Hydrolysis Products of Caryophyllene Oxide in Hops and Beer

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The hydrolysis products of caryophyllene oxide were analyzed in beer samples involving continuous liquid–liquid partitioning, cleanup, and GC/MS analysis. Clovanediol (**7**) was found in the samples in concentrations ranging from 50 to 700 ppb. A variety of hop oils obtained by steam distillation also were analyzed by GC/MS. Compounds **1**–**5** were identified in the hop oils. Sensory profiles of seven individual compounds and their mixtures were evaluated by a descriptive sensory panel. Overall, these compounds could be described as contributing to cedar, lime, floral, and spicy aroma notes to beer. The estimated threshold value for the hydrolysis mixture of caryophyllene oxide is 3.4 ppm in beer.

INTRODUCTION

Hop essential oils are important sources of flavoring components and hop aroma in beer. Terpenes are the major components in fresh hop oils, and they are easily oxidized upon storage (Lam et al., 1986; Tressel et al., 1978). The fate of these oxygenated compounds in the brewing process is of great interest in hop flavor chemistry. One of the important sesquiterpenes in aroma hops is α-humulene, which is oxidized to humulene epoxides upon storage. Under hydrolytic conditions, humulene epoxides undergo hydrolysis and isomerization reactions and produce a large number of compounds (Yang and Deinzer, 1992). The reaction products also have been found in beer samples and may contribute significantly to the flavor of these brews (Yang et al., 1993).

In addition to α-humulene, β-caryophyllene is an important sesquiterpene found in hop essential oils, and its major oxidation product is caryophyllene oxide. In weakly acidic aqueous solution under reflux, caryophyllene oxide forms two major hydrolysis products—clovane diol (**7**) and a vicinal diol (**6**). Four other isomerization products, including a ketone (**1**), an aldehyde (**3**), and two unsaturated cyclic alcohols (**4**, **5**), also were isolated and identified in the reaction mixture (Yang and Deinzer, 1993).

To determine whether the hydrolysis reactions of caryophyllene oxide take place during brewing, commercial and pilot beer samples were analyzed by previously described analytical methods (Yang et al., 1993). A variety of hop essential oils were analyzed to identify the reaction products of caryophyllene.

Many derivatives of caryophyllene oxide are flavoring compounds that have also been used for production of cosmetic and industrial goods [e.g., Brunke and Rojahn (1988)]. The isomerization products of caryophyllene oxide have been used to introduce a woody odor in perfumes (Mussinan et al., 1980). It was, therefore, of interest to determine the contributions, if any, to beer aroma of the hydrolysis products of caryophyllene oxide. Individual compounds were isolated, and their mixtures were evaluated by a descriptive sensory panel.

EXPERIMENTAL PROCEDURES

Materials. Caryophyllene oxide (95%, Aldrich, Deerfield, IL) was refluxed for 3 h in aqueous solution containing 3% ethanol buffered at pH 4. After extraction with pentane and dichloromethane, compounds **1**–**7** (Table I) were isolated and purified by flash chromatography and HPLC as described previously (Yang and Deinzer, 1993). The purity of the compounds was 95–100% (GC/FID). These compounds were used as analytical standards and for sensory evaluations.

To prepare fractions with different polarities, the reaction product mixture was extracted with pentane, which gave the less polar mixture A, containing compounds **1**–**6**. The remaining aqueous phase was extracted further with dichloromethane, which gave mixture B, containing mainly compound **7** and small amounts of **6**. Mixture AB contains all of the hydrolysis products and was prepared by extracting the hydrolysis mixture with dichloromethane.

Analytical Procedure. Beer samples were extracted for 24 h with dichloromethane. The organic phase was washed with an aqueous solution of 5% sodium bicarbonate. After the solvent was removed, the concentrate was cleaned up with aluminum oxide with activity grade II to remove nonvolatile material and then analyzed by GC/MS. Hop oils were obtained by steam distillation and directly injected into the GC column for analysis. An external standard mixture was used.

Sensory Evaluation. Seven compounds (**1**–**7**) isolated from the hydrolysis mixture of caryophyllene oxide were evaluated individually. Mixtures A, B, and AB also were evaluated under the same conditions as the individual compounds. The compounds or mixtures were dissolved in ethanol at a concentration of 50 mg/mL (stock solution) and then added to beer or water for evaluation. In a preliminary session the concentration level was determined, so that every panelist could comfortably detect and evaluate the aroma compounds in the samples; 20 ppm was chosen for the beer samples and 5 ppm for water samples. The goal of the sensory evaluation was to provide general descriptors of the hydrolysis products of caryophyllene oxide. The final aroma profile of the compounds or mixtures was based on data collected during descriptive panel sessions.

The sensory threshold of mixture AB was tested to examine the total aroma contribution of the hydrolysis products of caryophyllene oxide to beer. The Patton–Josephson method (Patton and Josephson, 1975) was used to estimate the threshold value.

For more details about the pilot brew, analytical procedure, sensory evaluation, and threshold testing method, the reader is referred to a previous publication (Yang et al., 1993).

RESULTS AND DISCUSSION

Reactions of Caryophyllene Oxide under Hydrolytic Conditions. The epoxide ring is the most reactive functional group in caryophyllene oxide. In acidic solution, ring opening of the protonated epoxide undergoes either S_N1 or S_N2 reaction, which largely determines the direction

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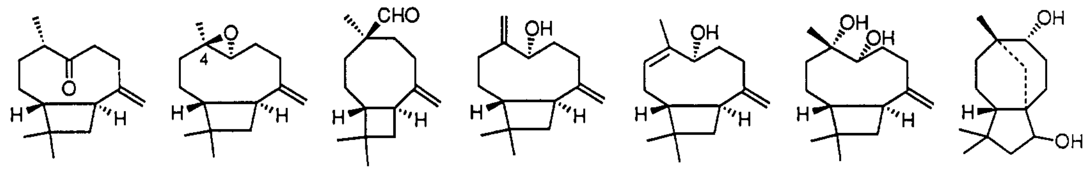
Table I. Reaction Products of Caryophyllene Oxide

compd	I_k[a]	area %[b]	IUPAC name
1	1941	3.4	4,11,11-trimethyl-8-methylenebicyclo[7.2.0]undecan-5-one
2	1987	10	4,12,12-trimethyl-9-methylene-5-oxatricyclo[8.2.0.$0^{4,6}$]dodecane (caryophyllene oxide)
3	2004	2.4	4,10,10-trimethyl-7-methylenebicyclo[6.2.0]decane-4-carbaldehyde
4	2292	4.3	11,11-dimethyl-4,8-dimethylenebicyclo[7.2.0]undecan-5-ol
5	2331	16	4,11,11-trimethyl-8-methylene-3-bicyclo[7.2.0]undecen-5-ol (caryophyllenol I)
6	2706	3.3	4,11,11-trimethyl-8-methylenebicyclo[7.2.0]undecane-4,5-diol
7	2942	39	4,4,8-trimethyltricyclo[6.3.1.$0^{1,5}$]dodecane-2,9-diol (clovane diol)

[a] The retention indices were determined under the following GC conditions: Carbowax, 30 m, 0.32 mm i.d., 0.25-μm film; 120 °C (2 min) to 250 °C at 2 °C/min. [b] Peak area percentage (GC/FID).

Chart I. Chemical Structures of Caryophyllene Oxide and Its Hydrolysis Products



1 2 3 4 5 6 7

Table II. Reaction Products 1–5 of Caryophyllene Oxide in Hop Essential Oils (Milligrams per Milliliter)

sample ID	1	2	3	4	5
Hallertauer Pellets, 1987	0.70	24		4.1	4.7
Hallertauer Tradition, 1991		1.2			
Spalter Select, 1991		1.6			
Hallertauer, 1991		1.6			
Saazer, 1985		4.7		1.7	1.5
Saazer, 1991		1.0		0.13	
Washington Mt. Hood, 1991		12	2.1	2.5	3.8
Idaho Mt. Hood, 1991	0.36	15	0.28	1.9	2.8
Cascade Pellets, 1991		1.0		0.17	0.21
77–01(6) (New Zealand), 1991		2.6			0.42
RRL(H)84 (India), 1991	0.36	2.4		0.89	0.89
Pride of Ringwood (Argentina), 1991	0.27	9.9			1.8

of ring opening: whether the C–O bond breaking takes place at the less sterically hindered carbon atom or at the position in which a positive charge is more easily stabilized (Pocker et al., 1988; Parker and Isaacs, 1959). From product structures, as shown in Chart I, it can be concluded that all of the major reactions involve the epoxide ring. The reactions can be classified into hydrolysis reactions and isomerization. The major reaction product was clovanediol (**7**), with an overall yield of 59% of the isolated compounds by weight. Clovanediol was produced by nucleophilic attack of the exocyclic double bond on the epoxide ring, followed by a cyclobutyl carbinyl ring expansion and an addition of a water molecule. Compound **6**, totaling 14% of the isolated compounds, could be formed through an S_N2 displacement reaction by water on the more substituted C-4 position of the epoxide ring. Although the hydrolysis reactions of caryophyllene oxide formed the major products, isomerization cannot be ruled out. Compounds **4** and **5** are apparently elimination products produced after the formation of a carbocation with a positive charge at C-4. This carbocation was formed directly from epoxide ring opening rather than from an addition–elimination mechanism. The formation of compounds **1** and **3** resulted from rearrangement reactions of caryophyllene oxide. The above-mentioned carbocation also could be the intermediate in the rearrangement (Yang and Deinzer, 1993).

Reaction Products in Hop Oil. All of the rearrangement products **1** and **3–5** were found in hop oils (Table II). Clovanediol (**7**) was found in peppermint (*Mentha piperita*) oil (Treibs and Lossner, 1960) and in the oleoresin of *Dipterocarpus pilosus* (Gupta and Dev, 1971), but it was not detected in the hop oils analyzed here. Caryophyllenol I (**5**) was also identified in clove oil (Uchida et al., 1986). Compounds **1**, **3**, and **4** were found in the reaction product mixtures of caryophyllene oxide, but they have not been reported as hop oil components.

Table III. Clovanediol in Beer

beer brewed with Hallertauer hop pellets	677 μg/L
Blitz-Weinhard's premium light ale	51.4 μg/L
Coors pilot brew with Hersbrucker Late hops	124 μg/L

Reaction Products in Beer. Among the six reaction products, only clovanediol (**7**) was identified in the beer samples analyzed (Table III). Although found in fairly high concentration, the presence of clovanediol in beer has not been reported before. Tressl et al. (1978) claimed to have found caryophyllenol in beer (5 ppb) at I_k = 2033. When the data obtained from the pure compound **5** are compared, the listed mass numbers are not in agreement with the mass spectrum of **5** and the GC retention index also does not match the I_k (2332) of **5**.

Clovanediol (**7**) in beer is unlikely to come from any brewing ingredients other than hops. This also was indicated by the analytical results of unhopped beer samples. Caryophyllene oxide was present in relatively high concentrations in the Hallertauer hop pellets, but it was not detected in the beer samples. Therefore, caryophyllene oxide must undergo hydrolysis and produce clovanediol during the brewing process. This was found as the major hydrolysis product of caryophyllene oxide in the model study (Yang and Deinzer, 1993). Experimental results showed that in addition to compound **7**, caryophyllene oxide produced compounds **1–6** in small amounts even at ambient temperatures in aqueous solution at pH 4. No chemical changes for compounds **1** and **3–6** were observed under hydrolytic conditions. These compounds are very likely present in beer at a few parts per billion or sub parts per billion concentration levels. This is below the detection limit of the analytical method applied. The concentration of clovanediol in beer is dependent on the hop variety, hopping rate, brewing conditions, and time for aging.

Aroma Profile of the Reaction Products. *Compounds in Water.* The overall intensity ratings for all of the compounds ranged from 5 to 8. Cedar and lime were the predominantly used descriptors followed by floral, herbal, and spicy (Figure 1). The remaining descriptors (rubber, lemon, pine, and pineapple) were only used once or twice for all 10 samples studied. Compounds 1 and 2

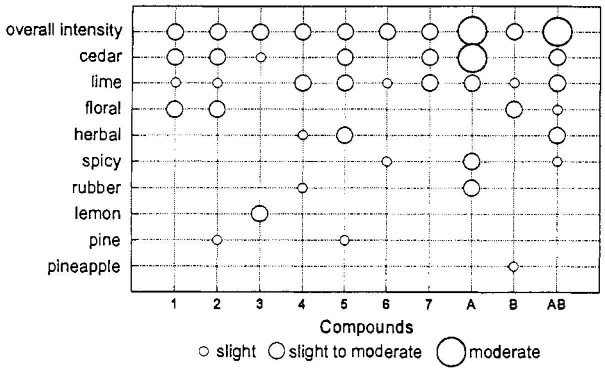


Figure 1. Sensory profile of hydrolysis products of caryophyllene oxide in water (5 ppm).

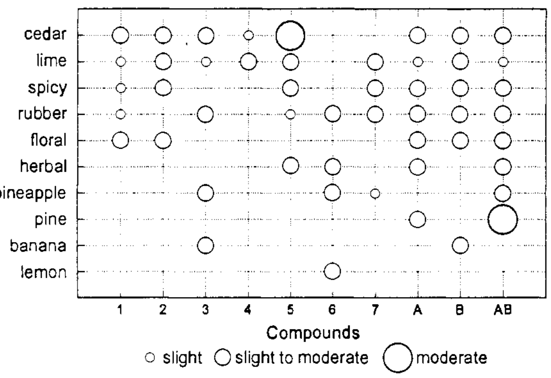


Figure 2. Sensory profile of hydrolysis products of caryophyllene oxide in beer (20 ppm).

were both characterized as having slight-to-moderate cedar and floral aromas and slight lime aroma (Figure 1). In addition, compound **2** was described as having a slightly floral note. Compound **3** had slight-to-moderate lemon and slight cedar notes. Compound **4** had slight-to-moderate lime aroma and slight herbal and rubbery notes. Compounds **5** and **7** had slight-to-moderate cedar and lime notes; in addition, compound **5** had herbal and pine character. Compound **6** had only slight lime and spicy notes. Mixture A was distinct from mixture B on the basis of a moderate cedar and slight-to-moderate lime aroma: a slight lime aroma was described as having slight-to-moderate floral and pineapple notes. Mixture AB was described as having slight-to-moderate cedar, lime, and herbal notes as well as slight floral and spicy notes.

Compounds in Beer. The descriptors most often used to describe the aroma of the compounds and their mixtures in beer were cedar, lime, spicy, rubber, and floral followed by herbal, pineapple, pine, banana, and lemon (Figure 2). Compounds 1–5 and mixtures A, B, and AB had lime and cedar notes ranging from slight to slight-to-moderate for compounds 1 and 2 and mixtures A, B, and AB. In addition, compounds **5** and **7** were rated as spicy. Eight of the 10 samples (excluding compounds 2 and 4) had rubber notes ranging from slight to slight-to-moderate in intensity. Herbal and pineapple were used for 4 of the 10 compounds, whereas pine and banana were used for only 2 compounds. Compound **6** was the only one to have slight-to-moderate lemon aroma. Compounds 1 and 2 (except for rubber) and mixtures A and B had a common set of descriptors: cedar, lime, spicy, rubber, and floral; their intensities ranged from slight to slight-to-moderate (Figure 2). Mixture A was described as having slight-to-moderate herbal and pine notes, whereas mixture B had a banana aroma. The profile of mixture AB in beer was similar to the profile of mixture AB in water: Mixture A had more "spicy-herbal" character, while mixture B had more "tropical-fruit" character. Compound **3** had slight-to-moderate cedar, rubber, pineapple, and banana notes with a slight lime aroma. Compound **4** was described as having slight cedar and slight-to-moderate lime notes. Mixture B was heavier in the "spicy-herbal" and slight rubber, herbal, pineapple, and lemon notes. Compound **7** was described as having slight-to-moderate lime, spicy, and rubber aromas and also a slight pineapple character. Compounds **6** and **7** were the only compounds without any cedar aroma. Mixture AB was the most complex one, having moderate pine intensity, slight-to-moderate cedar, spicy, rubber, floral, herbal, and pineapple notes, and also a slight lime aroma.

The estimated threshold value for the hydrolysis product mixture of caryophyllene oxide was 3.4 ppm in beer. 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 caryophyllene oxide in the beer samples analyzed here may not exceed the threshold value. These compounds, however, together with a large number of hydrolysis products of other oxygenated compounds in hop oil, such as humulene epoxides, could contribute to the aroma of the beer.

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Supplementary Material Available: Mass spectra of compounds 1–7 (4 pages). Ordering information is given on any current masthead page.

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