The photochemistry of *trans*-isohumulone, a bitter flavouring component of beer

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Abstract: Methanolic solutions of *trans*-isohumulone (2), a major bitter flavouring component in beer, were irradiated with UV light of 313 nm wavelength and yielded four primary products containing an enolized cyclic β -triketone moiety: *cis*-isohumulone (3), humulone (1), *dehydro*-isohumulone (7), and *dehydro*-humulinic acid (5). The last of these products results from loss of the 4-methyl-3-pentenoyl side chain of *trans*-isohumulone. Nine volatile products derived from this side chain were identified and quantitated. The identifications of all photoproducts were confirmed by independent preparation of authentic samples. No evidence of either intramolecular or intermolecular 2+2 cyclo-addition was observed. This work clarifies previous contradictory reports of the products of isohumulone photolysis and provides an example of unexpected photochemistry of an alkenyl-substituted enolized cyclic β -triketone.

Key words: trans-isohumulone, photo-degradation, cyclic β-triketone.

Résumé : La *trans*-isohumulone (2), un des constituants de la bière qui lui donne son goût amer, en solution dans le méthanol a été soumise à une irradiation UV à 313 nm et a conduit à la formation de quatre produits primaires qui contiennent une portion β -tricétonique cyclique énolisée: la *cis*-isohumulone (3), l'humulone (1), la *déhydro*isohumulone (7) et l'acide *déhydro*humulinique. Le dernier de ces produits résulte de la perte de la chaîne latérale 4-méthylpent-3-énoyle de la *trans*-isohumulone. On a aussi identifié la nature et quantifié neuf produits volatils dérivés de cette chaîne latérale. Les identifications de tous les photoproduits ont été confirmées par la préparation d'échantillons authentiques. On n'a pas pu mettre en évidence de cycloadditions 2+2 intramoléculaire ou intermoléculaire. Ces résultats permettent de clarifier des rapports antérieurs contradictoires relatifs à la photolyse de l'isohumulone et ils fournissent un exemple de photochimie inattendue d'une β -tricétone cyclique énolisée et substituée par une chaîne alcényle.

Mots-clés : trans-isohumulone, photodégradation, β-tricétone cyclique.

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Introduction

Beer is brewed by the yeast-mediated fermentation of an aqueous solution of malted barley to which hops (flowers of the plant *Humulus lupulus*) have been added. The hops are a source of water soluble, bitter-tasting compounds (known as alpha acids) of which humulone (1) is the major component. During the brewing process, the alpha acids undergo thermal isomerization to so-called iso-alpha acids that are responsible for the bitter flavour of beer (1). Thermal conversion of the hop alpha-acid humulone (1) to the iso-alpha acids *cis*-isohumulone (3) and *trans*-isohumulone (2) is illustrated in Scheme 1.

The NMR and UV spectra of isohumulone in various solvents reveal it to be enolized, and three tautomers are, in principle, possible. The tautomer illustrated in Scheme 1 is drawn throughout this paper for consistency, but this should not be taken to imply that the other tautomers are not present in solution. The enolized cyclic β -triketone of isohumulone is acidic with a p*K*a of 3.1 in aqueous ethanolic solutions (2), and thus is only partially ionized.^{2,3}

Beer develops an off-flavour when exposed to light because of the formation of trace amounts of 3-methyl-2butene thiol (4), and it has been shown that the iso-alpha acids must be present for this to occur (3–5). This thiol has a taste threshold in beer of about 10^{-11} mol/L, and so extremely small amounts formed by photolysis of iso-alpha acids can generate an off-flavour (6–8). In the brewing industry, beer so afflicted is termed "light-struck" and more colloquially as "skunky", since the thiol has an odor similar to that associated with skunks.

In beer, degradation of the iso-alpha acids is initiated either by direct absorption of UV light or indirectly by sensitization. In the latter case, components of the beer such as flavins mediate the light-struck reaction through absorption of both UV and visible light. The light-struck reaction can

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²The pKa was confirmed experimentally (details provided in the Supplementary Data).

³Supplementary data for this article are available on the journal Web site (canjchem.nrc.ca) or may be purchased from the Depository of Unpublished Data, Document Delivery, CISTI, National Research Council Canada, Ottawa, ON K1A 0R6, Canada. DUD 3767. For more information on obtaining material refer to cisti-icist.nrc-cnrc.gc.ca/irm/unpub_e.shtml.

Scheme 1.



be inhibited by appropriate packaging to block or filter light (hence the common use of green tinted or brown glass for beer bottles). Alternatively, the beer can be brewed with isoalpha acids that have been extracted from hops and chemically modified. For example, protection from the light-struck reaction can be achieved by the use of iso-alpha acids in which the alkene or carbonyl function of the 4-methyl-3pentenoyl side chain has been reduced (9, 10). While the mechanism of the light-struck reaction is not entirely known, the evidence suggests it proceeds as indicated in Scheme 2.

Interest in understanding and hence suppressing the lightstruck reaction in beer has led to a number of studies of the photochemistry of the iso-alpha acids. In many of these studies *trans*-isohumulone is used as a model, since it can be produced and isolated as a single stereoisomer from natural humulone extracted from hops (11). In one such study, photolysis of *trans*-isohumulone to high conversion was reported to generate *dehydro*-humulinic acid (**5**) in nearly quantitative yield (12, 13), as illustrated in Scheme 3.

However, it has also been reported that a variety of products result from high conversion photolysis of *trans*isohumulone, or of an iso-alpha-acid mixture, with a significant product being the decarbonylated *dehydro*-isohumulone (**6**), as illustrated in Scheme 4 (14–16).

It is well-known that enones (17-19) and enolized β diketones (20-25) undergo efficient 2+2 photocycloaddition reactions with alkenes. This reaction is especially efficient for systems containing the enone and the alkene function within the same molecule. It is therefore surprising that no products of photocycloaddition have been reported for isohumulone. This fact, and the inconsistencies in the reported products of photolysis of isohumulone, led us to examine in detail the products of the photochemistry of *trans*isohumulone. The result of this study is reported here. In view of its importance in the brewing industry, we have also examined the mechanism of this reaction. The results of that work will be reported separately.

In a further report, the headspace over an aqueous solution



(Reported but unobserved product)

Scheme 5.

(4)







FMN = Flavin Mononucleotide

containing a mixture of iso-alpha acid potassium salts and a flavin sensitizer has been examined after exposure of the solution to visible light (26). This study attempted to determine the fate of any side chains cleaved from isohumulone and found several, which are illustrated in Scheme 5.

The formation of 2-methyl-2-butene, isoprene, and 4methyl-3-pentenal are consistent with the intermediacy of a

Scheme 6.



4-methyl-3-pentenoyl radical. The other observed volatile compounds illustrated in Scheme 5 are not. These other products appear to be consistent with degradation of the other side chains of the iso-alpha acid, suggesting further sensitized degradation of the primary enolized cyclic β -triketone products.

Results and discussion

Identification of the enolized cyclic β-triketonecontaining photoproducts of *trans*-isohumulone

Solutions of *trans*-isohumulone in methanol were exposed to light of 313 nm wavelength. At conversions of less than 15%, four nonvolatile photoproducts containing the enolized cyclic β -triketone moiety were generated, as illustrated in Scheme 6.

Each product was isolated by semi-preparative HPLC and characterized by one-dimensional (¹H NMR and ¹³C NMR) and two-dimensional (gCOSY, gHSQC, and gHMBC) NMR spectroscopy, as well as electron ionization and chemical ionization mass spectrometry (GC-MS).³ The identifications were confirmed by independent preparation of an authentic sample of each compound. These results confirm the literature reports that *dehydro*-humulinic acid (**5**), *cis*-iso-humulone (**3**), and humulone (**1**) are photoproducts of *trans*-isohumulone (**2**) (12–16). No evidence for the formation of the *decarbonylated-dehydro*-isohumulone (**6**) was found (14,

Fig. 1. Variation with time of *trans*-isohumulone concentration and total photoproduct concentration (enolized cyclic β -triketone products only) for an UV light irradiation conducted in methanol.



Fig. 2. Variation with time of the concentration enolized cyclic β -triketones produced for the UV light irradiation of *trans*-isohumulone in methanol.



16). In the previous reports where (6) was identified as a product, the identification appears to have been based on HPLC-MS evidence; the product was apparently not isolated such that its ¹H and ¹³C NMR spectra could be recorded, which may account for the observation.

We have also examined the photochemistry of *trans*isohumulone in aqueous ethanolic solutions as well as in aqueous solutions buffered at a pH of 2.1 and 4.7.³ In all cases only these four nonvolatile photoproducts were observed when conversions were kept below 15%. This suggests that both the enol and enolate forms of *trans*isohumulone exhibit similar photochemistry and suggests that the tautomeric form adopted by isohumulone is also not a critical factor in its photochemistry.³ Futhermore, no evidence of products of intramolecular or intermolecular 2+2 cycloaddition was observed for *trans*-isohumulone.

Yields of the enolized cyclic β -triketone products formed by UV light irradiation of *trans*-isohumulone

The conversion of *trans*-isohumulone and the yields of the enolized cyclic β -triketone products were determined as a function of irradiation time. The results are shown in Figs. 1 and 2.

The data illustrated in Figs. 1 and 2 suggest that at conversions below 15% the photochemistry of *trans*-iso-humulone follows zero-order kinetics and that the four

Table 1. Yields of enolized cyclic β -triketones formed in the UV light irradiation of *trans*-isohumulone in methanol solution.

Enolized cyclic β-triketone photoproduct	Yield (%)
<i>cis</i> -Isohumulone	50 ± 4
Humulone	20 ± 1
dehydro-Isohumulone	22 ± 1
dehydro-Humulinic acid	6 ± 0.5
Total	98 ± 6.5

enolized cyclic β -triketone products isolated are primary products. At conversions above 15% the conversion of *trans*-isohumulone becomes less efficient and the products themselves decline in yield. This suggests that at these conversions the concentrations of the products are high enough for them to absorb light directly, or to quench the *trans*isohumulone excited state, and hence undergo secondary photolysis. We were unable to identify the products of secondary photolysis because of their complexity and the low yield of each component. Some previous studies have led to reports of the formation of complex mixtures of products when isohumulone is photolysed to high conversion (13– 16).

The gradients of the initial linear portions of the reaction profiles, shown in Figs. 1 and 2, allow the determination of the yield of each primary product based on converted *trans*-isohumulone. These yields are shown in Table 1.

The sum of the yields in Table 1 reveals that all of the converted *trans*-isohumulone has been accounted for, and that there are no other major primary photoproducts, apart from those derived from the cleavage of the pentenoyl side chain en route to *dehydro*-humulinic acid.

Identification and yields of the *trans*-isohumulone side chain photoproducts

The formation of *dehydro*-humulinic acid from the photolysis of *trans*-isohumulone must also yield one or more products resulting from the cleaved 4-methyl-3-pentenoyl side chain, as indicated in Scheme 7.

Deoxygenated diethyl ether solutions of *trans*isohumulone were exposed to light of 313 nm wavelength and directly analyzed by GC-MS at low conversions. The yields of the primary enolized cyclic β -triketone photoproducts were also determined by GC-MS and are given in Table 2.

The product yields shown in Table 2 are comparable with those determined when methanolic solutions of *trans*isohumulone were photolysed and analyzed by HPLC (Table 1). This indicates that the change in solvent did not influence the reaction outcome. The identities of the photoproducts derived from the cleaved 4-methyl-3-pentenoyl side chain were determined by GC-MS. Their identities were confirmed by comparison with independently prepared authentic samples. The product structures are given in Fig. 3.

Each of the products is a branched chain of either five carbons (compounds **8**, **9**, **11**, **12**, and **13**) or six carbons (compounds **10**, **14**, **15**, and **16**) and appear therefore to be products formed both prior to and following decarbonylation of an initially produced 4-methyl-3-pentenoyl radical. Several of these photoproducts contain oxygen (compounds **11**,

Scheme 7.



Table 2. Yields of primary enolized cyclic β -triketone products produced from UV light irradiation of *trans*-isohumulone in diethyl ether solution.

Enolized cyclic β -triketone photoproduct	Yield (%)
cis-Isohumulone	50 ± 3
Humulone	19 ± 2
dehydro-Isohumulone	21 ± 2
dehydro-Humulinic acid	5.8 ± 0.6
Total	96 ± 7





12, 13, 14, 15, and 16), which was not present in the 4methyl-3-pentenoyl side chain. This observation is consistent with a radical intermediate trapped by reaction with molecular oxygen. The structures of the remaining products are consistent with hydrogen atom transfer (from ether, to oxygen, or via disproportionation, etc.) both before and after decarbonylation of the initially produced 4-methyl-3pentenoyl radical. The various pathways are summarized in Scheme 8.

To our knowledge, there is only one published investigation of the volatile products of *trans*-isohumulone photochemistry; this involved analysis of the headspace of a riboflavin sensitized reaction of *trans*-isohumulone and led Scheme 8.



volatile product structures 8-16 are illustrated in Figure 3

to the identification of compounds **8**, **9**, and **10** (26). The yields of each of the products **8–16** found in our work were calculated relative to the amount of *dehydro*-humulinic acid formed and are presented in Table 3.

The dominant fate of the 4-methyl-3-pentenoyl side chain intermediate appears to be the addition of oxygen, despite measures taken to remove the dissolved oxygen from the solution prior to irradiation (The freeze-pump-thaw method of degassing is a well-known procedure often utilized for oxygen sensitive reactions.) (27). If this is the case, the relative yields of these products would increase in solutions containing ambient concentrations of dissolved oxygen. To test this, irradiations of *trans*-isohumulone were conducted with 313 nm light using diethyl ether solutions containing ambient levels of dissolved oxygen. The product yields obtained presented in Table 4.

The data in Table 4 show that within the experimental error, a mass balance was achieved for side chain product formation in the ambient oxygen containing solution. The incomplete recovery of the side chain derived compounds in deoxygenated solutions may reflect reaction of radical intermediates with the solvent to give undetected compounds. In the presence of oxygen, the radicals are trapped and these side reactions are avoided. Since no new volatile products were observed in the ambient oxygen solution, it is reasonable to conclude that the oxidation products formed in both solutions resulted from reaction of a radical intermediate with molecular oxygen.

Conclusions

As noted in the Introduction to this paper, previous reports of the products of photolysis of trans-isohumulone are contradictory. For example, in one study dehydro-humulinic acid (5) was reported as the only product (12, 13), while in another decarbonylated *dehydro*-isohumulone (6) was identified as a major product (14-16). In these reports, the products are not isolated or fully characterized, nor are their yields reported. In the work reported here the primary products formed by direct UV light irradiation of transisohulumone have been isolated and identified by ¹³C and ¹H NMR spectroscopy and by mass spectroscopy, their yields have been determined, and a complete mass balance has been obtained. We find that *dehydro*-isohumulinic acid (5) is not the sole product. Rather, three modes of reaction occur. These are isomerization, dehydration, and side chain cleavage to give humulone (1), cis-isohumulone (3), dehydroisohumulone (7), as well as *dehydro*-humulinic acid (5). No

Table 3. Percent yields of *trans*-isohumulone side chain derived products in deoxygenated diethyl ether solution.

Side chain product	Yield (%)
2-Methyl-2-butene	1.0 ± 0.2
Isoprene	0.9 ± 0.2
4-Methyl-3-pentenal	2.3 ± 0.3
3-Methyl-2-buten-1-ol	8.4 ± 0.9
3-Methyl-2-butenal	3.9 ± 0.5
3-Hydroxy-3-methylbutenal	11 ± 1
trans-4-Hydroxy-4-methyl-2-pentenoic acid	23 ±2
cis-4-Hydroxy-4-methyl-2-pentenoic acid	12 ± 1
5,5-Dimethylfuranone	12 ± 1
Total product yield	75 ± 8

Table 4. Percent yields of the identified side chain products from *trans*-isohumulone photolysis in diethyl ether in the presence of ambient oxygen.

Side chain product	Yield (%)
2-Methyl-2-butene	
Isoprene	_
4-Methyl-3-pentenal	_
3-Methyl-2-buten-1-ol	11 ± 1
3-Methyl-2-butenal	8.0 ± 0.7
3-Hydroxy-3-methylbutenal	14 ± 1
trans-4-Hydroxy-4-methyl-2-pentenoic acid	27 ± 3
cis-4-Hydroxy-4-methyl-2-pentenoic acid	16 ± 1
5,5-Dimethylfuranone	17 ± 1
Total product yield	93 ± 8

evidence was found for the formation of decarbonylated dehydro-isohumulone (6). Also, and surprisingly given the normal reactivity of enones when irradiated with UV light in the presence of alkenes, no evidence was found for products of intra- or intermolecular 2+2 photocycloaddition. The formation of dehydro-humulinic acid requires the loss of the 4methyl-3-pentenoyl side chain of trans-isohumulone. Previous studies of the fate of this 4-methyl-3-pentenoyl side chain were performed using mixtures of iso-alpha acids under conditions where secondary photochemistry could occur. In the work reported here, the primary products of side chain cleavage have been identified, their yields have been determined, and a complete mass balance has been obtained. In addition to the formation of dehydro-humulinic acid, we found that the side chain cleavage leads to products derived from the interception of 4-methyl-3-pentenoyl radicals and methylbutenyl radicals. This is compatible with the observation that isohumulones and light lead to the formation of very low concentrations of 3-methyl-2-butene thiol in a beer matrix and suggests that the thiol is produced by interaction of a photochemically generated methylbutenyl radical with a sulfur donor.

Experimental

General

All the solvents used for irradiations, spectrometry, and chromatography were obtained from the Sigma-Aldrich chemical company and were of spectrophotometric quality.

UV irradiations of *trans*-isohumulone were conducted with a 450 W Hanovia medium pressure mercury lamp, surrounded by an aqueous solution of potassium chromate (1.44 \times 10^{-3} mol/L) and sodium carbonate (1.25 × 10^{-2} mol/L) (27). Methanolic solutions were purged with nitrogen gas for 20 min prior to light exposure and maintained under nitrogen during the irradiation to minimize oxygen contamination. The solutions were stirred throughout the irradiation procedures. Samples were withdrawn from the irradiation vessel via syringe through a Teflon stopcock. Diethyl ether was freshly distilled immediately prior to use. Solutions prepared in diethyl ether were degassed by the freeze-pumpthaw method (27). Sampling of these ether solutions was performed at the conclusion of the irradiation to minimize molecular oxygen contamination and loss of volatile compounds.

Qualitative analyses of products

Semi-preparative HPLC separations were accomplished with a Waters instrument, employing a 10 mm × 300 mm Supelco C₁₈-nucleosil semi-preparative column. Samples were introduced with a 500 uL sample loop and separated with an eluent mixture of 80% acetonitrile, 20% water and 0.1% concentrated phosphoric acid. The NMR spectra were obtained in CDCl₃ with a Varian Mercury 400 NMR spectrometer at 400.09 MHz for ¹H and 100.69 MHz for ¹³C. The ¹H and ¹³C NMR assignments were correlated with the two-dimensional NMR techniques of gCOSY, gHSQC, and gHMBC.

Quantitative analyses of products

Samples were analyzed using an isocratic Waters HPLC system with a tunable absorbance detector set to 270 nm. Samples were introduced via a 10 uL injection loop and separated with a 250 mm × 4.6 mm Supelco ODS nucleosil analytical column. The eluent system, composed of a mixture of 600 mL of acetonitrile, 400 mL of distilled water, and 2 mL of concentrated phosphoric acid. Calibration curves were prepared for each analyte using five different concentrations (ranging from 1% to 25% of the starting transisohumulone concentration). The GC-MS analyses were performed with a Varian Saturn 4-D GC-MS, equipped with a DB-5 capillary column and a splitless injector. The temperature program of the GC began at 50 °C for 2 min, with the temperature increased to 180 °C at 15 °C/min, then to 200 °C at 4 °C/min and finally to 230 °C/min at 1 °C. The mass spectrometer scanned over the mass range of 33 to 550 mass units at 1 s intervals. Samples were analyzed in freshly distilled, spectrophotometric grade diethyl ether. Calibration curves were prepared for the analytes, using five different concentrations (ranging from 0.5% to 50% of the typical dehydro-humulinic acid concentration).

Preparation of compounds

Humulone

Humulone was isolated from a commercially available non-isomerized hop extract provided by from the Research and Development department of the Labatt Brewing Company. The procedure used is a literature method (11).

¹H NMR δ : 0.97 (d, J = 7.0 Hz, 3H), 1.01 (d, J = 7.0 Hz, 3H), 1.53 (s, 3H), 1.69, (s, 6H), 1.74 (s, 3H), 2.15 (m, J =

7.0 Hz, 1H), 2.49 (dq, J = 7.0 Hz, 2H), 2.77 (dd, J = 7.0 Hz, 2H), 3.06 (dq, J = 7.0 Hz, 2H), 5.00 (t, J = 7.0 Hz, 1H), 5.14 (t, J = 7.0 Hz, 1H). ¹³C NMR δ : 17.9, 22.7, 25.4, 25.7, 26.1, 30.2, 46.0, 84.2, 110.1, 113.7, 115.4, 116.2, 135.2, 137.7, 175.8, 186.9, 191.1, 198.2. GC-MS: 363 (76%), 345 (78%), 327 (6%), 307 (3%), 295 (6%), 266 (3%), 223 (3%), 197 (3%), 166 (3%), 137 (4%), 109 (7%), 97 (7%), 85 (5%), 69 (100%), 57 (16%).

trans-Isohumulone³

Humulone was photo-isomerized by a literature method (11) utilizing UV light from a medium pressure mercury lamp that had passed through a uranium oxide glass filter. The course of the irradiation was monitored by analytical HPLC. Analysis by HPLC and GC-MS indicated the purity to be greater than 97% after recrystallization.

¹H NMR δ : 0.88 (d, J = 6.6 Hz, 3H), 0.91 (d, J = 6.6 Hz, 3H), 1.47 (s, 3H), 1.51 (s, 3H), 1.63 (s, 3H), 1.67 (s, 3H), 2.08 (m, 6.6, 1H), 2.27 (dt, J = 7.1 & 15 Hz, 1H), 2.52 (dt, J = 7.1 & 15 Hz, 1H), 2.55 (d, J = 7.1 Hz, 2H), 2.98 (dd, J = 6.8 & 6.9 Hz, 1H), 3.27 (d, J = 7.2 Hz, 2H), 5.08 (t, J = 7.1Hz, 1H), 5.14 (t, J = 7.1 Hz, 1H). ¹³C NMR δ : 18.2, 18.7, 22.5, 23.1, 23.6, 25.9, 26.3, 38.8, 44.3, 55.8, 91.2, 110.6, 114.7, 120.3, 135.2, 136.4, 195.7, 198.2, 205.3, 207.1. GC-MS: 363 (52%), 345 (44%), 327 (3%), 293 (15%), 277 (7%), 266 (5%), 238 (3%), 209 (5%), 197 (9%), 109 (3%), 97 (8%), 85 (5%), 69 (66%), 57 (11%), 41 (100%).

cis-Isohumulone

Humulone was isomerized to *cis*-isohumulone by a literature method (28). The material was isolated as a light yellow oil from the reaction mixture and was purified by semipreparative HPLC.

¹H NMR δ : 0.94 (d, J = 6.2 Hz, 3H), 0.96 (d, J = 6.2 Hz, 3H), 1.52 (s, 3H), 1.56 (s, 3H), 1.59 (s, 3H), 1.63 (s, 3H), 2.13 (m, J = 6.2 Hz, 1H), 2.41 (dt, J = 7.2 Hz, 1H), 2.47 (dt, J = 7.1 Hz, 1H), 3.30 (d, J = 7.1 Hz, 2H), 3.20 (dd, J = 7.1 Hz, 1H), 3.30 (d, J = 7.0 Hz, 2H), 5.01 (t, J = 7.0 Hz, 1H), 5.12 (t, J = 7.0 Hz, 1H). ¹³C NMR δ : 18.0, 18.3, 22.6, 22.7, 25.6, 59.9, 26.3, 37.1, 46.0, 50.3, 55.5, 87.7, 110.7, 114.7, 119.8, 119.9, 135.4, 136.9, 195.5, 200.6, 206.3, 206.9. GC-MS: 363 (84%), 346 (51%), 295 (11%), 266 (7%), 197 (19%), 137 (4%), 109 (3%), 97 (5%), 85 (9%), 69 (80%), 57 (14%), 41 (100%).

Dehydro-humulinic acid

A sample of *trans*-isohumulone was used to prepare *dehydro*-humulinic acid by a literature method (29).

¹H NMR δ: 0.95 (d, J = 7.0 Hz, 3H), 0.96 (d, J = 7.0 Hz, 3H), 1.63 (s, 3H), 1.70 (s, 3H), 2.11 (m, J = 7.0 Hz, 1H), 2.58 (d, J = 7.1 Hz, 1H), 3.02 (d, J = 7.0 Hz, 2H), 5.21 (t, J = 7.0 Hz, 1H). ¹³C NMR δ: 17.7, 23.8, 25.2, 25.6, 48.9, 110.8, 116.7, 121.7, 136.2, 158.1, 161.3, 193.5, 198.1. GC-MS: 265 (100%), 247 (9%), 209 (10%), 190 (13%), 175 (6%), 152 (10%), 95 (8%), 81 (13%), 79 (13%), 69 (19%), 55 (21%).

Preparation of side chain products

PCC oxidations of primary alcohols to aldehydes

The pyridinium chlorochromate reagent was prepared by a literature method (30).

3-Methyl-2-butenal

About 0.5 g of 3-methyl-2-buten-1-ol was dissolved in 5 mL of CH_2Cl_2 and added to a suspension of 1.5 g of the PCC reagent in 20 mL of CH_2Cl_2 . The mixture was stirred at room temperature for about 2 h. Three 25 mL portions of diethyl ether were used to wash the mixture. The organic extracts were combined, dried with MgSO₄, and the solvent removed by rotary evaporation at room temperature. Isolation of the compound and final purification were accomplished by vacuum distillation.

¹H NMR δ : 1.95 (s, 3H), 2.12 (s, 3H), 5.54 (d, *J* = 7.1 Hz, 1H), 9.67 (d, *J* = 7.2 Hz, 1H). ¹³C NMR δ : 21.3, 27.6, 124.8, 158.7, 192.6. GC-MS: 84, 69, 55.

4-Methyl-3-pentenal

About 0.6 g of 4-methyl-3-penten-1-ol was dissolved in 5 mL of CH_2Cl_2 and added to a suspension of 1.5 g of the PCC reagent in 20 mL of CH_2Cl_2 . The mixture was stirred at room temperature for about 2 h. Three 25 mL portions of diethyl ether were used to wash the mixture. The organic extracts were combined, dried with MgSO₄, and the solvent removed by rotary evaporation at room temperature. Isolation of the compound and final purification were accomplished by vacuum distillation.

¹H NMR δ : 1.63 (s, 3H), 1.74 (s, 3H), 3.21 (d, J = 7.1 Hz, 2H), 5.31 (d, J = 7.1 Hz, 1H), 9.82 (s, 1H). ¹³C NMR δ : 18.7, 25.3, 36.8, 115.8, 138.4, 201.4. GC-MS: 98, 83, 70, 54, 44.

3-Methyl-3-hydroxybutanal

The 3-methyl-3-hydroxybutanal was prepared from 3methyl-2-butenal by a literature method (31). Isolation of the compound and final purification were accomplished by semi-preparative HPLC.

¹H NMR δ : 1.42 (s, 6H), 2.54 (s, 2H), 4.21 (s, 1H), 9.78 (s, 1H). ¹³C NMR δ : 27.2, 54.8, 79.1, 205.4. GC-MS: 102, 87, 85, 73, 58, 44.

4-Hydroxy-4-methyl-2-pentenoic acid derivatives

The 4-hydroxy-4-methyl-2-pentenoic acid derivative compounds were prepared by a literature method from UV irradiation of propargylic acid in isopropanol (32). The resulting yields of each product were approximately 26% for *trans*-4hydroxy-4-methyl-2-pentenoic acid, 14% for *cis*-4-hydroxy-4-methyl-2-pentenoic acid, and 9% for 5,5-dimethylfuranone. The products were separated and purified by semipreparative HPLC.

trans-4-Hydroxy-4-methyl-2-pentenoic acid

¹H NMR δ : 1.31 (s, 6H), 4.92 (s, 1H), 5.82 (d, J = 15 Hz, 1H), 7.28 (d, J = 15 Hz, 1H), 11.03 (s, 1H). ¹³C NMR δ : 27.8, 69.7, 123.6, 158.4, 172.3. GC-MS: 130, 115, 113, 85, 71, 59, 45.

cis-4-Hydroxy-4-methyl-2-pentenoic acid

¹H NMR δ : 1.49 (s, 6H), 5.48 (s, 1H), 5.87 (d, J = 12 Hz, 1H), 6.43 (d, J = 12 Hz, 1H), 10.91 (s, 1H). ¹³C NMR δ : 29.2, 76.4, 121.2, 153.3, 170.8. GC-MS: 130, 115, 113, 85, 71, 59, 45.

5,5-Dimethyl furanone

¹H NMR δ : 1.27 (s, 6H), 5.82 (d, J = 5.7 Hz, 1H), 7.28 (d, J = 5.8 Hz, 1H). ¹³C NMR δ : 27.4, 90.8, 122.1, 165.2, 175.2. GC-MS: 112, 97, 95, 70, 68.

dehydro-Isohumulone preparation

A 500 mg sample of *trans*-isohumulone was dissolved in 50 mL of freshly distilled anhydrous diethyl ether. A 1.5 mL aliquot of acetic anhydride and 0.5 mL of triethylamine were added and the mixture was stirred at room temperature for 3 h. The mixture was washed with three portions of sodium bicarbonate solution (1.25 g in 300 mL) and with three 100 mL portions of water. The organic layer was dried with magnesium sulphate, and the solvent removed by rotary evaporation. *Dehydro*-isohumulone was separated as an oil from the reaction mixture and further purified by semi-preparative HPLC.

¹H NMR δ: 0.88 (d, J = 7.0 Hz, 3H), 0.90 (d, J = 7.0 Hz, 3H), 1.60 (s, 6H), 1.71 (s, 6H), 2.12 (m, J = 7.0 Hz, 1H), 2.92 (d, J = 7.0 Hz, 2H), 3.47 (d, J = 7.0 Hz, 2H), 3.52 (d, J = 7.0 Hz, 2H), 4.94 (t, J = 7.0 Hz, 1H), 5.24 (t, J = 7.0 Hz, 1H). ¹³C NMR δ: 18.3, 22.6, 22.7, 24.1, 25.9, 40.3, 52.4, 113.9, 118.7, 125.8, 133.9, 134.2, 137.9, 153.7, 158.4, 176.7, 195.4, 198.6. GC-MS: 345 (100%), 247 (7%), 147 (3%), 119 (7%), 91 (9%), 77 (7%), 69 (20%), 53 (14%).

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