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Floral Hop Aroma in Beer

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Four beers were analyzed by gas chromatography–mass spectrometry for hop-derived compounds. Two were commercial American beers, one brewed with 60% Cascade and 40% Cluster hops and the second with a mixture of European hop varieties. The other two beers were pilot brews, one made exclusively with Hallertauer hops and the other with Cluster. Linalool was found consistently in all beers. Geraniol and geranyl isobutyrate were found in high concentration in the Cascade beer and to a lesser extent in the Cluster beer. Neither compound was detected in the other two beers. Levels of geraniol and geranyl isobutyrate are much higher in Cascade hops than in most other varieties. Flavor threshold data for geraniol and linalool indicate they are responsible for the floral aroma/taste of beer brewed with Cascade hops.

In our continuing investigations of hop aroma in beer (Peacock et al., 1980) we have found it useful, from sensory panel work, to elaborate on different types of hop aroma in beer. We found that beer brewed with Cascade hops has a floral aroma that is distinctly different from the aroma of beer brewed with traditional European aroma hops. The hop aroma of beers brewed with these latter varieties has a more spicy, herbal quality and is more complex than this floral aroma. The purpose of this work is to identify the compounds responsible for the floral aroma/taste note and to determine which hop varieties will impart this characteristic to beer.

EXPERIMENTAL SECTION

Beer analyses were carried out by a previously published method (Peacock et al., 1980).

Hop Analyses. Oils were isolated from hops or concentrated pellets by the method of Likens and Nickerson (1967). The oils were chromatographed on a 0.25 mm × 30 m glass capillary column coated with Carbowax 20M, temperature programmed from 60–190 °C at 5 °C/min, with helium as the carrier gas. A Hewlett-Packard Model 5830A GC with a Model 18835B capillary inlet system was used with a flame ionization detector in conjunction with a Hewlett-Packard 18850A GC terminal for data reduction and peak quantification.

GC–MS. All hop oil peaks reported were identified by GC–MS using the same GC conditions as above. Mass spectral data were acquired on a Finnigan Model 4023 quadrupole mass spectrometer.

Flavor Components. Linalool and geraniol were purchased from Aldrich Chemical Co., Milwaukee, WI, and used without further purification. Geranyl isobutyrate was synthesized by stirring equal molar amounts of geraniol and isobutyryl chloride with an excess of sodium carbonate in refluxing toluene for 1 h. The solution was cooled and washed with water, and the toluene was removed by distillation. The crude product was purified by column chromatography on silica gel. The purified product was shown to be 97% pure by GC with no detectable geraniol. NMR and IR spectra were consistent with the structure of geranyl isobutyrate. The mass spectrum was identical with that of a published reference spectra (Stenhagen et al., 1974).

Flavor threshold determinations were conducted using the ascending method of limits test (Meilgaard, 1980). At each test period the judges received sets of six triangles, each consisting of two controls and one spiked or test sample. A commercial American beer, brewed mostly with European aroma hops, was used as the base or control. The selected control was intentionally low in these floral notes, as thresholds of the floral compounds are dependent on the beer used. Since a panelist's ability to discriminate depends on familiarity with the compound, the panel was served samples with each compound repeatedly until the group threshold no longer decreased. The lowest threshold obtained for each compound at one sitting is reported in Table I.

Fermentation of Geranyl Isobutyrate. One hundred microliters of geranyl isobutyrate was added to 1 L of a 6% glucose solution with 1 g of commercial yeast nutrient and 50 mg of brewer's yeast. The fermentation was done at room temperature with a fermentation air lock for 1 month, at which time all glucose was metabolized. The "beer" was extracted with ether, the extract dried over anhydrous magnesium sulfate, and the ether then evaporated under a stream of dry nitrogen to a volume of 0.5 mL. Geraniol and geranyl isobutyrate were identified by GC–MS and quantified by GC as described for the hop oil samples. The ratio of geraniol to geranyl isobutyrate in the fermented sample was 3:17, or 15% of the ester was converted to geraniol. An identical sample was fermented at 7 °C and resulted in 0.6% conversion.

RESULTS AND DISCUSSION

The analyses of various beers and results from taste panel studies (Table II) indicate that linalool, geraniol, and to a lesser extent geranyl isobutyrate are the compounds responsible for most of the floral aroma/taste of beer brewed with Cascade hops. Linalool and geraniol both are well-known floral scents in the perfume industry. Linalool is a major component of lavender oil, and geraniol is the major component of oil of rose.

The contribution of linalool to the floral flavor profile of all four beers is substantial. The estimated concentration of 200 ppb of linalool in the beers analyzed is almost 8 times its sensory threshold in beer. This compound undoubtedly is flavor active in beer. The concentration of geraniol in the Cascade beer is also many times its sensory threshold; therefore, geraniol must be an important aroma/taste constituent of Cascade-brewed beer. The concentration of geranyl isobutyrate in even the Cascade beer is well below its sensory threshold. The sensory contribution of geranyl isobutyrate, though of minor importance, may add to the overall complexity of the floral aroma/taste profile.

The presence of linalool and geraniol in the wort during fermentation and storage would necessitate the formation

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Table I. Floral Component Thresholds in Beer[a]

			Linalool					
judge	5 ppb	10 ppb	20 ppb	40 ppb	80 ppb	160 ppb	BET[b]	log
1	−	−	+	+	+	+	14.14	1.15
2	−	−	−	+	+	+	28.28	1.45
3	+	−	−	−	+	+	56.56	1.75
4	−	+	+	+	+	+	7.07	0.85
5	−	−	−	+	−	+	113.14	2.05
6	−	−	−	−	+	+	56.56	1.75
7	+	+	+	+	+	+	3.54	0.55
8	−	−	+	+	+	+	14.14	1.15
9	−	−	+	+	+	+	14.14	1.15
10	−	−	+	+	+	+	14.14	1.15
11	−	−	+	+	+	+	14.14	1.15
12	+	+	−	−	+	+	56.56	1.75
13	−	−	−	+	+	+	28.28	1.45
14	+	+	+	+	+	+	3.54	0.55
15	+	−	−	+	+	−	226.27	2.35
16	+	−	−	+	+	−	226.27	2.35
17	+	−	+	−	+	+	56.56	1.75
								24.35

24.35/17 = 1.43 group BET = antilog 1.43 = 27 ppb

Histogram of Individual BET's

		11				
		10		17		
		9		12		
14		8	13	6		16
7	4	1	2	3	5	15
3.54	7.07	14.14	28.28	56.56	113.14	226.27 ppb

			Geraniol					
judge	5 ppb	10 ppb	20 ppb	40 ppb	80 ppb	160 ppb	BET	log
1	+	−	−	−	+	+	56.56	1.75
2	+	+	+	+	−	+	113.14	2.05
3	−	−	+	−	−	−	226.27	2.35
4	+	−	+	−	+	−	226.27	2.35
5	−	+	+	+	+	+	7.07	0.85
6	+	+	−	+	+	+	28.28	1.45
7	+	−	+	+	+	+	14.14	1.15
8	+	−	−	+	+	+	28.28	1.45
9	−	+	+	+	+	+	7.07	0.85
10	−	−	−	−	+	+	56.56	1.75
11	−	−	−	−	−	+	113.14	2.05
12	+	−	+	+	+	−	226.27	2.35
13	+	−	+	+	+	+	14.14	1.15
14	−	−	+	+	+	+	14.14	1.15
15	−	+	+	−	+	+	56.56	1.75
16	+	+	+	+	+	+	3.54	0.55
								25.00

25.00/16 = 1.56 group BET = antilog 1.56 = 36 ppb

Histogram of Individual BET's

		14		15		12
	9	13	8	10	11	4
16	5	7	6	1	2	3
3.54	7.07	14.14	28.28	56.56	113.14	226.27 ppb

			Geranyl Isobutyrate					
judge	62 ppb	125 ppb	250 ppb	500 ppb	1000 ppb	2000 ppb	BET	log
1	−	+	−	−	−	−	2830	3.45
2	+	−	+	−	−	+	1410	3.15
3	−	−	+	−	+	+	710	2.85
4	−	+	+	+	+	+	90	1.95
5	−	−	−	−	+	+	710	2.85
6	+	+	+	+	+	+	50	1.70
7	+	−	−	+	+	+	350	2.54
8	+	+	−	−	+	+	710	2.85
9	−	−	+	−	−	−	2830	3.45
10	−	+	−	−	+	+	710	2.85
11	+	+	+	+	+	+	50	1.70
12	−	+	−	+	+	+	350	2.54
13	+	+	−	−	+	+	710	2.85
14	−	−	−	+	+	+	350	2.54
15	−	+	−	−	−	−	2830	3.45
16	+	+	+	+	+	+	50	1.70
								42.42

42.42/16 = 2.65 group BET = antilog 2.65 = 450 ppb

Table I (*Continued*)

Histogram of Individual BET's

				13		
				10		
16			14	8		15
11			12	5		9
6	4		7	3	2	1
50	90	180	350	710	1414	2830 ppb

[a] These thresholds were determined by the ascending method of limits test (Meilgaard, 1980). [b] BET = best estimated threshold.

Table II. Hop Floral Aroma/Taste Components Characterized in Beers[a]

compound	Cluster beer, ppb	Hallertauer beer, ppb	Cascade beer, ppb	import mixture beer, ppb	threshold in beer, ppb
linalool	200	200	200	200	27[b]
geraniol	75	n.d.[d]	200	n.d.	36[c]
geranyl isobutyrate	25	n.d.	150	n.d.	450

[a] The flavor panel could easily distinguish between these beers. [b] Previously reported as 80 ppb (Meilgaard, 1975). [c] Previously reported as 100–200 ppb (Meilgaard, 1980). [d] n.d. = none detected.

Table III. Beer Aroma Components in Hops

hop variety	linalool[a]	geraniol[a]	geranyl isobutyrate[a]	mL of oil/100 g of hop	% α-acids[b]
Cascade[c]	0.85	0.27	1.56	1.56	7.6
Cluster[c]	0.44	0.24	0.60	0.55	12.3
Tettnanger[c]	0.75	0.03	n.d.	1.00	6.3
Hersbrucker[c]	0.50	n.d.[d]	n.d.	0.75	7.4
Hallertauer[c]	0.41	n.d.	n.d.	0.75	7.7
Perle[c]	0.26	n.d.	n.d.	0.80	7.7
Northern Brewer[c]	0.28	0.06	0.12	1.48	13.6
Shin-shu-wase	0.39	0.37	1.08	0.73	4.7
Talisman	0.31	0.37	0.95	0.64	6.1
Brewer's Gold	0.41	<0.01	n.d.	2.93	9.6
Backa	0.83	0.13	n.d.	0.60	3.6
Fuggle	0.47	<0.01	n.d.	1.28	5.4
Hallertauer	0.43	0.14	n.d.	0.39	4.8
Saazer	0.32	0.03	n.d.	1.08	6.6
Northern Brewer	0.30	0.04	n.d.	2.01	10.3
Cascade	0.51	0.14	2.11	1.10	6.2

[a] Reported as percent of oil. [b] Hop bitterness component; reported by the spectrophotometric method of the American Society of Brewing Chemists (1976). [c] Concentrated pellets. [d] n.d. = none detected.

of small amounts of their acetate esters in the final product. These esters were not detected in any of the beers and are unlikely to be major aroma contributors. In minute amounts they may, however, add to the complexity of the aroma.

Table III lists hop varieties with the amounts of linalool, geraniol, and geranyl isobutyrate present in their oils. The relatively large concentrations of these compounds found in beer suggests that, if hops are the only source, the transfer of these compounds from the hops to the finished beer during brewing is relatively efficient. This is not surprising considering that terpenoid alcohols and esters are somewhat water soluble as compared to the terpene and sesquiterpene hydrocarbons, which are found in greater abundance in hop oils but not in beer (Peacock et al., 1980).

As can be seen in Table III, the amount of linalool in the hop oil (or beer, Table II) does not vary as much with hop variety as does the geraniol or geranyl isobutyrate concentration. This suggests that varietal differences in the floral aroma/taste are predominantly geraniol–geranyl isobutyrate dependent even though linalool contributes significantly to the overall aroma profile.

It is likely that much of the geraniol found in beer actually originates from the hydrolysis of geranyl isobutyrate rather than from just geraniol in hops. Methyl esters from hop oil are believed to be transesterified during fermentation to give methanol and the corresponding ethyl esters (Nickerson and Likens, 1966; Tressl et al., 1978). Therefore, transesterification or hydrolysis of geranyl isobutyrate in the wort is likely. The fact that the geraniol to geranyl isobutyrate ratio is high in beers brewed with Cascade and Cluster hops, but is substantially lower in the hop oils, supports this idea. Furthermore, geranyl isobutyrate when fermented in a 6% glucose solution at room temperature yielded 15% geraniol. Under the same conditions at 7 °C, only 0.6% of the ester was hydrolyzed. The amount of hydrolysis of geranyl isobutyrate during fermentation, of course, would be extremely variable from brewer to brewer. Different results may also be expected depending on the yeast strain used and the temperature of fermentation. Wort boiling and aging of the beer are both likely to cause further hydrolysis . It was observed that 20–30% of the geranyl isobutyrate in fresh hops was hydrolyzed to geraniol after a 1-year refrigerated storage period. Since much of the geranyl isobutyrate in hops is likely converted to geraniol during brewing, the overall geraniol–geranyl isobutyrate content of the hop oil contributes the principal varietal difference in the floral hop aroma/taste.

The linalool/geraniol–geranyl isobutyrate ratio of the hops of Table III varies considerably between different varieties. Judges of the flavor panel, especially those with

Table IV. Relative Floral Aroma/Taste Intensity of Beers Spiked with Floral Components[a]

aroma	floral intensity bland beer[b] (17 tasters) $\bar{X}^d$	t	$P_{(1)}^e$	significant[f]	floral intensity floral beer[c] (13 tasters) $\bar{X}^d$	t	$P_{(1)}$	significant[f]
control	4.35				4.69			
100 ppb of geraniol	5.88	2.22	<0.025	+	5.54	1.31	<0.10	–
100 ppb of geraniol + 100 ppb of linalool	6.76	3.49	<0.0025	+	6.46	2.72	<0.005	+
100 ppb of linalool	5.76	2.04	<0.05	+	4.92	0.35	<0.25	–
100 ppb of geraniol + 200 ppb of geranyl isobutyrate	5.12	1.12	<0.25	–				

taste	floral intensity bland beer[b] (17 tasters) $\bar{X}^d$	t	$P_{(1)}^e$	significant[f]	floral intensity floral beer[c] (12 tasters) $\bar{X}^d$	t	$P_{(1)}^e$	significant[f]
control	3.94				4.17			
100 ppb of geraniol	5.65	3.00	<0.005	+	5.25	1.86	<0.05	+
100 ppb of geraniol + 100 ppb of linalool	6.00	3.61	<0.0025	+	6.33	3.72	<0.0025	+
100 ppb of linalool	5.00	1.86	<0.05	+	4.67	0.86	<0.25	–
100 ppb of geraniol + 200 ppb of geranyl isobutyrate	4.59	1.14	<0.25	–				

[a] Panelists were served beers and asked to rank floral aroma/taste intensity on a scale of 0–9 with 0 = no floral aroma/taste, 5 = average, and 9 = pronounced floral aroma/taste. Two-way analysis of variance was used to calculate error sum of squares. Standard error, $\Sigma(x_1 - \bar{x}_2)$, of difference between means of control and spiked sample was used in the t test. [b] Commercial beer with little floral or hop aroma/taste. [c] Commercial beer with mild but detectable floral aroma/taste. [d] Mean of observations. [e] One sided. [f] At 5%.

Table V. Calculation of Floral Index

hop variety	linalool flavor potential[a]	geraniol flavor potential[b]	geranyl isobutyrate flavor potential[c]	Σ^d	floral[e] index	floral index/% α-acids
Cascade[f]	31.5	7.5	16.7	55.7	86.9	11.4
Cluster[f]	16.3	6.7	6.5	29.5	16.2	1.32
Tettnanger[f]	27.8	0.8		28.6	28.6	4.54
Hersbrucker[f]	18.5			18.5	13.9	1.88
Hallertauer[f]	15.2			15.2	11.4	1.48
Perle[f]	9.6			9.6	7.7	1.00
Northern Brewer[f]	10.4	1.7	1.3	13.4	19.8	1.46
Shin-shu-wase	14.4	10.3	11.6	36.3	26.5	5.64[h]
Talisman	11.5	10.3	10.2	32.0	20.5	3.36
Brewer's Gold	15.2			15.2	44.5[g]	4.64
Backa	30.7	3.6		34.3	20.6	5.72[h]
Fuggle	17.4			17.4	22.3	4.12
Hallertauer	15.9	3.9		19.5	7.6	1.58
Saazer	11.9	0.8		12.7	13.7	2.08
Northern Brewer	11.1	1.1		12.2	24.5	2.38
Cascade	18.9	3.9	22.6	45.4	49.9	8.05

[a] Linalool flavor potential = percent of linalool in the oil divided by 0.027 (0.027 ppm is its theshold in beer). [b] Percent geraniol divided by 0.036. [c] ($^1/_3$% geranyl isobutyrate divided by 0.036) + ($^2/_3$% geranyl isobutyrate divided by 0.45). [d] The sum of the three flavor potentials. [e] Σ(X mL of oil/100 g of hops). [f] Concentrated pellets. [g] This figure is inflated because of the unusually high amount of oil from this hop. This oil yield is typical of Brewer's Gold. [h] These figures are high because of the low α-acid content of the hops from Table III.

a greater sensitivity for geraniol, have noticed that the aroma and taste of geraniol in beer at low concentrations (20 ppb) is much different than that at 100 ppb. They have also noticed that the aroma/taste of geraniol is much different that that of linalool. The varietal variation of floral aroma/taste in beer is considered to be mostly geraniol dependent, but the presence of an equivalent amount of linalool is needed as a flavor modifier to balance the geraniol "perfume" flavor.

In Table IV the differences in the flavor/aroma of floral components in beer are demonstrated. A two-way analysis of variance of the results from taste testing of four or five treatments by 12–17 tasters was made. The error from the analysis was used to determine the standard error of the differences between the means, which in turn was used in t tests between the controls and each spiked sample in the four sets. One-sided P values (Table IV) indicate that detection of aroma/taste of 100 ppb of geraniol plus 100 ppb of linalool is due to chance only 0.5% or less of the time. The aroma/taste of 100 ppb of geraniol and 100 ppb of linalool tested separately in bland beer (i.e., no aroma attributable to geraniol or linalool) is significant at least at the 5% level, whereas the aroma of these two components in floral beer cannot be considered statistically significant. Similarly, taste of geraniol is barely significant in floral beer at the 5% level. This is not surprising since the floral beer used is known to contain a hop variety (Cascade) that contains large amounts of both geraniol and linalool. Geranyl isobutyrate seems to depress the aroma/taste of geraniol below a level that is statistically significant. In general, one could expect the floral aroma/taste profile of a beer brewed with, for example, Cascade or Shin-shu-wase hops (low linalool/geraniol–geranyl isobutyrate ratio) (Table III) to be different from the floral aroma/taste of a beer with Tettnanger (high ratio).

An attempt was made (Table V) to quantify the amount of floral hop aroma/taste one could expect in a finished beer brewed with the same amount of various hops. By the formula explained in the table, each of the flavor components is weighted according to its sensory threshold concentration in beer. In the case of geranyl isobutyrate, the flavor potential is calculated assuming that one-third of that present in the hop is converted to geraniol in the beer. These contributions are added and multiplied by the yield of oil from the hops to give the column denoted as "floral index". We believe this index gives a fairly accurate measure of the amount of floral aroma/taste one can expect in a beer brewed with a standard amount of one of these hops.

The column on the extreme right of Table V is the floral index weighted to a standard amount of α-acids to give an estimation of the relative amount of floral hop aroma/taste one could expect in beers brewed to a standard bitterness by using the various hops. This index may be of more use to a brewer than the floral index. Using the floral index divided by % α-acids column, a brewer can estimate the relative intensity of the overall floral aroma/taste of a beer to a standard bitterness as a function of the hop variety used. A beer brewed with a hop variety with a high value in this column would have a more intense floral aroma/taste than a beer brewed to the same bitterness level with a hop with a lower value. Of course, subtle changes in the floral aroma/taste profile will occur as the linalool/geraniol–geranyl isobutyrate ratio changes in the beers.

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Dihydrochalcone Sweeteners. Synthesis and Sensory Evaluation of a Homoserine–Dihydrochalcone Conjugate with Low Aftertaste, Sucrose-like Organoleptic Properties

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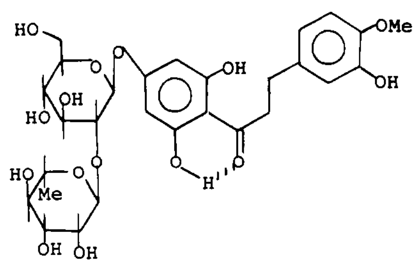
Neohesperidin dihydrochalcone (NHDHC; 1), known since 1963 as a potently sweet compound (340 ± 60 times the sweetness of sucrose), is plagued by a non-sucrose-like, lingering, sweet aftertaste. An analogue, 4, involving substitution of the neohesperidosyl moiety of 1 with homoserine ether functionality, is demonstrated to be potently sweet (400 ± 30) and to be the *first* sweet dihydrochalcone to have diminished aftertaste. Three additional amino acid–dihydrochalcone conjugates, 6, 7, and 8, were synthesized and evaluated. Differences in sensory properties were rationalized by changes in hydrophilic–hydrophobic balance as quantitated by the chromatographic parameter k'.

Eighteen years have now elapsed since the original report (Horowitz and Gentili, 1963) on the potent sweetness of the grapefruit-derived flavanoid neohesperidin dihydrochalcone (NHDHC; 1). The pleasant taste of 1 is unfortunately beset with one severe handicap, a long-lingering menthol–licorice-like sweetness, quite unlike that of sucrose, the consumers standard. Within the last 8 years, we have been intensively studying the molecular topography of 1, attempting to design and synthesize analogues which retain NHDHC's potent sweetness but



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lack its non-sucrose-like aftertaste (DuBois et al., 1977a,b 1981). We report here the synthesis and sensory evaluation of the *first* NHDHC analogue with significantly decreased aftertaste. In addition, we report the synthesis and sensory

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