

# Riboflavin-sensitized photooxidation of isohumulones and derivatives

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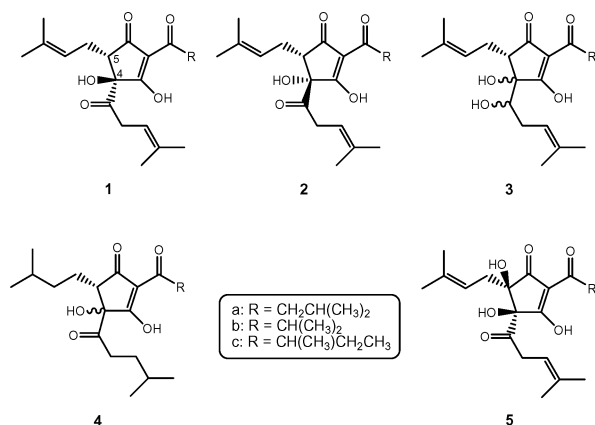
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Isohumulones, the bitter principles in beer, are decomposed by light-induced reactions, thereby adversely affecting beer quality. Upon absorption of visible light, riboflavin is excited and interacts with isohumulones, as well as with oxidized and reduced derivatives thereof. Reaction kinetics were investigated by laser flash photolysis at 355 nm and at 440 nm, and analysis of kinetic data afforded detailed insights into the reaction mechanism.

## Introduction

The occurrence of a particular off-flavor in beer exposed to light, known as lightstruck flavor (LSF), has been attributed to the formation of 3-methylbut-2-ene-1-thiol (MBT) or 'skunky thiol'. Kuroiwa and Hashimoto demonstrated that model systems, composed of riboflavin, isohumulones (also called iso- $\alpha$ -acids), and a sulfur source (cysteine), led to LSF on irradiation with visible light (350–500 nm).<sup>1</sup> The flavor threshold has since been a matter of discussion, as not only the reliability of taste panels may differ, but also the nature of the matrix may lead to varying evaluations.<sup>2–5</sup> Still, concentrations of a few ng MBT per liter are sufficient to make beers unpalatable. A formal mechanism for formation of MBT has been suggested by Kuroiwa and Hashimoto,<sup>6</sup> but, although all observations are in agreement with this proposal, detailed fundamental insight is still lacking.

The presence of isohumulones (typically between 15 and 25 mg L<sup>-1</sup> in lager beers) is pivotal in the formation of LSF. These beer bittering agents consist of a mixture of *trans*-isohumulones (**1a–c**) and *cis*-isohumulones (**2a–c**) (usual ratio 3 : 7) formed from humulones ( $\alpha$ -acids, two isomers and one homologue) present in hops (*Humulus lupulus* L.) by thermal isomerization during boiling in the brewing kettle. *trans*-Isohumulones (**1a–c**, Scheme 1; the denotation *trans* refers to the relative configuration of the hydroxyl group at C(4) and the prenyl side chain at C(5)) have been reported to be less stable than *cis*-isohumulones.<sup>7</sup>



**Scheme 1** Structures of *trans*-isohumulones (**1a–c**), *cis*-isohumulones (**2a–c**), dihydroisohumulones (**3a–c**), tetrahydroisohumulones (**4a–c**) and humulinones (**5a–c**).

Moreover, isohumulones are susceptible to light-induced decomposition either on direct irradiation in the UV-B wavelength region or on exposure to visible light in the presence of particular photoreactive light-absorbing species. Previously, we studied the photodegradation of isohumulones following a 308 nm laser flash *via* identification of the free radicals thus formed by time-resolved electron paramagnetic resonance spectroscopy (TR-EPR).<sup>8</sup> Light is initially absorbed by the enolized  $\beta$ -triketo chromophore and intramolecular triplet energy transfer to the  $\alpha$ -hydroxycarbonyl group induces a Norrish Type I  $\alpha$ -cleavage leading to a 4-methylpent-3-enoyl radical. Fast decarbonylation results in formation of a 3-methylbut-2-enyl radical, which can be trapped by a thiyl radical to give MBT. Dihydroisohumulones (**3a–c**), in which the photolabile  $\alpha$ -hydroxyketone is reduced to a 1,2-diol moiety, do not undergo the Norrish reaction rendering them lightproof. These findings are corroborated by a detailed analysis of reaction products arising from photolysis of *trans*-isohumulones and derivatives.<sup>9</sup>

However, LSF is most evident on exposure of beer to wavelengths corresponding to the blue part of the visible electromagnetic spectrum. Since isohumulones are transparent in this wavelength region, intervention of one or more light-absorbing compounds is a prerequisite to induce photodegradation. Riboflavin (RF; vitamin B2), present in beer in concentrations of several hundreds of micrograms per liter,<sup>10</sup> is known to be photoreactive under these conditions. RF exhibits two absorption bands with maxima at 375 and 446 nm, while the high molar absorptivities ( $>10^4$  M<sup>-1</sup> cm<sup>-1</sup>) at these wavelengths are indicative of  $\pi$ - $\pi^*$  transitions.<sup>11</sup> Due to the very short lifetime of the singlet state (<sup>1</sup>RF\*) of riboflavin (*ca.* 5 ns), the triplet state (<sup>3</sup>RF\*) mostly accounts for photoreactivity.<sup>12</sup> As the triplet energy level of RF (210 kJ mol<sup>-1</sup>) is much lower than that of the isohumulones (300 kJ mol<sup>-1</sup>), direct energy transfer is excluded.<sup>13</sup> On the other hand, <sup>3</sup>RF\* is a very strong electron acceptor, thereby leading to photooxidation of a variety of electron-donating substrates.<sup>14,15</sup> Furthermore, the interference of the highly oxidizing riboflavin radical cation ( $E = +2.28$  V) in photooxidation reactions has been suggested.<sup>16</sup>

We have studied electron transfer from isohumulones to <sup>3</sup>RF\* in model systems by observing transients after laser flash photolysis at 355 nm and at 440 nm. Photooxidation of isohumulones may initiate a reaction chain, which ultimately results in formation of MBT in beer. Reduced isohumulones including dihydroisohumulones (**3a–c**) and tetrahydroisohumulones (**4a–c**), as well as humulinones (**5a–c**), oxidized derivatives of isohumulones, were included in the study.

## Experimental

Dicyclohexylammonium (DCHA) salts of *trans*-isohumulones (**1a-c**) were prepared starting from a commercially available aqueous solution (100 ml; ca. 30% w/v) of the potassium salts of the isohumulones (**1a-c**, **2a-c**) (Botanix, Eardiston, Near Tenbury Wells, Worcestershire, England), which consisted of a mixture of *trans*-isohumulones (**1a-c**) and *cis*-isohumulones (**2a-c**). After acidification to pH 1 (1 M HCl) and extraction with ethyl acetate (200 ml), the organic phase was dried (MgSO<sub>4</sub>) and solvent removal *in vacuo* resulted in an oily residue (24 g), which was redissolved in ethyl acetate (200 mL) and treated with dicyclohexylamine (DCHA) (50.7 g, 0.28 mol). The DCHA salts of *trans*-isohumulones precipitated selectively.<sup>17</sup> Recrystallization from methanol-water (4 : 1, v/v; 50 mL) afforded pure DCHA salts of *trans*-isohumulones (10.1 g) as white flakes. Dihydroisohumulones (**3a-c**) and tetrahydroisohumulones (**4a-c**) were commercially available as aqueous solutions of the potassium salts (ca. 35% w/v and 10% w/w, respectively) (Botanix, Eardiston, Near Tenbury Wells, Worcestershire, England). The sodium salts of humulinones (**5a-c**) were prepared according to a literature procedure by dissolving humulones (2 g) in a biphasic system of diethyl ether (150 mL) and a saturated sodium hydrogen carbonate solution (150 mL).<sup>18</sup> After a catalytic amount of cumenylhydroperoxide was added, the reaction mixture was left for 2 days under ambient light conditions. The sodium salts of humulinones were formed at the interface between the two liquid phases and collected as white crystals (1.08 g, 51%).

Riboflavin (Sigma, Bornem, Belgium) (125 μM) was dissolved in a mixture (10 mL; 1 : 1 v/v) of acetonitrile and aqueous citrate (pH 4.6) or phosphate buffer (pH 7.0). Salts of isohumulones or derivatives were added in concentrations varying from 125 μM up to 1 mM. *N,N,N',N'*-Tetramethyl-*p*-phenylenediamine (Fluka, Bornem, Belgium) was added in concentrations varying from 62 to 250 μM to solutions, containing 125 μM riboflavin and 1.25–100 mM of the hop-derived compounds in a mixture (10 mL; 1 : 1 v/v) of acetonitrile and aqueous phosphate buffer (pH 7.0). All solutions were protected from light and degassed with nitrogen prior to use.

Two laser flash systems were used. A Spectra Physics (Stahnsdorf, Germany) GCR three-pulsed Nd:YAG laser (1064 nm) was frequency tripled to 355 nm (4–10 mJ per pulse) and used as an excitation source for the RF solution. The analytical light beam was generated by a pulsed xenon lamp (LAX 1450, Müller Elektronik-Optik, Moosinning, Germany), while absorption spectra of transients were obtained in the wavelength region of 400–750 nm by an optical multichannel analyser (OMA II, EG&G, Princeton Instruments, Princeton, NJ, USA). A 440 nm laser flash was generated by absorbing the third harmonic of a pulsed Q-switch Nd:YAG laser by a dye laser (Spectron Laser Systems, Rugby, UK) using Coumarin 120 (Lambda Physik, Germany) as dye. This laser flash served as the excitation source for solutions of mixtures of RF and the compounds of interest. The analytical light beam (pulsed xenon lamp (Applied Photophysics, Leatherhead, UK)) was sent through a monochromator and detected by a R928 photomultiplier. The energy of the laser was 2.3 mJ per pulse. Absorbances were measured at 297 K.

## Results and discussion

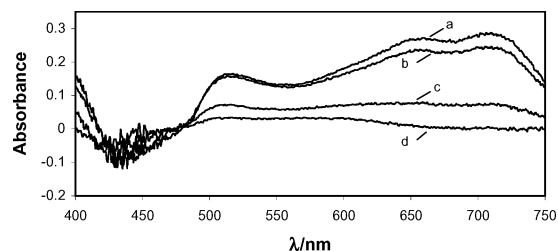
### Electron transfer from the salts of isohumulones and derivatives to triplet-excited riboflavin

Transient absorption spectra following a 355 nm laser flash of riboflavin are shown in Fig. 1. The transient absorption trace showed three maxima, while photobleaching occurred around 440 nm due to ground-state absorption.<sup>19</sup> The absorption maximum at 720 nm is attributed exclusively to <sup>3</sup>RF\*, since its decay is stimulated by addition of appropriate triplet quenchers.<sup>20</sup>

**Table 1** Bimolecular rate constants  $k$  (L mol<sup>-1</sup> s<sup>-1</sup>) for the interaction of isohumulones and derivatives with triplet-excited riboflavin in model systems

Substrate	MeCN–buffer pH 4.6 <sup>a</sup>	MeCN–buffer pH 7.0 <sup>a</sup>
<b>1a-c + 2a-c</b>	$1.7 \times 10^8$	$1.7 \times 10^8$
<b>1a-c</b>	$2.0 \times 10^8$	$1.5 \times 10^8$
<b>3a-c</b>	$1.8 \times 10^8$	$1.7 \times 10^8$
<b>4a-c</b>	$2.3 \times 10^8$	$1.9 \times 10^8$
<b>5a-c</b>	$1.6 \times 10^8$	$1.2 \times 10^8$

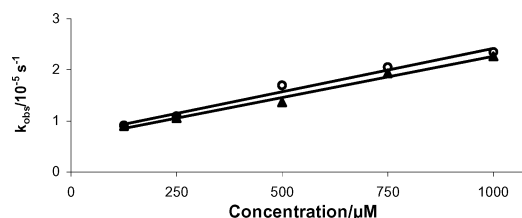
<sup>a</sup> v/v, 1 : 1.



**Fig. 1** Transient absorption spectra of riboflavin in an acetonitrile–water mixture (1 : 1, v/v) following excitation by a 355 nm laser flash at different time intervals (a: 0.1 μs; b: 1 μs; c: 10 μs; d: 50 μs; for conditions, see Experimental section).

Excitation at 440 nm also furnishes triplet-excited riboflavin, hence this wavelength is particularly suited for visible-light irradiation in the presence of isohumulones and derivatives, these being fully transparent at 440 nm. Five-membered-ring hop derivatives have  $pK_a$  values around 3<sup>21</sup> and, as the pH of lager beers is usually between 4.2 and 4.4, isohumulones (**1a-c**, **2a-c**) and their derivatives occur predominantly in their anionic forms.<sup>22</sup> Therefore, the compounds were studied as salts.

Addition of varying concentrations of the potassium salts of isohumulones (**1a-c**, **2a-c**) to mixtures of acetonitrile and aqueous citrate (pH 4.6) or phosphate (pH 7.0) buffer affected the decay of <sup>3</sup>RF\* considerably, while the decay rate was found to be proportional in both cases to the concentrations of the salts (Fig. 2). The bimolecular rate constant for the reaction of isohumulones with <sup>3</sup>RF\* at pH 4.6 and 7.0 did not differ. The value of  $1.7 \times 10^8$  L mol<sup>-1</sup> s<sup>-1</sup> was determined from the slope of the linear plot of the pseudo-first-order rate constant for decay of <sup>3</sup>RF\*, observed at 720 nm, as a function of the concentration of the salts. Since <sup>3</sup>RF\* induces electron transfer, eventually followed by proton exchange,<sup>11</sup> it is inferred that a one-electron oxidation of the potassium salts of isohumulones (**1a-c**, **2a-c**) has occurred. The role of particular structural changes (reduced and oxidized derivatives of isohumulones) could give information on the nature of this redox reaction. Bimolecular rate constants for their interaction with <sup>3</sup>RF\* are given in Table 1.



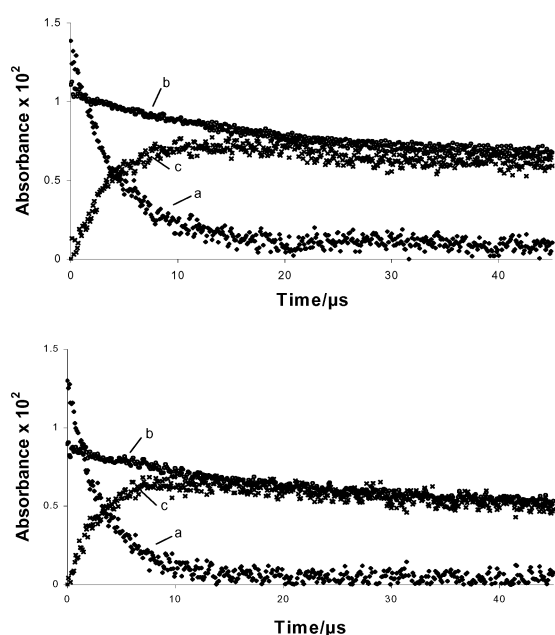
**Fig. 2** Linear plot of the pseudo-first-order rate constant ( $k_{\text{obs}}$ ) for decay of triplet-excited riboflavin, observed at 720 nm, as a function of the concentration of the potassium salts of isohumulones (**1a-c**, **2a-c**) (pH 4.6 (○) and pH 7.0 (▲)).

In dihydroisohumulones (**3a-c**), a vicinal diol replaces the photolabile  $\alpha$ -hydroxycarbonyl group and, in tetrahydroisohumulones (**4a-c**), the side-chain double bonds are reduced. Humulinones (**5a-c**) are oxidized at C(5), hence, radical formation at this fully substituted carbon is precluded. These

modifications had only negligible effects on the reaction kinetics. It was, therefore, deduced that electron abstraction involved exclusively the  $\beta$ -tricarbonyl chromophore, which obviously is common to all compounds investigated. This conclusion is in agreement with spin trapping experiments on electrochemical oxidation of salts of isohumulones and derivatives.<sup>23</sup> The values for **5a–c** are somewhat different, probably due to the perturbed reactivity of the  $\beta$ -tricarbonyl by the hydroxyl at C(5).

### Formation of reduced riboflavin radicals

It was found that decay of  $^3\text{RF}^*$  in the presence of either **1a–c** or **3a–c** resulted in formation of a transient species with an absorption maximum around 520 nm, corresponding to the reduced riboflavin radical ( $\text{RFH}^\cdot$ ), formed upon instantaneous protonation of the riboflavin radical anion ( $\text{RF}^{\cdot-}$ ) in the buffered systems used. Although absorbances of  $^3\text{RF}^*$  and  $\text{RFH}^\cdot$  are competitive at 520 nm, formation of  $\text{RFH}^\cdot$  could be monitored by subtracting the absorption vs. time profile at 720 nm,  $A^{720}$  (absorption by  $^3\text{RF}^*$ ), multiplied by the initial ratio  $A^{520}/A^{720}$  (for normalization), from the absorption vs. time profile at 520 nm,  $A^{520}$  (Fig. 3).<sup>16,24</sup>



**Fig. 3** Absorption vs. time profiles observed at 720 nm (a) and 520 nm (b) following excitation by a 440 nm laser flash of an acetonitrile-phosphate buffer (pH 7.0) (1 : 1, v/v) solution containing 0.125 mM riboflavin and 1 mM of **1a–c** (upper panel) or an acetonitrile-citrate buffer (pH 4.6) (1 : 1, v/v) solution containing 0.125 mM riboflavin and 1 mM of **3a–c** (lower panel). Formation of  $\text{RFH}^\cdot$  at 520 nm (c) is monitored by subtracting trace (a) from trace (b).

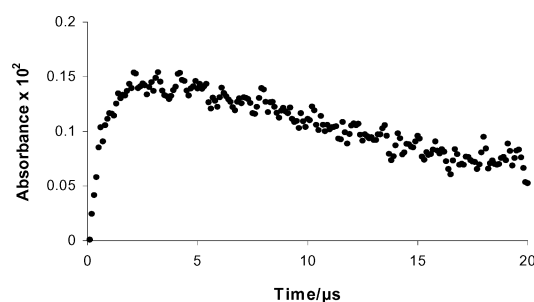
### Electron transfer from *N,N,N',N'*-tetramethyl-*p*-phenylenediamine to radicals derived from *trans*-isohumulones and dihydroisohumulones

Although spectroscopic data indicate formation of  $\text{RFH}^\cdot$ , unequivocal proof could not be given for the presence of radicals derived from isohumulones or their derivatives. However, the existence of these radicals was indirectly shown by reaction with an electron donor such as *N,N,N',N'*-tetramethyl-*p*-phenylenediamine (TMPD), followed by monitoring the absorbance of the TMPD radical cation ( $\text{TMPD}^{\cdot+}$ ) thus formed.

Varying concentrations of TMPD were added to buffered solutions (pH 7.0) of riboflavin and *trans*-isohumulones (**1a–c**) or dihydroisohumulones (**3a–c**) and transient absorption features, typical of the  $\text{TMPD}^{\cdot+}$  (maximum absorbances at 565 and 610 nm), were evident. Although the rate constant for

interaction of TMPD with  $^3\text{RF}^*$  is  $2.0 \times 10^9 \text{ L mol}^{-1} \text{ s}^{-1}$ , it is unlikely that direct electron transfer occurs under the given conditions.<sup>25</sup> As higher salt concentrations lead to increased observed rate constants, formation of  $\text{TMPD}^{\cdot+}$  originates from reaction of radicals derived from **1a–c** or **3a–c** with TMPD.

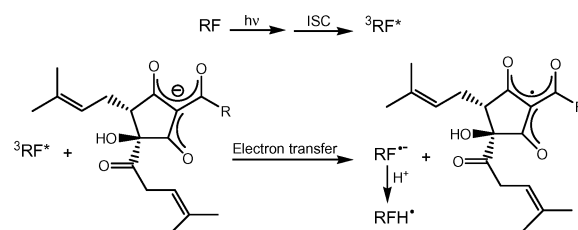
Formation and decay of  $\text{TMPD}^{\cdot+}$  following a 440 nm laser flash were monitored at 565 nm, as shown for reaction with radicals derived from dihydroisohumulones (**3a–c**) (Fig. 4). The rate constant for  $\text{TMPD}^{\cdot+}$  formation was determined to be  $1.8 \times 10^9$  and  $1.6 \times 10^9 \text{ L mol}^{-1} \text{ s}^{-1}$  for reaction with radicals, derived from **1a–c** and **3a–c**, respectively, from plotting the observed formation constant ( $k_{\text{obs}}$ ), as a function of TMPD concentrations.



**Fig. 4** Transient absorption trace arising from the formation of the *N,N,N',N'*-tetramethylphenylenediamine (TMPD) radical cation, monitored at 565 nm, after reaction of TMPD (125  $\mu\text{M}$ ) with radicals produced on photooxidation of dihydroisohumulones (**3a–c**) (100 mM) by triplet-excited riboflavin.

### Thermodynamic feasibility of electron transfer from the salts of *trans*-isohumulones and derivatives to triplet-excited riboflavin

Direct energy transfer from  $^3\text{RF}^*$  to the salts of isohumulones (**1a–c**, **2a–c**), dihydroisohumulones (**3a–c**), tetrahydroisohumulones (**4a–c**) or humulinones (**5a–c**) is precluded due to unfavorable triplet energy differences of ca. 90  $\text{kJ mol}^{-1}$ .<sup>13</sup> However, the change in standard free energy,  $\Delta G^0$ , for the electron transfer reaction, as depicted in Scheme 2, is calculated to be  $-47.1 \text{ kJ mol}^{-1}$  from the redox potentials of the reactants and  $\Delta E_{0,0}$ , the 0–0 excitation energy of  $^3\text{RF}^*$ .<sup>26</sup>



**Scheme 2** Mechanism of an electron transfer reaction from the anions of *trans*-isohumulones (**1a–c**) to the triplet excited state of riboflavin ( $^3\text{RF}^*$ ).

$$\Delta G^0_{\text{electron transfer}} = -nF(E_{\text{red}} - E_{\text{ox}}) - \Delta E_{0,0}$$

The energy gap between ground and triplet-excited state levels of riboflavin amounts to 210  $\text{kJ mol}^{-1}$ .  $E_{\text{red}}$  and  $E_{\text{ox}}$  are the ground-state half-wave potentials of the reductant (**1a–c**) and oxidant (RF), respectively. The redox potential of the RF/ $\text{RFH}^\cdot$  couple is  $-0.29 \text{ V vs. SHE}$ ,<sup>11</sup> while oxidation peak potentials of the salts are determined to be 1.4 V vs. SHE.<sup>23</sup> The highly exothermic character is in accordance with a thermodynamically favorable electron transfer.

### Conclusion

Electron transfer from the salts of isohumulones (**1a–c**, **2a–c**) and salts of reduced and oxidized derivatives to triplet-excited riboflavin was evidenced by kinetic analysis of transient absorp-

tion spectra of riboflavin following excitation by a laser flash at 440 nm. Formation of radicals derived from isohumulones (**1a-c**, **2a-c**) is most likely the initial step in the decomposition to a 3-methylbut-2-enyl radical, which is a key intermediate in the pathway leading to the lightstruck flavor in beer. Tetrahydroisohumulones (**4a-c**) are equally reactive, but, remarkably, dihydroisohumulones (**3a-c**) are also oxidized by triplet-excited riboflavin. This result contradicts the commonly held belief that these compounds are lightproof. As radical formation is excluded at C(5) in humulinones (**5a-c**), it is clear that the  $\beta$ -tricarbonyl moiety, present in five-membered-ring hop-derived compounds, donates an electron to triplet-excited riboflavin. The oxidation mechanism is further confirmed by direct spectroscopic detection of the *N,N,N',N'*-tetramethyl-*p*-phenylenediamine radical cation after trapping of the radicals derived from photolysis of *trans*-isohumulones or derivatives.

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## References

- 1 Y. Kuroiwa and N. Hashimoto, Composition of sunstruck flavor substance and mechanism of its evolution, *Proc. Am. Soc. Brew. Chem.*, 1961, 28–36.
- 2 J. Irwin, L. Bordeleau and R. L. Barker, Model studies and flavor threshold determination of 3-methyl-2-butene-1-thiol in beer, *J. Am. Soc. Brew. Chem.*, 1993, **51**, 1–3.
- 3 H. Goldstein, S. Rader and A. A. Murakami, Determination of 3-methyl-2-butene-1-thiol in beer, *J. Am. Soc. Brew. Chem.*, 1993, **51**, 70–74.
- 4 J. Templar, K. Arrigan and W. J. Simpson, Formation, measurement and significance of lightstruck flavor in beer: a review, *Brew. Dig.*, 1995, May, 18–25.
- 5 P. Hughes, The lightstruck flavour problem, *Cerevisia*, 1999, **24**(2), 21–25.
- 6 Y. Kuroiwa and H. Hashimoto, Studies on hops with reference to their role in the evolution of sunstruck flavor of beer, *Rep. Res. Lab. Kirin Brew. Co. Ltd.*, 1961, **4**, 35–40.
- 7 L. De Cooman, G. Aerts, H. Overmeire and D. De Keukeleire, Alterations of the profiles of iso- $\alpha$ -acids during beer ageing, marked instability of *trans*-iso- $\alpha$ -acids and implications for beer bitterness consistency in relation to tetrahydroiso- $\alpha$ -acids, *J. Inst. Brew.*, 2000, **3**, 169–178.
- 8 C. S. Burns, A. Heyerick, D. De Keukeleire and M. D. E. Forbes, Mechanism for formation of the lightstruck flavor in beer revealed by time-resolved electron paramagnetic resonance, *Chem. Eur. J.*, 2001, **7**, 4553–4561.
- 9 A. Heyerick, Y. Zhao, P. Sandra, K. Huvaere, F. Roelens and D. De Keukeleire, Photolysis of hop-derived *trans*-iso- $\alpha$ -acids and *trans*-tetrahydroiso- $\alpha$ -acids: product identification in relation to the lightstruck flavour of beer, *Photochem. Photobiol. Sci.*, 2003, **2**, 306–314.
- 10 M. G. Duyvis, R. Hilhorst, C. Laane, D. J. Evans and D. J. M. Schmedding, Role of riboflavin in beer flavor instability: determination of levels of riboflavin and its origin in beer by fluorometric apoprotein titration, *J. Agric. Food. Chem.*, 2002, **6**, 1548–1552.
- 11 P. F. Heelis, The photophysical and photochemical properties of flavins (isoalloxazines), *Chem. Soc. Rev.*, 1982, **11**, 15–39.
- 12 C. Pac, K. Miyake, Y. Masaki, S. Yanagida, T. Ohno and A. Yoshimura, Flavin-photosensitized monomerization of dimethylthymine cyclobutane dimer: remarkable effects of perchloric acid and participation of excited-singlet, triplet, and chain-reaction pathways, *J. Am. Chem. Soc.*, 1992, **114**, 10756–10762.
- 13 J. D. Hastings, M. J. McGarrity, L. Bordeleau and J. D. Thompson, in *Abstracts of the Brewing Congress of the Americas*, September 1992, St. Louis, MI, USA, p. 97.
- 14 P. F. Heelis, B. J. Parsons, G. O. Phillips and J. F. McKellar, A laser flash-photolysis study of nature of flavin mononucleotide triplet-states and reactions of neutral form with amino acids, *Photochem. Photobiol.*, 1978, **28**, 169–173.
- 15 K. Kino, I. Saito and H. Sugiyama, Product analysis of GG-specific photooxidation of DNA via electron transfer: 2-aminoimidazolone as a major guanine oxidation product, *J. Am. Chem. Soc.*, 1998, **120**, 7373–7374.
- 16 L. Jian, W.-F. Wang, Z.-D. Zheng, S.-D. Yao, J.-S. Zhang and N.-Y. Lin, Reactive intermediates in laser photolysis of guanosine, *Res. Chem. Intermed.*, 1991, **15**, 293–301.
- 17 H. A. Thornton, J. Kulandai, M. Bond, M. P. Jontef, D. B. Hawthorne and T. E. Kavanagh, Preparation of *trans*-iso- $\alpha$ -acids and use of their dicyclohexylamine salts as a standard for iso- $\alpha$ -acids analysis, *J. Inst. Brew.*, 1993, **6**, 473–477.
- 18 M. Verzele and D. De Keukeleire, in *Chemistry and Analysis of Hop and Beer Bitter Acids*, Elsevier, Amsterdam, The Netherlands, 1991, pp. 52–53.
- 19 M. S. Grodowski, B. Veyret and K. Weiss, Photochemistry of flavins II. Photophysical properties of alloxazines and isoalloxazines, *Photochem. Photobiol.*, 1977, **26**, 341–352.
- 20 C.-Y. Lu, S.-D. Yao and N.-Y. Lin, Photooxidation of 2'-deoxyguanosine 5'-monophosphate (dGMP) by flavin adenine dinucleotide (FAD) via electron transfer: a laser photolysis study, *Chem. Phys. Lett.*, 2000, **330**, 389–396.
- 21 W. J. Simpson, Ionization behaviour of hop compounds and hop-derived compounds, *J. Inst. Brew.*, 1993, **4**, 317–326.
- 22 M. Verzele and D. De Keukeleire, in *Chemistry and Analysis of Hop and Beer Bitter Acids*, Elsevier, Amsterdam, The Netherlands, 1991, p. 93.
- 23 K. Huvaere, M. L. Andersen, K. Olsen, L. H. Skibsted, A. Heyerick and D. De Keukeleire, Radicaloid-type oxidative decomposition of beer bittering agents revealed, *Chem. Eur. J.*, 2003, **9**, 4693–4699.
- 24 C.-Y. Lu, W.-Z. Lin., W.-F. Wang, Z.-H. Han, S.-D. Yao and N.-Y. Lin, Riboflavin (VB<sub>2</sub>) photosensitized oxidation of 2'-deoxyguanosine-5'-monophosphate (dGMP) in aqueous solution: a transient intermediates study, *Phys. Chem. Chem. Phys.*, 2000, **2**, 329–334.
- 25 C.-Y. Lu and Y.-Y. Liu, Electron transfer oxidation of tryptophan and tyrosine by triplet states and oxidized radicals of flavin sensitizers: a laser flash photolysis study, *Biochim. Biophys. Acta*, 2002, **1571**, 71–76.
- 26 A. Gilbert and J. E. Baggott, in *Essentials of Molecular Photochemistry*, Blackwell, Oxford, England, 1991, p. 184.