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Hop Aroma in American Beer

Val E. Peacock, Max L. Deinzer,* Lois A. McGill, and Ronald E. Wrolstad

Two commercial American beers and two pilot-brew beers brewed with different hop varieties were analyzed by gas chromatography–mass spectrometry for hop-derived aroma compounds. The pilot brew made from Hallertauer mittelfrueh hops had significantly higher concentrations of α-terpineol, humulene epoxide I, humulol, *T*-cadinol, α-eudesmol, humulenol II, and 4,4-dimethylcrotonolactone than those of the pilot brew made from Washington Cluster hops. The commercial American beers analyzed were brewed with Oregon Cascade hops in one case and a mixture of Hallertauer (primarily), Tettnanger, and Styrian in the other. Caryolan-1-ol, nerolidol, humulene epoxide I, δ-cadinol, and α-eudesmol were present in the beer brewed with imported hop varieties, but they were absent in the beer brewed with the domestic hop. Sensory panel studies indicate that humulenol II may be a contributor to a fine hoppy aroma.

Certain European hop varieties command a higher price in the market place because of the "kettle-hop flavor" they impart to the finished beer. It has been well documented that the chemical makeup of the essential oils isolated from hops varies with the variety of the hop (Naya and Kotake, 1972; Likens and Nickerson, 1967; Buttery and Ling, 1967). About 90% of the mass of these essential oils is made up of terpene and sesquiterpene hydrocarbons, and the relative concentration of these hydrocarbons is the principal difference among hop oils from different cultivars. However, most, if not all, of these hydrocarbons are hydrated, polymerized, or steam distilled out of the wort during wort boiling (Shimazi et al., 1974; Tressl et al., 1979; Howard, 1965; Maule, 1967). The "kettle-hop flavor" may be caused not by the hydrocarbons but by the more water-soluble, oxygenated fraction of the hop oil. "Kettle hop" aroma/flavor is the nonbitter aromatic aroma/flavor note contributed by late additions of aroma hops to the brew kettle. Numerous compounds found in the oxygenated fraction of hop oils have been detected in finished beers (Shimazi et al., 1974; Sandra and Verzele, 1975; Micketts and Lindsay, 1978; Tressl et al., 1978a, 1979) but there is still much controversy as to which ones contribute to the hoppy aroma of beer. The purpose of this work is to address this question. We report the presence of certain oxygenated compounds, which are either absent or in substantially reduced concentration in beer brewed with domestic hop varieties, in finished beer brewed with a foreign aroma hop. We also discuss the relevance of each of these compounds to hop aroma in beer.

EXPERIMENTAL SECTION

Sample Preparation. Eight liters of each beer was vacuum distilled (0.02 torr) at 20 °C into a trap cooled in liquid nitrogen. Two grams of Dow Corning polydimethylsiloxane antifoam agent was added to the beer to suppress foaming during the distillation. Four 2-L distillation fractions were taken. The last fraction was collected until only the nonvolatile residue remained in the distillation flask. Each distillation fraction was then extracted three times with ether, and the combined ether washings were then back-extracted with purified water three times. This extraction eliminated most of the ethanol and acetic acid. The ether extracts of the distillation fractions were dried over $MgSO_4$, and the solvent was removed under reduced pressure to a volume of about 0.5 mL. Each distillation extract was fractionated by liquid chromatography (LC), using a Merck Lobar 240 × 10 mm prepacked silica gel column. A flow rate of 2 mL/min was used with 50% pentane/ether as eluant. Five fractions were collected: the first 10 mL after injection, 10 mL, 10 mL, 20 mL, and the next 20 mL. After evaporation of the solvent, gas chromatography–mass spectrometry (GC/MS) analysis was carried out on each LC fraction of each distillation fraction for a total of 20 analyses/beer.

Gas Chromatography (GC). An 80 m × 0.5 mm i.d. glass capillary coated with Carbowax 20M was used for the separation. The column temperature was programmed from 80 to 180 °C at 4 °C/min and was then held at 180 °C to the end of elution. Helium was the carrier gas.

GC/Mass Spectrometry (GC/MS). Mass spectra were acquired by using a Varian CH-7 single-focussing mass spectrometer with a glass jet separator and an ionizing voltage of 70 eV. The instrument was interfaced to a System Industries 150 data system for data acquisition and reduction.

Flavor Threshold Determinations. All sensory evaluation tests were conducted, using the standard preparation and serving procedures for the triangular testing method. Ninety-milliliter (3 oz) samples were carefully poured into coded 360-mL (12 oz) amber glasses. The three samples, two alike and one different, were placed on serving trays, using all six serving combinations. The trays were served to taste panelists seated in individual testing booths lighted with amber lights and containing a sink with water available. The 15–20 panelists were

Department of Agricultural Chemistry (V.E.P., M.L.D.) and the Department of Food Science and Technology (L.A.M., R.E.W.), Oregon State University, Corvallis, Oregon 97331.

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Table I. Hop Oil Components Found in Two American Beers

	A,[a] ppb	B,[b] ppb
linalool	200	200
α-terpineol	75	
carvacrol	2000	200
caryolan-1-ol		25
nerolidol		75
humulene epoxide I		100
T-cadinol	100	200
δ-cadinol		100
α-eudesmol		100
humulenol II	250	250
4,4-dimethylcrotonolactone	50	100

[a] Beer brewed with Oregon Cascade hops. [b] Beer brewed with a mixture of Hallertauer mittelfrueh hops (mostly), Tettnanger hops, and Styrian hops.

selected judges who had participated on hop flavor panels at least three or four times a week for 6 months.

A commercial beer with hops low in aroma was used for all threshold tests. The carrier beer was secured from a single source and was between 30–50 days old when "spiked" and tested. The spiked samples were prepared at least 24 h prior to testing. The unspiked controls were handled in the same manner as the spiked samples, except the test compound was not present in the carrier. The threshold concentrations reported are those at which a significant difference was detected with a 95% or higher probability level by using the triangular test methods and a minimum of 30 judgements from the taste panel.

Synthesis of Flavor Components. Humulenol II and humulene epoxide II were prepared (Damodaran and Dev, 1968) and purified (Nigam and Levi, 1964) by previously reported methods from humulene purchased from Tridom Chemical Corp., Hauppauge, NY. There was a small amount (5%) of humulenol I in the purified humulenol II used for the taste sensory panel work. The olfactory response was assumed to be similar.

4,4-Dimethylcrotonolactone was prepared by the previously reported iodolactonization of 4-methyl-3-pentenoic acid (King and Waight, 1974). It was purified by liquid chromatography on a silica gel column before use.

RESULTS AND DISCUSSION

Our initial work on hop aroma compounds in beer was done on two commercial American beers. Beer A was brewed primarily with Cascade hop and beer B was brewed with a mixture of domestic and European varieties, predominantly Hallertauer mittelfrueh. A delicate hop aroma was detectable in both beers; however, the hop character of the two was very different. Table I lists the hop-derived compounds detected in these two beers and their approximate concentrations. The hop oil makeup of these two commercial beers was also very different. These differences could, however, be due to different brewing processes or to the use of different hop varieties.

To eliminate variation of brewing conditions as a parameter, we obtained two pilot beers brewed under identical conditions from a commercial brewery. Only the hops used were different. One beer was brewed with 100% Cluster hop, a domestic hop variety, and the second was brewed with 100% Hallertauer mittelfrueh, a European variety. The results of our analyses for hop-derived compounds in these two beers are shown in Table II. Sensory panel evaluations by triangular tests detected significant differences (<0.05) in aroma among the pilot beers brewed without hops (control) and Cluster hops, the control and Hallertauer hops, and the Cluster hops and Hallertauer hops.

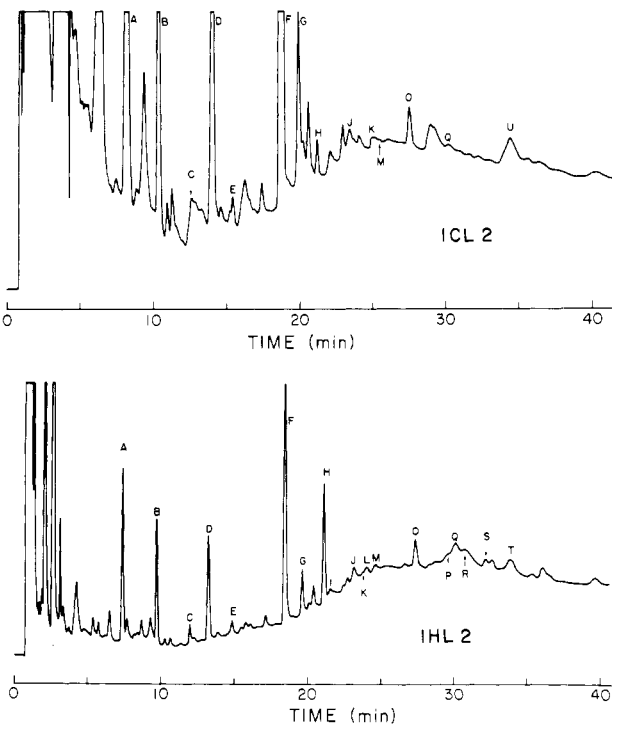


Figure 1. Gas chromatograms of beer fractions. 1CL2 is the second LC fraction of the first distillation fraction of the 100% Cluster pilot beer. 1HL2 is the corresponding fraction for the 100% Hallertauer pilot beer. Labeled peaks are (A) ethyl hexanoate, (B) ethyl octanoate, (C) linalool, (D) ethyl decanoate, (E) α-terpineol, (F) β-phenylethyl acetate, (G) ethyl dodecanoate, (H) BHT, (I) β-phenylethanol, (J) β-phenylethyl isobutyrate, (K) 2-acetylpyrrole, (L) isomaltose, (M) humulene epoxide I, (N) caryolan-1-ol, (O) BHA, (P) humulol, (Q) *T*-cadinol, (R) carvacrol, (S) α-eudesmol, (T) humulenol II, (U) B(57), M(170) not humulenol II.

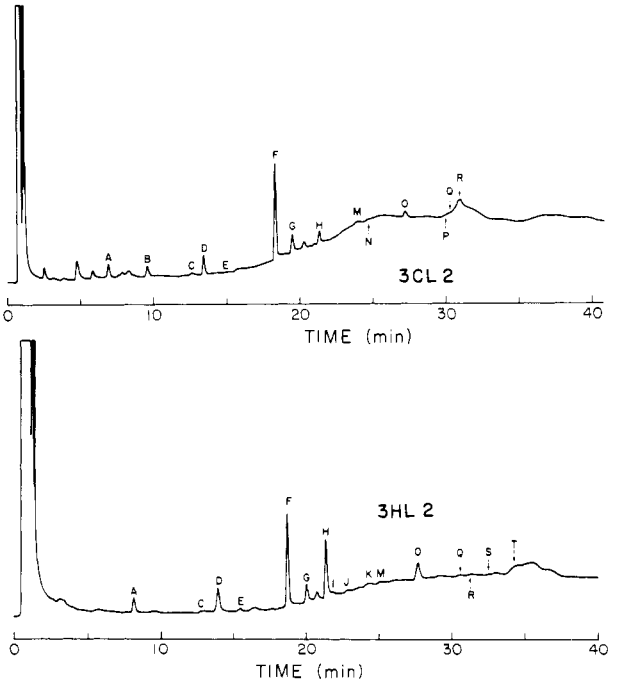


Figure 2. 3CL2 and 3HL2 are the second LC fraction of the third distillation fractions. Peaks are labeled as in Figure 1.

Gas chromatograms of some of the interesting distillates and liquid chromatography fractions are compared in Figures 1 and 2. The most obvious differences in the hop oil derivatives of the two pilot beers are that the beer

Table II. Hop Oil Components Found in Pilot Beers

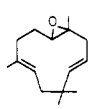
	Cluster[a]	Hallertauer[a]	threshold, ppb in beer	threshold, ppb in water
trans-linalool oxide		25		
linalool	200	200	80[b]	6[c]
α-terpineol	75	175	2000[b]	40[c]
humulene epoxide I	50	125		10[c]
caryolan-1-ol	50			
humulol	75	200		
T-cadinol	50	150		
carvacrol	500	500		
α-eudesmol		150		
humulenol II		500	500[d]	
4,4-dimethylcrotonolactone	200	2000	>40000[d,e]	

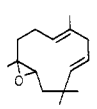
[a] Sensory panel evaluation detected a significant (<0.05) difference in aroma between the pilot beers brewed with these two hop varieties. [b] Meilgaard (1975). [c] Tressl et al. (1979). [d] This study. [e] Sandra and Verzele (1975).

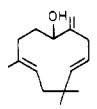
Table III. Hop Storage Data of Tressel et al. (1978b)

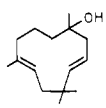
	ppm 1974	ppm 1977
trans-linalool oxide	1	20
4,4-dimethylcrotonolactone	1.5	70
humulene epoxide I	23	80
humulene epoxide II	33	250
humulol	11	88
T-cadinol	13	15
humulenol II	2	290
humulene	6520	185
linalool	25	45

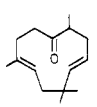
brewed with Hallertauer hops contains *trans*-linalool oxide, α-eudesmol, and humulenol II, which were not detected in the beer brewed with Cluster hops. Substantially more 4,4-dimethylcrotonolactone, α-terpineol, humulene epoxide I, humulol, and *T*-cadinol also were found in the beer brewed with Hallertauer hop.

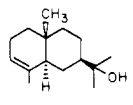

Humulene Epoxide II


Humulene Epoxide I


Humulenol II


Humulol


Humuladienone


α-Eudesmol

The much higher concentration of 4,4-dimethylcrotonolactone in the beer brewed with Hallertauer hop suggested that it may be a major aroma contributor. However, after our flavor panel work, we concluded in concurrence with Sandra and Verzele (1975) that 4,4-dimethylcrotonolactone is not a contributor to the hoppy aroma since its concentration in beer is far below its flavor threshold concentration. The compound when pure does not smell like hops, but it resembles the smell of tetrahydrofuran.

We have found, in agreement with others (Micketts and Lindsay, 1978; Tressl et al., 1978a), substantial quantities of linalool in all the commercial and pilot beers which we have analyzed. Its concentration is in the same range as its flavor threshold range in beer, and we, therefore, conclude linalool is sensory relevant in beer hop aroma. α-Terpineol, which is found in much lower concentrations, probably has much less sensory relevance to the hoppy aroma of beer than linalool has since its threshold concentration is much higher (Meilgaard, 1975).

Oxidation and hydration products of humulene make up a major part of the hop-derived compounds found in beer. Humuladienone was previously credited as a contributor to the hoppy aroma of beer (Shimazi et al., 1974). Sandra and Verzele (1975) could not find humuladienone, with an estimated sensitivity of 1 ppb, in the beers they analyzed. Tressl et al. (1978a) reported finding humuladienone in beer at a concentration of 10 ppb. Its reported flavor threshold is 100 ppb (Shimazi et al., 1974). We found no evidence of humuladienone in any of the beers we have analyzed. Since humuladienone cannot be uniformly detected in hoppy beers, its contribution to hop aroma in beer must be minimal. We have found humulenol II in hoppy beers in concentrations approximating its threshold, but we were unable to detect humulenol II in the pilot brew brewed with Cluster hops. We conclude, therefore, that humulenol II is *in part* responsible for the hoppy aroma of some beers. Tressl et al. (1978a) report finding humulenol II in a German beer in much higher

Table IV. Hop Oil Makeup (%) as a Function of Variety[a]

	Saaz[b]	Hallertauer[c]	Northern Brewer[d]	Yakima[e]	Shinshu-wase[f]
linalool	0.40	0.51	0.30	0.23	0.55
humulene	28.84	41.72	29.38	20.47	5.29
α-selinene[g]	Tr		0.62	0.87	1.98
humulene epoxide I	0.11	0.42	0.05		
humulene epoxide II	0.62	1.72	0.29	0.11	0.05
humulol	0.09	0.06	0.02		
α-eudesmol	0.22		0.07		
humulenol II	0.18	1.46	0.11		
T-cadinol	0.15	0.17	0.11		0.08
δ-cadinol	Tr	0.04	0.05		

[a] Reprinted with permission from Naya and Kotake (1972). Copyright 1972 Chemical Society of Japan. [b] Low volume premium Czechoslovakian hop. [c] High volume premium German hop. [d] English high α-hop ("bitterness" hop).[h] [e] U.S. Cluster hop ("bitterness" hop).[h] [f] Japanese hop ("bitterness" hop).[h] [g] The parent hydrocarbon of α-eudesmol. [h] Terminology used in the trade

concentrations than those reported here. Tressl et al. (1979) have also suggested that humulene epoxide I probably contributes to hop aroma in beer since its concentration in beer is far above its threshold value. There are no flavor panel data available on humulol, which makes it difficult to speculate on its sensory relevance in beer.

The work of Tressl et al. (1978b) suggests that the oxygenated humulenes are formed by air oxidation of humulene in the stored hops and that they are not biosynthesized by the plant. Examination of some of Tressl's data on hop storage (Table III) supports this opinion. Over a 3-year storage period, the concentrations of all the oxygenated humulenes substantially increased, with a corresponding decrease in the humulene concentration. The oxidation products Tressl observed are the ones that would be expected by air oxidation of humulene, according to the work of Pickett et al. (1977). Pickett states that upon heating of humulene to 65 °C or 100 °C in O_2, or by photolysis in O_2, humulene epoxide II is the major product formed (33%). Humulenol II is the second most abundant oxidation product (0.05%), and humuladienone is not formed in any detectable quantities. Chemical epoxidation of α-humulene with perbenzoic acid (Damodaran and Dev, 1968) or *m*-chloroperbenzoic acid (this work) yields about a 20:1 mixture of humulene epoxide II/humulene epoxide I. The observation of significant concentrations of humulene epoxide I and the absence of humulene epoxide II in the pilot brews as well as in the two commercial American beers may be due to acid catalysis (Nigam and Levi, 1964) of the humulene epoxide II to humulenol II, which was found in high concentrations. Humulene epoxide I, on the other hand, must not convert readily since it was present in the beer, while humulenol I was not detected. [In beer, with a pH of about 4, this rearrangement may be quite facile since it has been observed with HBr in pyridine (Nigam and Levi, 1964).] Humulenol I, the corresponding acid rearrangement product of humulene epoxide I, has, in fact, never been reported in beer or hops. Possibly some steric effect that slows epoxidation at the "I" site of humulene may hinder the rearrangement to the allylic alcohol. Dauben et al. (1975) reported that humulol is formed by the acid-catalyzed hydration of humulene. This is a likely pathway for humulol formation in hop oil and beer.

It is concluded that oxidation products of humulenes collectively are of major importance for a characteristic hoppy aroma which some hop varieties impart to beer. This hypothesis is supported by work on hop oils by Naya and Kotake (1972). Their results are presented in Table IV. Those hop varieties which are generally recognized in the industry for their "noble" aroma are contained in the left-hand side of the table. "Noble" aroma hops contain more humulene as well as higher amounts of the oxidation products of humulene than do those hop varieties which are generally not recognized for their aroma characteristics.

The data in Tables I and II suggest that the contribution of cadinols and α-eudesmol to the hoppy aroma in beer needs to be investigated further. The concentrations of these constituents also tend to be higher in the "noble" aroma hops although we are at a loss to explain why Naya and Kotake (1972) did not find α-eudesmol in Hallertauer mittelfrueh hops, as they did in some of the other aroma varieties (Table IV). Tressl et al. (1978b) noted that the concentrations of cadinols in hops increase only slightly with aging. These compounds may, therefore, be biosynthesized by the hop plant rather than transformed from the parent hydrocarbon through chemical transformation, i.e., oxidation under storage and oxidation or hydration during brewing. More work is needed on the identification of hop aroma constituents in the beer, the contribution they make to the flavor and aroma, and the mechanisms that produce them. This work will be continued in our laboratories.

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