Chemical Analysis and Sensory Evaluation of Hydrolysis Products of Humulene Epoxides II and III

Xiaogen Yang,[†] Cindy Lederer,[‡] Mina McDaniel,[‡] and Max Deinzer[*,†]

Department of Agricultural Chemistry and Department of Food Science and Technology,
Oregon State University, Corvallis, Oregon 97331

An analytical method was developed for authentic beer samples to identify hydrolysis and isomerization products of humulene epoxides II and III. This method involved a continuous liquid–liquid extraction, cleanup and concentration procedure, and analysis by GC/MS. Twelve compounds were identified in the concentration range from 0.002 to 2.8 mg/L in pilot brew and commercial beer samples. Seven of these compounds have not been found in beer before. A variety of hop essential oils, obtained by steam distillation, also was analyzed by GC/MS. In addition to humulene monoepoxides I–III, compounds 8 and 9 were found in the hop oils. Compound 9 has not been reported as a hop oil component previously. Sensory profiles of individual compounds and their mixtures produced by hydrolysis of humulene epoxides II and III were evaluated by a descriptive sensory panel. Overall, these compounds could be described as generally contributing cedar, lime, and spicy aroma notes to the beer. The estimated threshold value for the hydrolysis mixture of humulene epoxide II in beer is 2.3 ppm.

INTRODUCTION

The characteristic aroma of hops is determined by their essential oils. Much effort has been directed toward characterization of the sensory properties of individual hop components and the analysis of these compounds in beer. However, it is well-known that hop aroma changes during the brewing process and during hop storage. This probably is associated with chemical reactions of hop components. The reaction products can affect the flavor of beer. Under brewing conditions the oxygenated hop oil compounds have a better chance of surviving the boiling period and of undergoing reactions than do the hydrocarbons. Peacock and Deinzer (1988) studied the fate of hop oil components in beer and noted a rapid decrease of concentration of certain hop compounds, especially oxygenated components. Lam et al. (1986) also reported that most oxidation products of α-humulene survived the fermentation process poorly. This was suggested to be due to adsorption onto yeast (Moir et al., 1983). It is likely, however, that the concentration decrease results from chemical reactions of these compounds.

Model studies of humulene epoxides under hydrolysis conditions have been conducted in our laboratory (Yang and Deinzer, 1992). The results indicate that these epoxides can readily undergo hydrolysis and isomerization reactions and produce a large number of compounds under brewing conditions. To identify the reaction products of humulene epoxides in the brewing process, an analytical method was developed for authentic beer samples. Three pilot brews, three commercial beer samples, and a variety of hop essential oils were analyzed.

The total concentration of the reaction products of humulene epoxides II and III was generally much higher than that of the remaining starting compounds in beer. It was, therefore, of interest to examine the aroma of these reaction products. We report the results of these studies.

EXPERIMENTAL PROCEDURES

Reference Materials. Humulene epoxide II was synthesized by epoxidation of α-humulene using m-chloroperbenzoic acid

and purified by liquid chromatography to 99.7% (GC/FID). To synthesize humulene epoxide III, α-humulene was oxidized to the triepoxide stereochemical mixture, and this mixture was reduced to the monoepoxide in a solution of WCl$_3$/BuLi/THF. Humulene epoxide III was isolated and purified to 99% (GC/FID). Humulene epoxide II was hydrolyzed in aqueous solution containing 3% ethanol buffered at pH 4. The epoxide (0.2–0.4 mg/mL) was boiled for 3 h under reflux. The hydrolysis products were extracted by CCl$_3$F (Freon 11) and then by CH$_2$Cl$_2$ (DCM). The solvents were removed by a gentle nitrogen stream. Freon 11 extraction gave mixture A, which was composed of compounds 1–9 and 12; the DCM extraction gave mixture B, which contained compounds 11, 13, 15, and 17. Mixture AB, containing compounds 1–17 and approximately 17% (GC/FID) unidentified reaction products, was obtained by combination of mixtures A and B in the ratio of their extraction yields. Humulene epoxide III was hydrolyzed under the same conditions as described above, and individual compounds were isolated from the reaction mixture by flash chromatography and further purified by HPLC. The purity of most of the compounds was >95% according to GC/FID and NMR analysis. Compound 5 was approximately 87% pure, and compounds 6 and 7 coexisted in the ratio of 2:1. The detailed synthesis and isolation procedure was described in a previous paper (Yang and Deinzer, 1992).

Sample Preconcentration and Cleanup. Three liters of a beer sample was added to a continuous extraction apparatus containing 700 mL of CH$_2$Cl$_2$ and extracted for 24 h. The organic phase was then washed twice with 100 mL of 5% NaHCO$_3$ and dried over 30 g of Na$_2$SO$_4$. The solvent was evaporated in a Kuderna-Danish apparatus. The concentrate (0.5–1 mL) was added to a column (10 mm i.d.) packed with 3 g of alumina containing 3% water, 80–200 mesh (Fisher Scientific Co., Fair Lawn, NJ); 10 mL of CH$_2$Cl$_2$ containing 0.5 mL of ethanol was used as elution solvent. The eluate was then concentrated to 100–300 μL under a gentle N$_2$ stream.

GC/MS Analysis. GC/MS analyses were carried out on a Finnigan Model 4023 quadrupole mass spectrometer using a 30 m, 0.32 mm i.d. Carbowax fused silica capillary column (Alltech Associates, Inc., Deerfield, IL). Helium was used as the carrier gas at a flow rate of 25 cm/s. The beer extracts were injected with a split ratio of 1:50. The oven temperature was programmed as follows: 120 °C held for 2 min and then increased to 250 °C at a rate of 2 °C/min. The injector temperature was set at 220 °C and the transfer line at 275 °C. The ion source temperature was 140 °C; the electron energy was 70 eV. Compound identification was based on comparison of GC retention times and mass spectra using external standards.

[†] Department of Agricultural Chemistry.
[‡] Department of Food Science and Technology.

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Recovery Study. Ten microliters of a standard solution containing 4–80 µg/µL hydrolysis products was spiked into 3 L of unhopped pilot beer, followed by the same procedure as that used for the analysis of beer samples.

Pilot Brews. Hop essential oil was obtained by steam distillation from Hallertauer Hallertauer hop pellets (1987). The hop oil was further fractionated into hydrocarbons and oxygenated compounds with a 2.5 × 2.5 cm silica gel column (silica gel 60, 32–63 µm, 14% water) and eluted with pentane and diethyl ether. Three 5-gal batch pilot beers were brewed, one with 80 g of hop pellets, another with 240 µL of the hop oil, and the third with 150 µL of the oxygenated fraction of hop oil. An unhopped brew also was made as control. After hop pellets or hop oil fractions were added to the brew kettle, the wort was boiled for additional 10 min. The commercial beers used in this study were arbitrarily chosen.

Descriptive Sensory Panel. *Sample Preparation.* All of the hydrolysis products of humulene epoxides II and III were evaluated individually except compounds **2** and **16**, which are minor constituents of the hydrolysis products. The amounts of isolated **2** and **16** were not sufficient for sensory evaluation. Humulene epoxide II always exists in hops in larger amount than epoxide III (II:III = ca. 10:1). Product mixtures of humulene epoxide II were evaluated in the sensory panel. Mixture A contained the less polar fraction and mixture B the polar fraction of the hydrolysis products of humulene epoxide II. After the extraction solvent was removed by a nitrogen stream, all of the compounds or mixtures were dissolved separately in ethanol at a concentration of 50 or 100 mg/mL (stock solution).

Compounds Spiked in Beer. A bottle of beer (200 mL at 20 °C) was spiked with a stock solution. The cap was screwed on, and the bottle was inverted gently several times. The bottles were stored under refrigeration (10 °C) for a minimum of 12 h before evaluation. Individual samples of beer spiked with 20 ppm of each compound or compound mixture were evaluated. Compound mixture A was evaluated at the 40 ppm level. These spiking levels were chosen to be sure that every panelist could detect all of the compounds in beer.

Compounds Spiked in Water. A stock solution (50 mg/mL) of each compound was prepared, and 28 µL was dissoved in 6 mL of 95% ethanol. This solution (600 µL) was delivered into 355-mL amber-colored glasses and then diluted to 28 mL with spring water. All of the compounds spiked in water were evaluated at 5 ppm.

Sample Presentation. The beer bottles were gently inverted several times before uncapping. The spiked beer sample size was 20 mL, and the spiked water sample size was 28 mL. The samples were served in 355-mL amber-colored glasses, capped with aluminum lids and parafilm. Each sample had a random 3-digit number. The serving temperature was 20 °C, and the sample evaluation took place around a training table under white lights.

Trained Panel. The panel consisted of eight Oregon State University staff and graduate students. The goal of the sensory panel was to provide a general profile of the hydrolysis products of humulene epoxides II and III. The samples were not tested in a formal testing situation in individual booths with replications. The final aroma profile of the compounds was based on data collected during descriptive panel sessions. During the early sessions, panelists were encouraged to generate descriptors by comparing the aroma of the sample to the control and by describing the aroma that was different. Aroma reference standards were provided to help panelists identify what they were smelling (Table I). Panelists used a 16-point intensity scale for rating aroma intensities (0 = none, 1 = just detectable, 3 = slight, 7 = moderate, 11 = large, 15 = extreme).

Sample Evaluation. For training and discussion purposes, panelists were given from four to eight samples at a time: half of these samples were compounds spiked in water, and half were compounds spiked in beer. Samples were served in random order to each panelist, but the water-based samples were kept together as were the beer-based samples. In addition, the panelists were instructed to smell a control (20 mL of water and with 2% ethanol) or a control beer before evaluating their respective samples. The control was not spiked. After evaluation of the aroma reference standards, panelists were instructed to rate those descriptors in

Table I. Aroma Reference Preparation

attribute	reference preparation[a]
pineapple	primary aroma of a reference prepared by 3–4 chunks of fresh, frozen pineapple
lemon	primary aroma of a reference prepared by 1/8 of a Sunkist lemon
lime	primary aroma of a reference prepared by 1/8 of a lime
lime (oil)	primary aroma of a reference prepared by 1 drop on an aroma stick of lime standard (source: Avalon Farms, Corvallis, OR)
cedar	primary aroma of a reference prepared by drop on an aroma stick of cedar standard (source: Avalon Farms)
spicy	primary aroma of a reference prepared by 1 drop on aroma stick of eugenol (Aldrich Chemical Co., Inc., Milwaukee, WI) or 5 mL of cloves (Schilling, McCormick Co., Inc., Baltimore, MD)
floral	primary aroma of a reference prepared by 1 drop on aroma stick of 2-phenylethanol (Aldrich)
herbal	primary aroma of a reference prepared by 1 g of rosemary (Spice Island, Specialty Brands Inc., San Francisco, CA), 0.60 g of basil (The R. T. French Co., Rochester, NY), and 0.15 g of ground thyme (Crescent Mfg. Co., Seattle, WA)
rubber	primary aroma of a reference prepared by a rubber stopper in a glass
banana	primary aroma of a reference prepared by 3–4 slices of a ripe banana

[a] All references were served in 350-mL amber glasses covered with an aluminum foil lid.

the sample that were different from the control. The exception to this instruction was rating of overall intensity: panelists were instructed to rate the overall aroma of the sample, which included the malty and ethanol aromas from the beer.

Threshold Testing. To examine the total aroma contribution of the hydrolysis products to beer, the sensory threshold of the mixture was tested. The Patton and Josephson method (Patton and Josephson, 1957) was used to estimate the threshold value of the hydrolysis mixture of humulene epoxide II. A series of samples was prepared containing the hydrolysis mixture in a concentration of 1.88, 3.75, 7.5, 15.0, and 30 ppm. Five 20-mL samples were randomly presented to each panelist. The sample temperature was 20 °C. Twenty panelists evaluated each tray of five samples twice. Serving order was randomized among panelists. The panelists were asked to indicate if the compound was present or not present in each of the five samples. In addition to the samples, panelists were provided with a control sample which contained 20 mL of the medium beer. Panelists used the control for comparison to help detect different aromas. They were also provided with a reference of the compound mixture on an aroma stick. To determine threshold estimates, average threshold values were plotted against percent positive response, with the 50% level of positive response being selected as the average threshold for the mixture.

RESULTS AND DISCUSSION

Hydrolysis Reactions of Humulene Epoxides. Humulene epoxides II and III produce a large number of compounds when refluxed for 3 h in aqueous solution buffered at pH 4. Twelve major products (>1% of GC peak area) were isolated from the reaction mixture of humulene epoxide II and 16 from that of humulene epoxide III comprising 83% and 93%, respectively, of the GC peak area. Humulene epoxide I is much more resistant to hydrolysis since after 3 hours of refluxing, 80% of the starting material remained (Yang and Deinzer, 1992). It was observed also from the analytical results that the concentration of humulene epoxide I is generally lower than that of humulene epoxide II in hops but higher in finished beer [e.g., Tressl et al. (1978)]. The hydrolysis products can be easily divided into two groups by extraction of the mixture using pentane and subsequently

Table II. Humulene Epoxide II and III Hydrolysates

compd	A^a	B^a	IUPAC name
1	1.0	0.8	1,5,8,8-tetramethyl-12-oxa-5-tricyclo[7.2.1.0^{6,9}]dodecene
2	0.9	1.1	2,6,6,9-tetramethyl-4,8-cycloundecadien-1-one (humuladienone)
3	4.4	6.7	1,5,5,8-tetramethyl-12-oxabicyclo[9.1.0]dodeca-3,7-diene (humulene epoxide II)
4	1.8	16.2	3,7,10,10-tetramethyl-12-oxabicyclo[9.1.0]dodeca-3,7-diene (humulene epoxide III)
5	2.4	1.2	4,8,11,11-tetramethyl-8-tricyclo[7.2.0.0^{2,5}]undecen-4-ol
6		3.2	2,6,6,9-tetramethyl-8-tricyclo[6.3.0.0^{2,4}]undecen-5-ol
7		1.3	2,6,6,9-tetramethyl-9-tricyclo[6.3.0.0^{2,4}]undecen-5-ol
8	8.6	6.09	6,6,9-trimethyl-2-methylene-4,8-cycloundecadien-1-ol (humulenol II)
9	1.5	1.6	2,6,6,9-tetramethyl-2,4,8-cycloundecatrien-1-ol
10		2.1	2,6,6,9-tetramethyltricyclo[6.3.0.0^{2,4}]undecane-5,9-diol
11	14	13.5	4,8,11,11-tetramethyltricyclo[7.2.0.0^{2,4}]undecane-5,8-diol
12	19	14.3	(1RS,2RS)-2,6,6,9-tetramethyl-4,8-cycloundecadiene-1,2-diol
13	6.1	3.5	(1RS,2RS)-2,6,6,9-tetramethyl-4,8-cycloundecadiene-1,2-diol
14		2.3	2,6,6,9-tetramethyltricyclo[6.3.0.0^{2,4}]undecene-5,9-diol
15	2.5	7.3	1,5,5,8-tetramethyl-8-bicyclo[8.1.0]undecene-2,9-diol
16, 17	21	11.5	4,8,11,11-tetramethyltricyclo[6.3.0.0^{2,4}]undecane-5,9-diol

^{a} A, B, GC peak area percentage for hydrolysates of humulene epoxides II and III, respectively.

Scheme I
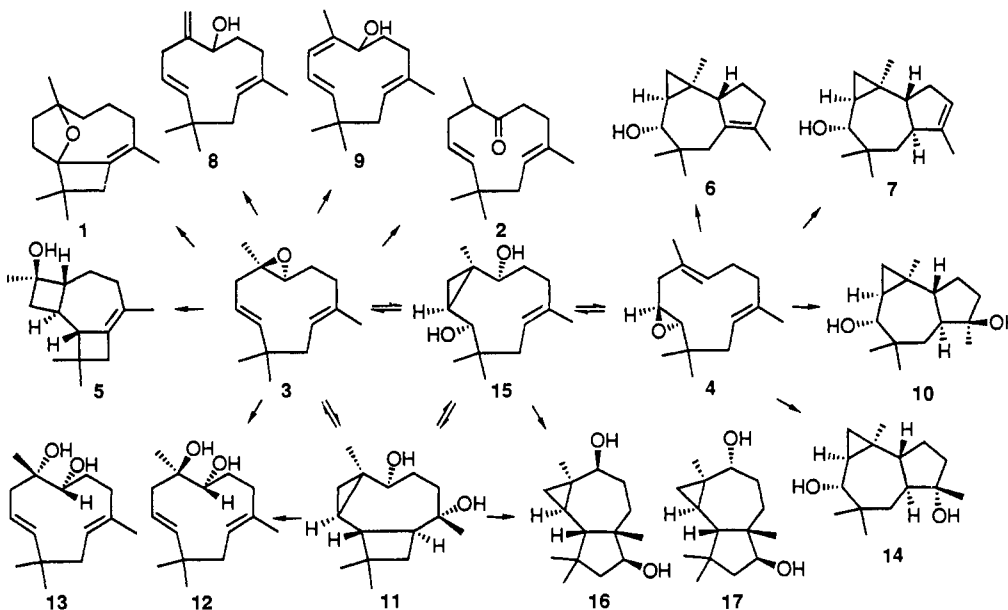
dichloromethane. The pentane group contained compounds 1–9, which are isomerization products with the formula $C_{15}H_{24}O$. It also contained compound 12. The dichloromethane group included compounds 10–17 with the formula $C_{15}H_{26}O_2$, which result from addition of a water molecule to the epoxide. Reversible interconversion among the terpenoids was discovered in previous studies (Yang and Deinzer, 1992). Because of the reversible isomerization between humulene epoxides II and III, all of the compounds formed from hydrolysis of humulene epoxide II also are present in the product mixture of humulene epoxide III, but the product ratios are not the same (Table II). For example, compounds 6, 7, 10, and 14 were found in the reaction products of humulene epoxide III in 1–4% (GC/FID) but were just detectable in the product mixture of humulene epoxide II. The reaction pathways are summarized in Scheme I.

Analytical Procedure. The reaction products of humulene epoxides are less volatile or sensitive to heat in aqueous solution. Therefore, continuous liquid–liquid extraction with dichloromethane was found to be suitable for isolation of these compounds from beer. The dichloromethane extract contains a large amount of nonvolatile material and was removed prior to concentration and GC analysis. Acidic compounds were washed with an aqueous solution of 5% $NaHCO_3$. Aluminum oxide proved to be

a more effective adsorbent for sample cleanup than silica gel and Florosil. Isomerization of humulene epoxides occurs on the surface of active aluminum oxide, unless the activity of the aluminum oxide is weaker than grade II (Damodaran and Dev, 1968). Therefore, the aluminum oxide used for cleanup was carefully controlled to maintain a grade II activity. A preconcentration factor of 10 000–30 000 with recovery rates of 80–130% was reached by following the analytical procedure.

Reaction Products in Beer. All of the identified hydrolysis compounds of humulene epoxides II and III were found in the pilot beers (Table III), except compounds 6, 7, 10, and 14 which are produced only by hydrolysis of humulene epoxide III. Interestingly, compound 11 had the highest concentration in all beer samples. The study of the reaction mechanism indicates that compound 11 is an important intermediate in the hydrolysis process. This compound produced the same products with nearly the same ratio as humulene epoxide II under hydrolytic conditions. The presence of large numbers of hydrolysis products of humulene epoxides in beer demonstrates that hydrolysis and isomerization are important reactions in the brewing process. The changing hop aroma in beer is probably associated with reactions of hop oil components.

Reaction Products in Hops. Compounds 8 and 9, together with humulene epoxides I–III, are identified in

Table III. Reaction Products (Micrograms per Liter) of Humulene Epoxides II and III in Pilot and Commercial Beer Samples

compd[a]	pellets[b]	hop oil[c]	oxy fr[d]	Coors[e]	Sierra[f]	Weinhard's[g]
1		8.3	9.1			
2	36	36	43			
3	270	5.3	1.9	17		
4	38					
5	18	8.3	7.5			
8	460	500	550	24	114	12
9	9.7	30	37			
11	2400	2600	2800	600	754	430
12		83	79	220		
13		45	36	27		
15	150	244	200	31	37	64
17	340	139	140	90	100	125
total, mg/L	3.7	3.8	3.9	1.0	1.0	0.63

[a] See Scheme I. [b] Pilot brew with Hallertauer Hallertauer hop pellets. [c] Pilot brew with hop oil extracted from Hallertauer Hallertauer hop pellets. [d] Pilot brew with hop oil oxygenated fraction from Hallertauer Hallertauer hop pellets. [e] Corrs pilot brew with 100% Hersbrucker hop. [f] Sierra Nevada pale ale. [g] Blitz Weinhard's premium light ale.

Table IV. Concentrations (Milligrams per Milliliter) of Humulene Monoepoxides I–III and Reaction Products 8 and 9 in Hop Essential Oils

sample ID	I	II	III	8	9
Hallertauer Pellets, 1987[a]	18	104	14	25	1.3
Hallertau, 1988	15	78	12	29	1.7
Hallertauer Tradition, 1991	0.88	5.3	0.41	0.68	0.036
Hallertauer Select, 1991	0.64	4.0	0.34	0.51	nd[b]
Saazer, 1985	5.5	24	4.2	10	0.49
Saazer, 1991	0.73	4.6	0.32	0.69	0.032
Washington Mt. Hood, 1991	7.4	33	4.2	13	0.98
Idaho Mt. Hood, 1991	6.1	37	3.7	8.1	0.42
Cascade Pellets, 1991	0.67	3.3	0.42	0.86	0.052
Cluster, 1991	1.0	4.2	0.96	46	3.3
UDA 21490, 1991	2.0	16	2.1	0.97	nd
New Zealand, 1991	1.5	8.9	1.2	1.7	0.13
India, 1991	2.4	7.4	1.7	4.3	0.19
Ringwood (Argentina), 1991	0.86	3.67	0.42	0.77	nd

[a] The hop pellets were used to make the pilot brews in this study. By the time of brewing, the oxygenated fraction of hop hoil in the pellets was increased to 63% from 30% at the time of analysis. [b] nd, not detected.

the hop essential oils analyzed (Table III). All other hydrolysis products of humulene epoxides were not detected in the hop oil samples. Humulenol II (8) occurs naturally with humulene (Damodaran and Dev, 1968) and is also found in hop oils (Table IV). Although compound 9 has been reported to be a lead tetraacetate oxidation product of humulene (Iwamuro et al., 1981), it has not been reported to be a hop component before. Humuladienone (2) and tricyclohumuladiol (11) have been reported as constituents of a Japanese hop (Naya and Kotake, 1969), but they have not been detected in any of the hop oils analyzed here.

Aroma Profile of the Hydrolysis Products. Humulene epoxide II has been suggested to contribute a hoppy aroma in beer (Tressl et al., 1978; Peacock and Deinzer, 1981), but the sensory profile of the epoxide has not been reported. Fukuoka and Kowaka (1985) evaluated the flavor of humulene epoxides I (hay-like) and II (moldy), humulenol II (weak sagebrush-like), humulol (hay-like), and caryophyllene oxide (cosmetic and methanol-like). They concluded that none of these compounds was the key component for the herbal flavor in beer. Together with the hydrolysis products of humulene epoxides, the general aroma profile of humulene epoxides I–III was examined in this study. Panelists agreed upon the following nine descriptors: cedar, spicy, herbal, floral, lime,
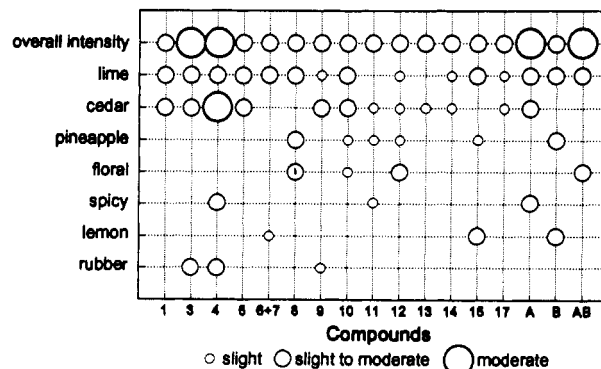
Figure 1. Sensory profile of hydrolysis products of humulene epoxides II and III in water (5 ppm).
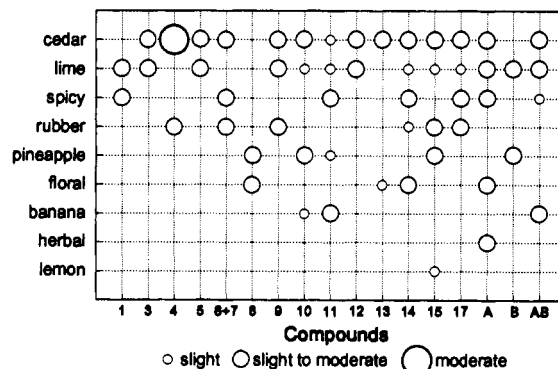
Figure 2. Sensory profile of hydrolysis products of humulene epoxides II and III in beer (20 ppm).

lemon, banana, pineapple, and pine. In addition, rubber, sweet, musty, and camphor–medicinal were added after the initial ballot was developed. The criterion for determining if a descriptor is meaningful is that the descriptor be used by at least three panelists.

Compounds in Water. The aroma of the compounds in water could be described as cedar, spicy, floral, lime, lemon, banana, pineapple, and rubber (Figure 1). Cedar, lime, and pineapple were frequently used descriptors in both water and beer. The camphor–medicinal descriptor was not suggested by the panelists for the compounds spiked in water. Compounds 1, 5, 9, and 17 had the same two descriptors, cedar and lime; compound 9 was also described by a rubber note. Compound 4 and mixture A shared the descriptors cedar, spicy, and lime; in addition 4 had a rubber note. Mixtures A and B only shared lime as a common descriptor: B could be described as having fruity character with lime, lemon, and pineapple notes, whereas A had lime notes but also distinctly different aroma notes of cedar and spicy. The aroma of humulene epoxide I in water could be described as cedar, lime, herbal, and honey–sweet.

Compounds in Beer. Overall, compounds in beer could be described as having cedar, spicy, herbal, floral, lime, lemon, banana, pineapple, and rubber aroma notes (Figure 2). Predominant descriptors were cedar, spicy, lime, pineapple, and rubber, followed by floral, banana, and, to a lesser extent, herbal and lemon notes. Rubber was used more often to describe compounds in beer than in water. All compounds, with the exception of 1 and mixture B, had a cedar aroma character ranging in intensity from 3 (slight) to 6.8 (moderate). Thirteen compounds were described as having lime notes with ratings from 2.6 (slight) to 4 (slight to moderate). Rubber, spicy, and pineapple were the next most used descriptors. Compounds 3 and 5, and 12 had similar profiles (cedar and lime). Compound

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1 was unique in having a camphor–medicinal note in addition to spicy and lime notes. Cedar, lime, banana, and pineapple were all used to describe compounds 10 and 11. Compound 11 also had a slight-to-moderate spicy aroma. Several descriptors were used for compound 14: cedar, spicy, floral, lime, and rubber. Lime was the only common descriptor shared by mixtures A and B: cedar, spicy, herbal, floral, and lime were used to describe A, whereas B descriptors were lime, pineapple, and rubber. The aroma profile for the mixture AB included cedar, spicy, lime, and banana notes. The aroma of humulene epoxide I in beer could be described as cedar, pineapple, lime, and spicy (data not shown).

The major aroma characteristics of the hydrolysis products of humulene epoxides II and III can be summarized as citrus, tropical fruity, woody, spicy, and floral, which provides the beer with a highly valued hop aroma. The estimated threshold value for the hydrolysis mixture of humulene epoxide II in beer was 2.3 ppm. The concentration ratios of the hydrolysis compounds depend on the reaction conditions. Therefore, the threshold value of the hydrolysis mixture is suitable only for estimating the extent of the aroma contribution of the hydrolysis products to beer flavor. The total concentration of the identified hydrolysis products of humulene epoxide II in the pilot brews exceeded the threshold value (Table III). These compounds, therefore, must contribute to the aroma of the beer. The concentration of the identified compounds in the commercial beer samples was close to the threshold value (Table III). The hydrolysis products of humulene epoxide II also were important aroma contributors, together with a large number of hydrolysis products of other oxygenated compounds in hop oil, such as caryophyllene oxide, which we have been studying, and the results will be reported later.

To exceed the threshold value of 2.3 ppm, the lowest concentration of humulene, which survives the brewing process and converts to the hydrolysis products, is 230 mg/100 g of hops, assuming a hopping rate of 100 g/L. Generally, only aroma hops provide a high concentration of humulene.

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Supplementary Material Available: Mass spectra of reaction products of humulene epoxides II and III (8 pages). Ordering information is given on any masthead page.

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