The Photolysis of *trans*-Isohumulone to Dehydrohumulinic Acid, a Key Route to the Development of Sunstruck Flavour in Beer

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trans-Isohumulone (2) is photolysed to dehydrohumulinic acid (5) by a Norrish type 1 cleavage, affording a precursor of 3-methylbut-2-ene-1-thiol, the compound responsible for the sunstruck flavour of beer.

The hop α-acids, *e.g.* humulone (1), are very sensitive to visible and u.v. light. Thus, irradiation of compound (1) at wavelengths >300 nm readily affords *trans*-isohumulone (2) in a totally regio- and stereo-specific fashion.[1] This process has been made amenable to the large-scale production of beer bitter substances.[2] Due to its blue-shifted absorption the photoproduct (an iso-α-acid) is stable during the irradiation period needed for its formation. However, at shorter wavelengths it

invoked.[3] We now report on the behaviour of *trans* isohumulone (2) upon direct excitation.

Results and Discussion

Evidence for photoreaction, when *trans*-isohumulone (2) was irradiated at 300 nm, was provided by the appearance of a new

$R^1 = CH_2CHMe_2$

$R^2 = CH_2CH{=}CMe_2$
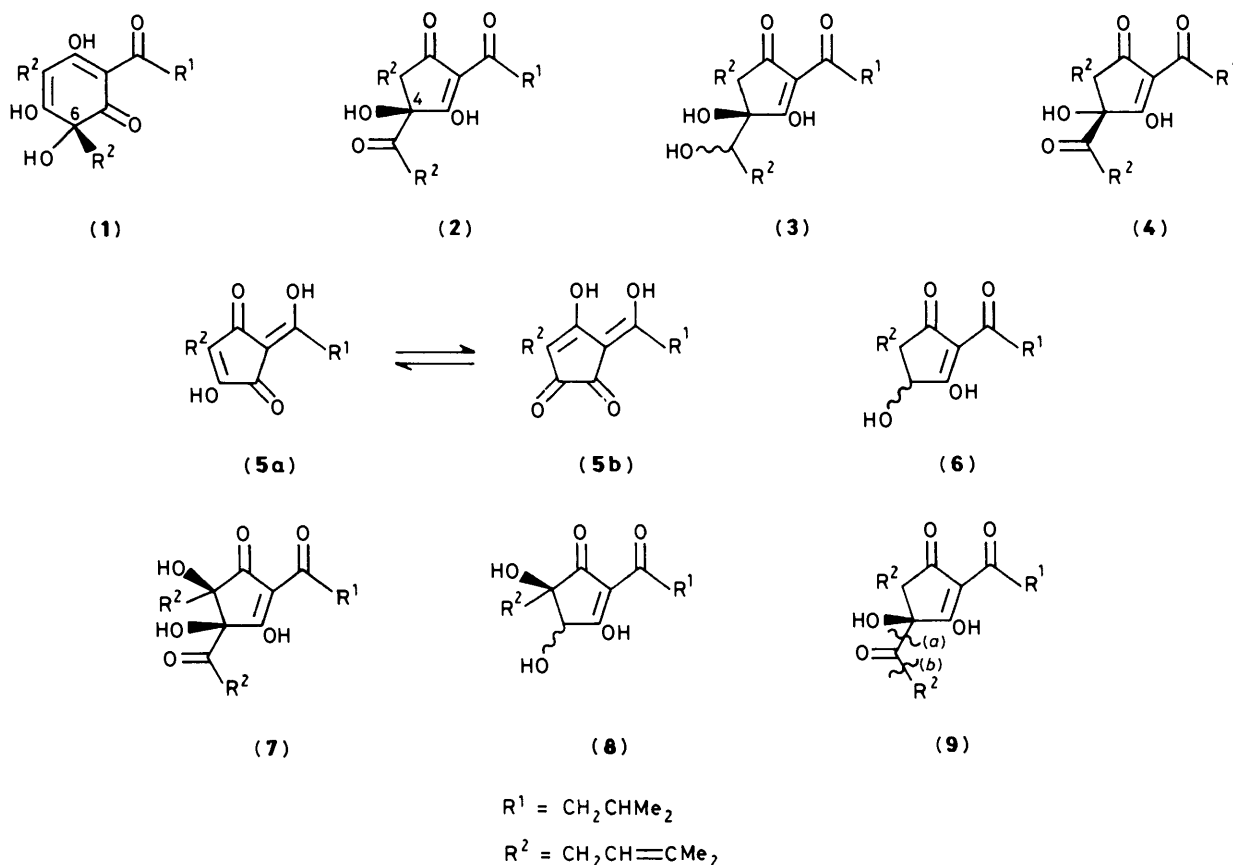
displays photoreactivity. This is also the case in the riboflavin photosensitised action of sunlight on beer, leading to rapid development of the so-called sunstruck off-flavour.[3] The involvement of iso-α-acids is proved by the light stability of beer that has been brewed with reduced or ρ-iso-α-acids, *e.g. trans*-ρ-isohumulones (3). It has been shown that 3-methylbut-2-ene-1-thiol is mainly responsible for the off-flavour.[3,4] Although the reaction mechanism is not exactly known, the interaction of the unsaturated acyl side-chain with a thiol radical has been

absorption band at 252 nm with a shoulder around 280 nm (Figure). The growth of a broad absorption band with low intensity between 300 and 320 nm was also noticed. H.p.l.c. analysis (see Experimental section for conditions) demonstrated the formation of a reaction product having a lower retention time than *trans*-isohumulone. Furthermore, a second component appeared at the tail of the *trans*-isohumolone peak. The respective components were characterised by ¹H n.m.r. after preparative h.p.l.c. separation. The partially resolved peak was
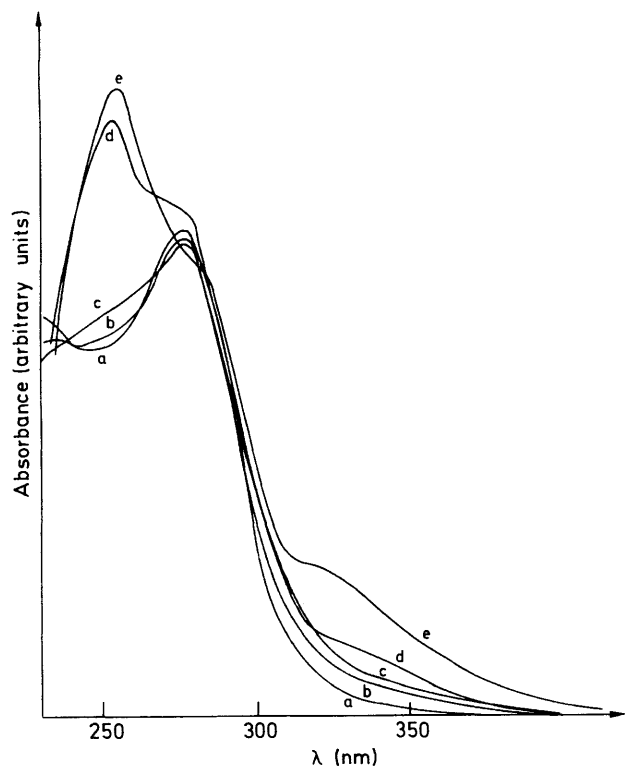
Figure. Changes in u.v. absorbance during irradiation of *trans*-isohumulone (2): (*a*) before irradiation; (*b*) after 15 min; (*c*) after 1 h; (*d*) after 2.25 h; and (*e*) after 3.5 h

composed of a mixture of *trans*-isohumulone (2) and *cis*-isohumulone (4). The early peak, the new photoproduct (k′ 1.6), was identified as dehydrohumulinic acid (5) (see Experimental section for structural data). The chemical yield was optimised to 62%. The quantum yield, measured by ferrioxalate actinometry,[5] amounted to 0.03.* At longer irradiation times several minor compounds could be detected albeit not exceeding a few percent.

The structure of dehydrohumulinic acid (5) was confirmed by independent synthesis. It has been reported that (5) could be prepared either by oxidation of humulinic acids (6) with bismuth(III) oxide, by alkaline degradation of humulinone (7) or by dehydration of oxyhumulinic acids (8).[6,7] Thus, the reaction product, obtained from (6) according to the known procedure,[6] was identical with the photoproduct (5) in all respects. It occurs as a mixture of two enol tautomers and represents a rare case of exocyclic enolization in five-membered ring hop compounds.[8] A further complication may arise from E/Z isomerism around this particular enol double bond. The predominance of (5a) (>80%†) reflects on one hand the opportunities for hydrogen bond formation and on the other hand the destabilising effect of a 1,2-dione functionality.

The formation of both photoproducts (4) and (5) from compound (2) can be rationalised by light-induced α-cleavage of the carbonyl group in the side-chain at C-4. Two fragmentations can be envisaged: either ketol α-cleavage [(*a*) in formula (9)] or α-cleavage in the acyclic β,γ-enone part [(*b*) in formula (9)]. In both instances a stabilised radical pair is formed. Ready recombination accounts for the low product quantum yield, while the mixture of isohumulones results from epimerisation of

the ketyl radical centre obtained *via* splitting (*a*). The ratio of *trans*- to *cis*-isohumulone is *ca.* 3:1. This is unusual, since in mixtures of isohumulones from other sources either the more stable *cis*-isohumulone predominates or equal amounts of both are present.[9] Establishment of a photoequilibrium is hampered by the simultaneously occurring photodegradation. The ketyl radical, derived either directly *via* route (*a*) or after decarbonylation of the acyl radical from route (*b*), leads to dehydrohumulinic acid (5) by consecutive α-hydrogen elimination and tautomeric rearrangement. It was not possible to quench the photoprocess to any appreciable extent. On the other hand, irradiation of (2) at higher energy (254 nm) led to a very complex reaction mixture containing no major photoproduct. It is suggested that the primary process is a Norrish type 1 photofragmentation arising from the (n,π*) manifold.[10]

The formation of dehydrohumulinic acid (5) provides the first proof for direct photodegradation of *trans*-isohumulone (2). This finding is related to the development of sunstruck flavour in beer. The ketyl radical, formed from (9) *via* splitting (*a*), affords, after decarbonylation, the corresponding allyl radical, which is also directly accessible by cleavage of (9) *via* process (*b*). Both radicals are precursors of 3-methylbut-2-ene-1-thiol obtained after trapping by a thiol radical. The flavour threshold concentration of this compound is so low (<1 p.p.b.) that the photoprocess (quantum yield of 0.03), reported here, should contribute significantly to the offending phenomenon.

Experimental

trans-Isohumulone (2) (31.8 mg, 0.088 mmol), obtained *via* literature procedures,[3] was irradiated in deaerated methanol (250 ml) at 300 nm (Rayonet photoreactor containing 8 RUL-3000 Å lamps) through Pyrex. The reaction was monitored by u.v. spectroscopy (Unicam SP 1750 ultraviolet spectrophotometer). After 3.5 h the solvent was evaporated off and the residue chromatographed on a Lichroma tubing column (25 × 0.46 cm) filled with demineralised 5 μm ROSIL-C_{18}-D (RSL-Alltech Europe, Eke, Belgium) and eluted at 1 ml min⁻¹ with CH_3CN–H_2O–H_3PO_4 (80:20:1) (Varian 5020 LC liquid chromatograph equipped with a 10 μl Valco 7000-psi injector and a Varian UV-50 variable wavelength detector operated at 280 nm). Dehydrohumulinic acid (5) was isolated and recrystallised from ethanol (23.2 mg, 62%), m.p. 136 °C (Found: C, 68.6; H, 7.5. $C_{15}H_{20}O_4$ requires C, 68.2; H, 7.6%); $λ_{max}$.(MeOH–HCl, 0.1M) 252 (23 000 dm³ mol⁻¹ cm⁻¹), 275 (21 600), and 320sh nm; $λ_{max}$.(MeOH–NaOH, 0.1M) 234 (14 700), 277 (31 000), and 297 (31 200) nm; $δ_H$(Bruker WH 360 n.m.r. spectrometer, 360 MHz, $CDCl_3$) 0.98 (6 H, d, J 6.5 Hz, Me_2CH), 1.72 (6 H, s, $Me_2C=C$), 2.10 (1 H, m, Me_2CH), 2.60 (2 H, d, J 7.0 Hz, CHCH_2CH), 3.04 (2 H, d, J 7.0 Hz, CHCH_2CO), 5.23 (1 H, t, J 7.0 Hz, $CH=C$), 6.9 (1 H, br, enol, OH), and 12.4 (1 H, br, enol OH); m/z (AEI MS-50 mass spectrometer, electron impact, 70 eV) 264 (M^+, 100%), 209 (36), 196 (30), and 152 (50).

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* This value agrees well with the quantum yield for photodegradation of the isohumulones, reported in ref. 4. However, the reaction was not fully characterised, nor was a reaction product identified.

† Estimated from the broadening of some ¹H n.m.r. signals.

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