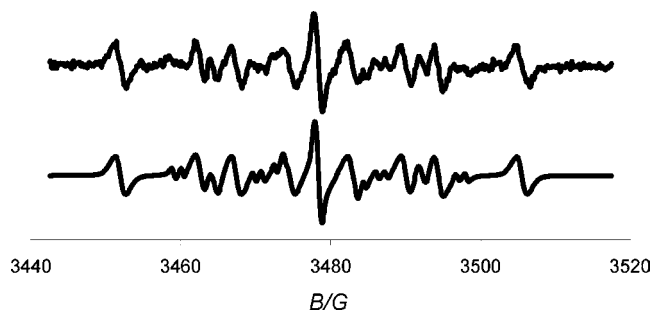
<sup>a</sup> RA = relative abundance.**Figure 2.** Experimental (upper trace) and simulated (lower trace) spin patterns of radicals derived from dihydroisohumulones (**2**) after photooxidation by <sup>3</sup>RF\* and subsequent spin trapping by DMPO under an oxygen atmosphere (for conditions, see Materials and Methods).**Table 2.** Relevant Data from Simulations of the EPR Spectra, Recorded on Prolonged Exposure Times, of an Oxygen-Saturated Solution of RF, DMPO, and Isohumulones (**1**) or Dihydroisohumulones (**2**)<sup>a</sup>

time (min)		1					2				
20	RA (%)	51	33	7	5	4	17	10	70	3	
	a <sub>N</sub> (G)	13.7	14.0	15.4	15.9	14.8	15.6	15.8	13.7	13.9	
	a <sub>H</sub> (G)	10.5	22.4			18.0	22.9			10.7	
	a <sub>H</sub> <sup>γ</sup> (G)	1.3								1.2	
30	RA (%)	18	30	18	29	5	34	19	41	6	
	a <sub>N</sub> (G)	13.7	14.0	15.4	15.9	14.8	15.5	15.8	13.7	13.9	
	a <sub>H</sub> (G)	10.6	22.6			17.9	22.9			10.7	
	a <sub>H</sub> <sup>γ</sup> (G)	1.3								1.3	

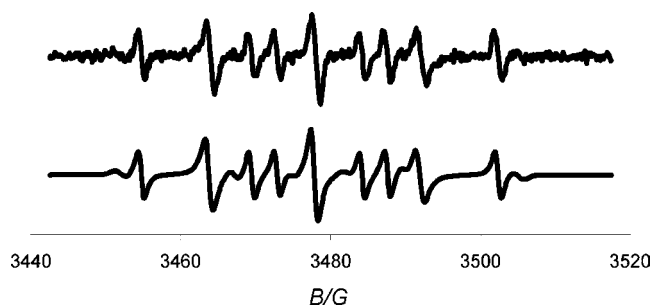
<sup>a</sup> RA = relative abundance.

doublets, and proper simulations revealed hyperfine coupling constants of a<sub>N</sub> ~ 15.5 G and a<sub>H</sub> ~ 22.9 G (**Table 1**). Additionally, a three-line signal with a<sub>N</sub> ~ 15.8 G was detected under these conditions. Similar signals were observed in an oxygen atmosphere after prolonged irradiation (>25 min) (**Figure 2**), while only one spin adduct (a<sub>N</sub> ~ 13.7 G, a<sub>H</sub> ~ 10.7 G, and a<sub>H</sub><sup>γ</sup> ~ 1.2 G) formed immediately after the irradiation was started (**Table 2**).

Isohumulones (**1**) gave a more complex EPR pattern (**Figure 3**). Visible light irradiation for 30 min under a nitrogen atmosphere showed four spin adducts (**Table 3**). Saturating the solution with oxygen produced an even more complex profile. Five species produced on prolonged exposure (20 min) could

**Figure 3.** Experimental (upper trace) and simulated (lower trace) spin patterns of radicals derived from isohumulones (**1**) after photooxidation by <sup>3</sup>RF\* and subsequent spin trapping by DMPO under a nitrogen atmosphere (for conditions, see Materials and Methods).**Table 3.** Relevant Data from Simulations of the EPR Spectra, Recorded at Varying Irradiation Times, of a Nitrogen-Degassed Solution of Isohumulones (**1**), RF, and a Spin Trap (DMPO and MNP, Respectively)<sup>a</sup>

time (min)		DMPO			MNP			
2	RA (%)	54	33	13	44	50	6	
	a <sub>N</sub> (G)	15.5	15.8	13.9	16.4	14.1	16.4	
	a <sub>H</sub> (G)	22.6					4.4	
5	RA (%)	51	27	22	51	41	8	
	a <sub>N</sub> (G)	15.5	15.9	13.9	16.4	14.1	16.5	
	a <sub>H</sub> (G)	22.6					4.4	
10	RA (%)	72	28		43	52	5	
	a <sub>N</sub> (G)	15.5	15.9		16.4	14.1	16.4	
	a <sub>H</sub> (G)	22.6					4.5	
20	RA (%)	72	28		38	59	3	
	a <sub>N</sub> (G)	15.5	15.9		16.4	14.1	16.4	
	a <sub>H</sub> (G)	22.6					4.6	
30	RA (%)	50	32	8	10	37	60	3
	a <sub>N</sub> (G)	15.5	15.8	13.9	13.6	16.4	14.1	16.4
	a <sub>H</sub> (G)	22.7			10.7			4.6
	a <sub>H</sub> <sup>γ</sup> (G)				1.2			

<sup>a</sup> RA = relative abundance.**Figure 4.** Experimental (upper trace) and simulated (lower trace) spin patterns of radicals derived from tetrahydroisohumulones (**3**) after photooxidation by <sup>3</sup>RF\* and subsequent spin trapping by DMPO under a nitrogen atmosphere (for conditions, see Materials and Methods).

be identified, while initially, only one signal was observed (a<sub>N</sub> ~ 13.7 G, a<sub>H</sub> ~ 10.6 G, and a<sub>H</sub><sup>γ</sup> ~ 1.3 G). Relevant data on irradiation under an oxygen atmosphere are presented in **Table 2**.

Tetrahydroisohumulones (**3**) led to significantly different EPR spectra on light exposure (**Figure 4**). The most prevalent species detected at prolonged irradiation (>20 min) was a triplet with a<sub>N</sub> ~ 14.1 G, while a triplet of doublets (a<sub>N</sub> ~ 15.1 G, a<sub>H</sub> ~ 23.0 G) disappeared after an irradiation time of more than 20 min (**Table 4**). Another species consisted of a triple doublet with a<sub>N</sub> ~ 14.7 G and a<sub>H</sub> ~ 18.1 G. Similar findings ensued from irradiation of oxygen-rich solutions, although spin adducts

**Table 4.** Relevant Data from Simulations of the EPR Spectra, Recorded at Varying Irradiation Times, of a Nitrogen-Degassed Solution of Tetrahydroisohumulones (**3**), RF, and a Spin Trap (DMPO and MNP, Respectively)<sup>a</sup>

time (min)		DMPO			MNP		
2	RA (%)	43	12	45	73	27	
	$a_N$ (G)	14.7	15.0	14.1	14.4	16.6	
	$a_H$ (G)	18.1	23.0				
5	RA (%)	42	12	46	67	30	3
	$a_N$ (G)	14.8	15.1	14.1	14.4	16.6	16.6
	$a_H$ (G)	18.1	22.9				4.6
10	RA (%)	44	12	44	52	40	8
	$a_N$ (G)	14.8	15.1	14.1	14.2	16.6	16.5
	$a_H$ (G)	18.1	23.2				4.8
20	RA (%)	37	8	55	49	48	3
	$a_N$ (G)	14.7	15.1	14.1	14.2	16.6	16.5
	$a_H$ (G)	18.1	22.8				4.7
30	RA (%)	32	68		51	42	7
	$a_N$ (G)	14.7	14.1		14.2	16.6	16.5
	$a_H$ (G)	18.1					4.7

<sup>a</sup> RA = relative abundance.**Table 5.** Relevant Data from Simulations of the EPR Spectra, Recorded on Prolonged Exposure Times, of an Oxygen-Saturated Solution of RF, DMPO, and Tetrahydroisohumulones (**3**) or Humulinone (**4**)<sup>a</sup>

time (min)		<b>3</b>			<b>4</b>		
20	RA (%)	24	8	68	52	29	19
	$a_N$ (G)	14.8	15.4	14.1	13.5	15.5	16.1
	$a_H$ (G)	18.1	22.7		10.8	22.4	
	$a_{H'}$ (G)				1.2		
30	RA (%)	31	69		72	18	10
	$a_N$ (G)	14.7	14.1		13.5	15.5	16.0
	$a_H$ (G)	18.1			10.9	22.3	
	$a_{H'}$ (G)				1.2		

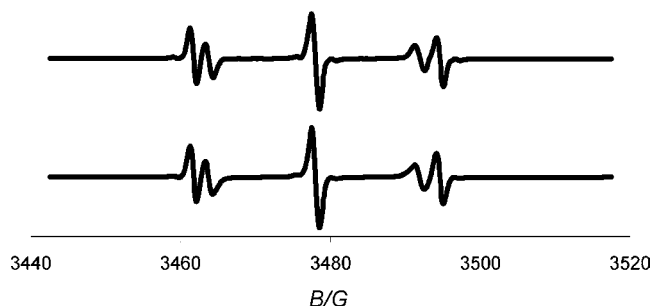
<sup>a</sup> RA = relative abundance.**Table 6.** Relevant Data from Simulations of the EPR Spectra, Recorded at Varying Irradiation Times, of a Nitrogen-Degassed Solution of Humulinone (**4**), RF, and a Spin Trap (DMPO and MNP, Respectively)<sup>a</sup>

time (min)		DMPO			MNP		
2	RA (%)	very weak signals			79	9	12
	$a_N$ (G)				16.5	14.7	16.4
	$a_H$ (G)						4.6
5	RA (%)	59	41		89	11	
	$a_N$ (G)	15.4	16.2		16.5	16.4	
	$a_H$ (G)	22.2				4.6	
10	RA (%)	58	42		84	16	
	$a_N$ (G)	15.5	16.2		16.5	16.5	
	$a_H$ (G)	22.2				4.6	
20	RA (%)	77	23		91	9	
	$a_N$ (G)	15.5	16.1		16.5	16.5	
	$a_H$ (G)	22.3				4.6	
30	RA (%)	62	24	14	91	9	
	$a_N$ (G)	15.6	16.1	13.2	16.5	16.5	
	$a_H$ (G)	22.3		10.5		4.6	
	$a_{H'}$ (G)			1.2			

<sup>a</sup> RA = relative abundance.

were only detected after 20 min (**Table 5**). Analogous spectra as for dihydroisohumulones (**2**) were observed, when humulinone (**4**) was examined (**Tables 5 and 6**).

Changing from acetonitrile/water to citrate buffer (pH 4.6)/acetonitrile solutions resulted invariably in the appearance of an additional EPR signal ( $a_N \sim 13.7$  G,  $a_H \sim 10.9$  G, and  $a_{H'} \sim 1.2$  G). This signal was also present, when a solution

**Figure 5.** Experimental (upper trace) and simulated (lower trace) spin patterns of radicals derived from isohumulones (**1**) after photooxidation by <sup>3</sup>RF\* and subsequent spin trapping by MNP under a nitrogen atmosphere (for conditions, see Materials and Methods).

containing RF and DMPO in citrate buffer/acetonitrile was exposed to visible light. In the buffered system, isohumulones (**1**) and humulinone (**4**) produced traces of a spin adduct, which was not observed in neutral solution ( $a_N \sim 14.6$  G,  $a_H \sim 18.0$  G, and  $a_N \sim 14.8$  G, respectively).

**Spin Trapping with MNP.** On irradiation of a solution of MNP in acetonitrile/water (v/v, 1:1), a major triplet ( $a_N \sim 16.5$  G) and a minor double triplet ( $a_N \sim 16.4$  G and  $a_H \sim 4.7$  G) were identified. However, no radicals could be observed, when the experiment was repeated in the presence of RF. Substituting water for a citrate buffer (pH 4.6) did not have a significant influence on spin adduct formation.

Addition of isohumulones (**1**) to neutral solutions of RF and MNP gave three nitroxyl species on irradiation with visible light under a nitrogen atmosphere (**Figure 5**). Two of these signals were detected, when only MNP was irradiated, while a spin adduct with  $a_N \sim 14.1$  G had not been observed previously (**Table 3**). Similar observations were made for dihydroisohumulones (**2**) ( $a_N \sim 15.1 \pm 0.1$  G) (**Table 1**) and tetrahydroisohumulones (**3**) ( $a_N \sim 14.3 \pm 0.1$  G) (**Table 4**), but the signal derived from humulinone (**4**) ( $a_N \sim 14.7$  G) (**Table 6**) disappeared within 5 min. Signals arising from irradiation of oxygen-saturated solutions were detected only after prolonged irradiation times (ca. 30 min), but no new signals were apparent.

Significant spectral changes did not occur, when samples were prepared in a citrate buffer/acetonitrile system. However, a new species arising from tetrahydroisohumulones (**3**) ( $a_N \sim 8.1$  G) was found, while only one spin adduct ( $a_N \sim 14.0$  G) was observed on irradiation under an oxygen atmosphere.

## DISCUSSION

Kuroiwa suggested that isohumulones (**1**) are prone to undergo decomposition by a formal Norrish type I  $\alpha$ -cleavage pathway, when exposed to visible light in the presence of flavins (**10**). Detailed insights into this Kuroiwa formalism lacked until it was shown by laser flash photolysis experiments that an exergonic one-electron transfer from the salts of the five-membered ring hop derivatives to <sup>3</sup>RF\* is the main photochemical event on the route to lighstruck flavor formation in beer (**16**). Moreover, it was demonstrated that the ionized  $\beta$ -tricarboxyl moiety releases an electron on (electrochemical) oxidation of isohumulones and derivatives, while the acidic form was unreactive under these conditions (**2**).

Dihydroisohumulones (**2**) do not possess the photolabile  $\alpha$ -hydroxyketone group; hence, they are considered to be light stable, whereas for tetrahydroisohumulones (**3**), a similar behavior as observed for isohumulones is expected, although reactivity originating in the side chain unsaturation is excluded.



As humulinone (**4**) is oxidized at C(5) with respect to isohumulones, radical formation at this carbon is abolished.

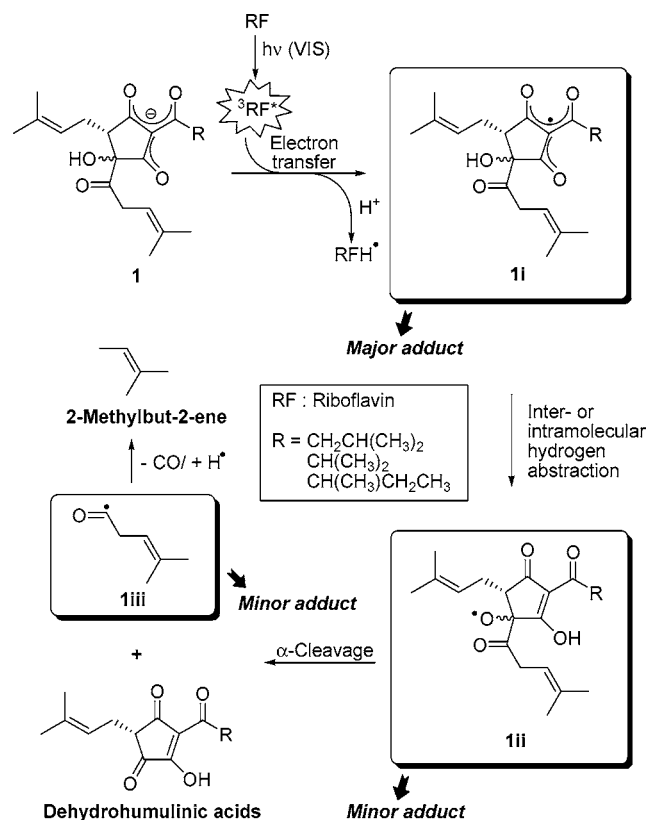
As radicals derived from isohumulones and derivatives were too short-lived to be observed directly by EPR spectroscopy, spin trapping experiments were most important in unraveling the nature of the radicals formed on electron transfer to  $^3\text{RF}^*$ . Because MNP mainly forms stable adducts with carbon-centered radicals, whereas carbon- and oxygen-centered radicals may be observed with DMPO as a trapping agent, the nature of the incipient radicals can be derived. Moreover, the values of the coupling constants  $a_N$  and  $a_H$ , which are obtained from computer simulations involving the EPR signals, provide valuable information on the structure of the radical being trapped.

On DMPO spin trapping under a nitrogen atmosphere, a similar signal ( $a_N \sim 15.5 \pm 0.5$  G;  $a_H \sim 22.5 \pm 0.5$  G) could be detected for all compounds confirming the finding that radicals arise from a reactive moiety common to all five-membered ring hop derivatives. Prolonged irradiation of isohumulones (**1**) and humulinone (**4**) led to trapping of a  $\text{RO}^\bullet$  radical, which typically exhibits an extra-coupling to a DMPO hydrogen (20). Because this radical was not observed at the beginning of irradiation, it is, most likely, formed by a secondary reaction. On the other hand, tetrahydroisohumulones (**3**) produced a major DMPO adduct with  $a_N \sim 14.7$  G and  $a_H \sim 18.1$  G, which is typical for a trapped acyl radical. A similar, although minor, compound, was identified, when isohumulones or humulinone were dissolved in a buffered system. On irradiation under an oxygen atmosphere, a  $\text{RO}^\bullet$  radical was observed only for compounds with unsaturated side chains (**1**, **2**, and **4**). Possibly, it is an intermediate in an ene type reaction with singlet oxygen, which should be readily formed on quenching of  $^3\text{RF}^*$  with ground state oxygen (21). The origin of the observed triplets ( $a_N \sim 16.0 \pm 0.2$  G and  $a_N \sim 14.0 \pm 0.1$  G) is unclear, as these species lack hyperfine couplings to the DMPO hydrogen. Scarce literature information attributes these signals to decomposition of the spin trap (22, 23).

Spin adducts with MNP afforded the most important information, as the absence of a hydrogen hyperfine coupling implies that a tertiary carbon radical had been trapped. Two EPR signals were attributed to photochemical degradation of MNP. The signal with an  $a_N$  value of  $16.5 \pm 0.1$  G resulted from trapping of a *tert*-butyl radical, generated by photochemical cleavage of MNP, while a minor species ( $a_N \sim 16.5$  G and  $a_H \sim 4.7$  G) was, most likely, formed via subsequent hydrogen abstraction from acetonitrile. Remarkably, photochemical degradation of MNP has not been observed, when only RF was present. Probably the *tert*-butyl radical recombined with  $^3\text{RF}^*$ . However,  $^3\text{RF}^*$  readily removes an electron from salts of isohumulones or derivatives leading to another radical to be trapped by MNP. The value of the coupling constant  $a_N$  for this spin adduct is in good agreement with the value observed on electrochemical oxidation of isohumulones and derivatives (2). Accordingly, the  $a_N$  values of 14.1 G for isohumulones (**1**) and 15.2 G for dihydroisohumulones (**2**) are attributed to MNP adducts of low-electron density triacylmethyl radicals (24). A similar value for  $a_N$  of 14.2 G was determined for tetrahydroisohumulones (**3**) in neutral solutions, although an additional species with  $a_N \sim 8.1$  G, corresponding to an acyl radical, was observed in the buffered system. Interestingly, the triacylmethyl radicals derived from humulinone (**4**) ( $a_N \sim 14.7$  G) disappeared rapidly, perhaps due to a perturbation of the reactivity by the hydroxyl at C(5).

From these findings, it was concluded that sensitized irradiation of isohumulones (and also of derivatives) provokes an electron transfer from the ionized  $\beta$ -tricyarbonyl to  $^3\text{RF}^*$  leading

**Scheme 2.** Spin Adducts Formed on Photooxidation of Isohumulones (**1**) by  $^3\text{RF}^*$



to the formation of reactive triacylmethyl radicals, which are readily added to the spin trap (**1i**; Scheme 2). Stabilization pathways for these species may include inter- or intramolecular hydrogen abstraction, resulting in an alkoxy radical (**1ii**, producing a minor spin adduct with DMPO), which eventually induces  $\alpha$ -cleavage. The  $\alpha$ -hydroxyketone group was found particularly vulnerable, as acyl radicals were observed on irradiation of isohumulones (**1**), tetrahydroisohumulones (**3**), and humulinone (**4**). The 4-methylpent-3-enoyl radical (**1iii**), arising from  $\alpha$ -oxyketone cleavage of isohumulones (**1**) [or humulinone (**4**)], is readily decarbonylated to form a stabilized 3-methylbut-2-enyl radical (25). This explains the low concentrations of unsaturated acyl radicals. The 3-methylbut-2-enyl radical, the main precursor for MBT, leads to formation of 2-methylbut-2-ene in the absence of a suitable sulfur source in the model system, while concurrent formation of the nonvolatile dehydrohumulinic acids after photooxidation of isohumulones corroborates the occurrence of the proposed radical intermediates (26). The 4-methylpentanoyl radical, derived from  $\alpha$ -cleavage of the  $\alpha$ -hydroxyketone group in tetrahydroisohumulones (**3**), decarbonylates slowly (8); therefore, a different and more prevalent spin adduct is formed. This result evidences the identification of 4-methylpentanal as a major degradation product of tetrahydroisohumulones on exposure to visible light in the presence of flavins (26).

In conclusion, although the beer matrix is too complex to carry out proper mechanistic investigations, studies in model systems on visible-light induced degradation of isohumulones (and derivatives thereof) have been most revealing. On irradiation of ionized isohumulones (**1**), dihydroisohumulones (**2**), tetrahydroisohumulones (**3**), and humulinone (**4**) in the presence of RF, five-membered ring radicals were readily formed. These radicals could only be detected by formation of adducts with suitable spin traps (DMPO or MNP), followed by EPR

spectroscopy. Values for different hyperfine couplings were obtained from computer simulations of the experimental spin patterns, allowing identification of both oxygen- and carbon-centered radicals. It was found that radicals formed on photochemical oxidation were similar to radicals observed on electrochemical oxidation of isohumulones and derivatives. An electron is abstracted from the ionized  $\beta$ -tricarboxyl moiety, which is common to all five-membered ring hop derivatives. It was clearly shown that dihydroisohumulones are decomposed by a similar mechanism and this finding compromises the use of these compounds to resist photoinduced degradation. Radicals derived from isohumulones may further decompose by  $\alpha$ -cleavage, thereby producing intermediates on the pathway to LSF development in beer. Furthermore, acyl radicals formed on degradation of tetrahydroisohumulones are precursors for low molecular weight aldehydes, which may account for unpleasant stale and cardboard off-flavors.

Extrapolation of these results to the real beer matrix is a topic of further research, which should indicate whether this model is valid, both for the development of LSF as for the generation of stale or cardboard off-flavors in beer.

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