

first identified by Shimazu et al. (1974) in a Japanese beer. Humulene epoxide I, humulene epoxide II, linalool, humulol, and humulenol II are strongly concentrated components. All aroma compounds shown in Table II have been identified in Spalter hops (Tressl et al., 1978) and most of them increase during the storage of hops. Damascenone and β -ionone have been found in a very low trace range. Both are important constituents and have thresholds of 0.009 and 0.008 ppb, respectively. All constituents, presented in Table II, have been characterized in some other German beers of the type "Pilsener", but in much lower concentrations (Friese, 1977).

The effect of kettle hop boiling and the influence of the yeast strain on hop aroma constituents in beer are still unknown.

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LITERATURE CITED

- Buttery, R. G., Black, D. R., *J. Food Sci.* **32**, 414 (1967).
 Drawert, F., Tressl, R., *Tech. Q., Master Brew Assoc. Am.* **9**, 72 (1972).
 Friese, L., Thesis, TU Berlin, 1977.
 Naya, Y., Kotake, M., *Tetrahedron Lett.*, 1715 (1967).
 Naya, Y., Kotake, M., *Tetrahedron Lett.*, 1645 (1968).
 Sandra, P., *Cerevisia* **1**, 37 (1976).
 Shimazu, T., Hashimoto, N., Kuroiwa, Y., *ASBC Proc.* **9**, 7 (1974).
 Tressl, R., Friese, L., Fifth International Ferm. Symposium, Berlin, 1976, p 381.
 Tressl, R., Friese, L., Fendesack, F., Köppler, H., *J. Agric. Food Chem.* **26**, 000 (1978).
 van Straten, S., de Vrijer, F., TNO Report R 4030, Central Institute for Nutrition and Food Research TNO, Zeist, The Netherlands, 1973.

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Studies of the Volatile Composition of Hops during Storage

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One hundred and forty constituents of Spalter hops (among them: terpenes, sesquiterpenes, esters, ketones, aldehydes, alcohols, lactones, ethers, and fatty acids) have been characterized and (semi)-quantified by means of distillation-extraction, liquid-solid chromatography, and capillary gas chromatography-mass spectrometry. After a storage period of 3 years, the changes of individual components were determined. Terpene and sesquiterpene hydrocarbons are decreased by polymerization and oxidation. To some extent they are transformed into hydrophilic components such as epoxides, alcohols, and aldehydes, which may be easily transferred to beer. In addition, components such as aldehydes and fatty acids, which are known as off-flavor constituents in beer, are formed.

More than 150 volatile constituents in different varieties of hops were characterized by several authors (Buttery et al., 1963, 1965; Naya and Kotake, 1968, 1970, 1971; Tressl and Friese, 1978b). According to Naya and Kotake (1972) hops can be divided into myrcene-rich varieties and into humulene-rich varieties. Varieties like Spalt, Hallertauer Mittelfrüh, and Saaz belong to the second group and are known to possess a desirable fine aroma. To date many investigators failed to prove the existence of hops aroma constituents in beer. In 1967, Buttery et al. identified ethyl dec-4-enoate and ethyl deca-4,8-dienoate in an American beer. Drawert and Tressl (1972) characterized a few hops aroma constituents in a German beer. According to Guadagni et al. (1966), hop oil possesses a very low threshold. One part per billion of the oxygenated fraction of hop oil can be perceived (by the senses). In German beer with a typical hoppy aroma, we characterized 30 to 40 hop flavor constituents by GC-MS (Tressl and Friese, 1976). Most of these constituents are only trace compounds in the original hops. They are derived from major components by chemical or biochemical reactions during processing. Therefore it is important to know about the

changes of individual aroma constituents during storage and processing of hops. By means of LSC-GC-MS we characterized and (semi)quantified more than 120 volatile constituents of Spalter hops and followed the changes of individual compounds after a storage period of 3 years. The amounts of terpene and sesquiterpene hydrocarbons decreased considerably, while the amounts of aldehydes, ketones, alcohols, fatty acids, and oxygenated terpenes and sesquiterpenes increased.

MATERIALS AND METHODS

Sample Preparation. Five kilograms of Spalter hops, harvested in 1974, were stored at 0 °C in a dark room. One hundred grams of homogenized hops was mixed with 1200 mL of distilled water, and the hop oil constituents were isolated by means of distillation-extraction with pentane-ether (1:1) for 4 h (Krüger and Baron, 1975). The extract was dried over Na₂SO₄, then concentrated to a volume of 2 mL and separated by adsorption chromatography.

Adsorption Chromatography. One hundred microliters of hop oil were separated on 5 g of silica gel (activity II-III, Merck 7734, column 200 × 0.9 mm i.d.). The hydrocarbons were eluted with 100 mL of (I) pentane, the oxygenated fraction with 100 mL of ether. Both samples were concentrated using a Vigreux distillation column and examined by GLC. The oxygenated components were cleanly separated on silica gel. Six fractions (40 mL each) were eluted with solvents of increasing

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Table I. Changes of Terpenes and Sesquiterpenes during the Storage of Hops

compd	LSC ^a	<i>I</i> _K (CW-20M) ^a	<i>M</i> _r	approx concn, ppm	
				1974	1977
1 α-pinene	I	1040	136	20	1.5
2 β-pinene	I	1121	136	90	1
3 myrcene	I	1166	136	1700	3
4 limonene	I	1208	136	29	2.5
5 <i>trans</i> -ocimene	I	1251	136	25	
6 α-copaene	I	1491	204	156	16
7 α- <i>trans</i> -bergamotene	I	1584	204	163	33
8 β-caryophyllene	I	1594	204	2430	45
9 β-farnesene	I	1646	204	570	
10 humulene	I	1668	204	6520	185
11 aromadendrene	I	1685	204	570	40
12 germacrene D	I	1710	204	50	10
13 α-murolene	I	1715	204	80	15
14 γ-cadinene	I	1752	204	120	15
15 γ-cadinene	I	1754	204	130	20
16 α-bisabolene	I	1767	204	490	3
17 α-cadinene	I	1784	204	50	7
18 calamenene	I	1822	202	90	17
19 α-calacorene	I	1901	200	140	10
20 cadalene	I	2203	198		10

^a LSC, liquid-solid chromatographic fraction; *I*_K, Kovats indices; LSC II, contained only small amounts of humulene.

polarity: (II) pentane (P); (II') pentane-methylene chloride (P/MC) (9:1); (III) P/MC (2:1); (IV) P/MC (1:2); (V) P/E (9:1); (VI) ether (E). The different fractions were concentrated to a definite volume and analyzed by GLC and by capillary gas chromatography-mass spectrometry.

The free fatty acids were determined according to the method of Tressl et al. (1978a).

Gas Chromatography. Investigations were performed with a Varian Aerograph 2740-1 with two FID, a linear temperature program, and an effluent splitter (10:1). Column 1: 3 m (2 mm i.d.) glass; 10% FFAP on Chromosorb WAW/DMCS 80-90 mesh; temperature program 80-250 °C, 2 °C/min. Column 2: 4 m (4 mm i.d.) glass; 8% OV-17 on Chromosorb WAW/DMCS, 60-80 mesh, temperature program 60-240 °C, 4 °C/min for preparative GC.

Capillary Gas Chromatography-Mass Spectrometry. A 50-m glass capillary column (0.28 mm i.d.) coated with CW-20M, temperature program 70-190 °C, 2 °C/min, in a Carlo Erba Fractovap 2101 AC was connected to a double-focusing mass spectrometer CH 5-DF of Varian MAT, Germany. The glass capillary column (3 mL of He/min) lead directly to the ionization chamber via a platinum-glass capillary connection. The ionization voltage was at 70 eV, the ion source temperature 200 °C, and the resolution 2000 (10% Tal). Mass spectra were recorded with Oscillophil of Siemens, Germany. Separated compounds were registered by the pressure measuring ion source device.

Reference Compounds. Reference samples were purchased if available or were gifts from Professor Bohlmann (Berlin), Dragoco (Holzminden), Firmenich (Geneva), Haarman and Reimer (Holzminden), Dr. Naya (Osaka), and Oril (Paris).

RESULTS AND DISCUSSION

Spalter Hops grown in Hallertau were harvested and stored at 0 °C in a dark chamber. After 2 months the hop oil was isolated and enriched by distillation-extraction, separated by liquid-solid chromatography into six fractions of increasing polarity, and investigated by capillary gas chromatography-mass spectrometry. More than 100 constituents have been characterized and (semi)quantified (Tressl, 1978a). After a storage period of 3 years, the investigation was repeated and the changes of individual aroma constituents were determined. The results are

shown in Tables I to III. The hop oil content decreased from 1.63 g/100 g of hops to 0.88 g/100 g of hops. The stored hops had an intensive odor of isovaleric and caproic acids. Hydrocarbons, esters, aldehydes, ketones, ethers, and alcohols were cleanly separated on silica gel. Trace constituents were isolated and enriched by preparative gas chromatography. The distillation-extraction, liquid-solid chromatographic, and gas chromatographic separations were carried out with internal standards. Therefore, most of the constituents were determined semiquantitatively.

The results for hydrocarbons are presented in Table I. It can be seen that the amounts of all terpenes and sesquiterpenes decreased considerably. They are obviously decomposed by oxidative reactions and by polymerization. The hydrocarbon content of the hop oil decreases from 88 to 7% and the oxygenated components amount from 12 to 93%. The content of esters keeps nearly constant. Only the loss of unsaturated components is significant. Most of the ketones shown in Table II increase considerably during storage of hops. 4-Methyl-2-pentanone, 3-methyl-2-pentanone, and 4-methyl-3-penten-2-one are derived from humulones and lupulones. 6-Methyl-5-hepten-2-one is known as an oxidative degradation product of sesquiterpenes, diterpenes, and carotinoids. Other ketones may be formed by oxidative degradation of fatty acids. On the other hand, the amounts of unsaturated ketones decreased. Hexanal, heptanal, nonanal, and some unsaturated aldehydes were determined only in stored hops. They are formed by oxidative degradation of linoleic, linolenic, and oleic acids. The strongly concentrated alcohol 2-methyl-3-buten-2-ol is derived from humulones as shown by model systems. Most of the oxygenated terpenes and sesquiterpenes increased considerably during storage. It is interesting that some of the aroma-contributing constituents increase 10- to 30-fold. They have been determined only as trace constituents in the fresh hops. Caryophyllene epoxide, humulene epoxide I, and humulene epoxide II increase considerably. The structures of five sesquiterpene epoxides, characterized in stored hops, are still unknown. Among terpene and sesquiterpene alcohols, linalool, α-terpineol, caryolan-1-ol, humulol, and humulenol-II are strongly concentrated. β-Ionone and damascenone were identified for the first time. Both are known as oxidative degradation products of β-carotene and possess very low thresholds. The results for fatty acids

Table II. Changes of Oxygenated Constituents during the Storage of Hops

compd	LSC	I_K (CW-20M)	M_r	approx concn, ppm	
				1974	1977
ester					
21 methyl hexanoate	III	1184	130	10	+
22 methyl heptanoate	III	1281	144	47	31
23 methyl 4-methyl- hex-2-enoate	III	1226	142	50	46
24 methyl 6-methyl- heptanoate	III	1338	158	15	18
25 methyl octanoate	III	1390	158	25	20
26 methyl nonanoate	III	1487	172	42	37
27 methyl nonenoate	IV	1521	170	36	10
28 methyl 7-methyl- nonanoate	III	1532	186	2	3
29 methyl 8-methyl- nonanoate	III	1543	186	8	10
30 methyl decanoate	III	1592	186	15	9
31 methyl dec-4-enoate	IV	1622	184	175	135
32 methyl deca-4,8- dienoate	IV	1685	182	72	22
33 methyl geranate	IV	1690	182	15	10
34 methyl undecenoate	IV	1718	198	3	2
35 methyl dodecenoate	IV	1810	212	5	3
36 methyl dodecadienoate	IV	1871	210	40	3
ketones					
37 4-methylpentan-2-one	V	994	100	10	290
38 3-methylpentan-2-one	V	1005	100	5	280
39 4-methyl-3-penten-2-one	V	1118	98	—	2
40 3-hepten-2-one	V	1243	112	—	5
41 2-heptanone	IV	1178	114	0.5	80
42 2-octanone	IV	1281	128	1.0	12
43 6-methyl-5-hepten-2-one	IV	1330	126	3	100
44 7-methyloctan-2-one	IV	1337	142	0.8	5
45 2-nonanone	IV	1386	142	39	110
46 2-decanone	IV	1490	156	42	133
47 decen-2-one	IV	1521	154	3.5	11
48 9-methyldecan-2-one	IV	1542	170	20	45
49 2-undecanone	IV	1595	170	127	370
50 undecen-2-one	V	1621	168	12	76
51 2-dodecanone	IV	1697	184	44	102
52 2-tridecanone	IV	1805	198	82	240
53 tridecen-2-one	IV	1819	196	22	2
54 2-tetradecanone	IV	1907	212	26	85
55 tetradecen-2-one	V	1918	210	8	5
56 tetradecadien-2-one	V	1950	208	15	—
57 2-pentadecanone	IV	2011	226	25	30
58 pentadecen-2-one	V	2024	224	25	—
59 pentadecadien-2-one	V	2053	222	40	—
60 2-hexadecanone	IV	2112	240	5	—
aldehydes					
61 hexanal	III	1070	100	0.5	110
62 2-hexenal	IV	1122	98	—	12
63 heptanal	III	1178	114	—	26
64 nonanal	III	1386	142	—	130
65 2,4-hexadienal	IV	1408	96	—	3
66 2,4-heptadienal	IV	1510	110	—	2
67 2-nonenal	IV	1524	140	—	10
68 furfural	IV	1444	96	—	7
69 benzaldehyde	III	1502	106	0.5	8
70 2-phenylacetaldehyde	III	1625	120	—	2
71 geranial	V	1714	152	—	3
alcohols					
72 2-pentanol	VI	1010	86	—	10
73 2-methyl-3-buten-2-ol	VI	1025	86	—	480
74 2-methyl-1-propanol	VI	1075	75	—	14
75 1-butanol	VI	1128	74	—	10
76 3-methyl-1-butanol	VI	1187	88	—	5
77 1-pentanol	VI	1230	88	—	3
78 1-hexanol	VI	1305	86	—	160
79 1-hexanol	VI	1337	102	—	2
80 2-hexen-1-ol	VI	1368	100	—	3
81 1-heptanol	VI	1441	116	—	5
82 1-octanol	VI	1447	116	—	20
83 1-octen-3-ol	V	1437	128	2	15
84 2-nonanol	VI	1511	144	3	10

Table II. (Continued)

compd	LSC	I_K (CW-20M)	M_r	approx concn, ppm	
				1974	1977
terpenoids, sesquiterpenoids, lactones, ethers					
85 <i>trans</i> -linalool oxide	VI	1432	170	1	20
86 <i>cis</i> -linalool oxide	VI	1461	170	1	1.5
87 pentylfuran	I	1231	128	—	5
88 hexenylfuran	I	1415	150	15	10
89 2,2,7,7-tetramethyl- 1,6-dioxaspiro[4.4]- nona-3-ene	V	1230	182	—	15
90 2,2,7,7-tetramethyl- 1,6-dioxaspiro[4.4]- nona-3,8-diene	V	1283	180		25
91 7.7-dimethyl-6.8- dioxabicyclo[3.2.1]- octane	V	1298	142	1	27
92 hop ether	V	1362	152	1.5	13
93 karahana ether	V	1370	152	0.5	5
94 4,4-dimethyl-2- buten-4-olide	IV	1583	112	1.5	70
95 sesquiterpene epoxide 1	IV	1958	220	—	30
96 sesquiterpene epoxide 2	IV	2029	220	—	50
97 caryophyllene epoxide	V	1964	220	5	60
98 sesquiterpene epoxide 3	V	1968	220	—	5
99 sesquiterpene epoxide 4	V	1978	220	—	4
100 humulene epoxide I	V	1993	220	23	80
101 humulene epoxide II	V	2017	220	33	250
102 sesquiterpene epoxide 5	V	2064	220	—	15
103 sesquiterpene epoxide 6	V	2155	220	—	48
104 linalool	VI	1543	154	25	45
105 terpinen-4-ol	VI	1585	154	1	5
106 α -terpineol	VI	1672	154	3	20
107 β -fenchyl alcohol	VI	1568	154	—	7
108 geraniol	VI	1832	154	0.5	8
109 nerolidol	VI	2020	222	3	10
110 γ -eudesmol	VI	1978	222	1	—
111 caryolan-1-ol	VI	2019	222	6	18
112 epicubenol	VI	2037	220	16	20
113 humulol	VI	2124	222	11	88
114 δ -cadinol	VI	2167	222	16	20
115 T-cadinol	VI	2136	222	13	15
116 α -cadinol	VI	2205	222	12	25
117 humulenol II	VI	2274	220	2	290
118 β -ionone	V	1912	192	—	+
119 damascenone	V	1801	190	—	+

Table III. Changes of Fatty Acids during the Storage of Hops

compd	I_K (CW-20M)	M_r	approx concn, ppm	
			1974	1977
120 2-methylpropionic acid	918	102	16	220
121 butyric acid	975	102		5
122 3-methylbutyric acid	1020	116	165	690
123 2-methylbutyric acid				
124 pentanoic acid	1080	116	0.5	20
125 4-methylpentanoic acid	1134	130	20	40
126 pentenoic acid	1159	114	0.2	13
127 hexanoic acid	1184	130	15	140
128 4-methyl-3-pentenoic acid	1231	128	1	40
129 3-hexenoic acid	1245	128		25
130 2-hexenoic acid	1262	128		40
131 heptanoic acid	1281	144	22	95
132 4-methyl-2-hexenoic acid	1326	142	5	15
133 4-methylhexenoic acid	1330	142		17
134 6-methylheptanoic acid	1338	158	5	78
135 octanoic acid	1390	158	4	46
136 nonanoic acid	1487	172	10	46
137 nonenoic acid	1521	170	1	15
138 8-methylnonanoic acid	1543	186		5
139 decanoic acid	1592	186	3	15
140 4-decenoic acid	1622	184	10	65
141 4,8-decadienoic acid	1685	182	3	22
142 undecenoic acid	1719	198		2
143 geranyllic acid	1688	182		2
144 benzoic acid	1604	136		5

are summarized in Table III. It can be seen that 2-methylpropionic, 2-methylbutyric, and 3-methylbutyric acids increase strongly during the storage of hops. They are derived by degradation of cohumulone, adhumulone, and humulone and are responsible for the unpleasant aroma of stored hops. Pentanoic, hexanoic, octanoic, 2-hexenoic, and 3-hexenoic acids are formed by oxidation of fatty acids. Most of the other compounds may be produced by hydrolytic cleavage of the corresponding methyl esters.

The results show that the composition of hop aroma constituents changes considerably during storage. Terpene and sesquiterpene hydrocarbons are decreased by polymerization and oxidation. To some extent they are transformed into hydrophylic components such as epoxides and alcohols, which may be easily transferred to beer. These reactions are comparable to processes during the kettle hop boiling. In addition, components such as aldehydes and fatty acids, which are known as off-flavor constituents in beer, are formed.

LITERATURE CITED

Buttery, R. G., Lundin, R. E., Ling, L., *Chem. Ind.* **28**, 1225 (1963).

- Buttery, R. G., Black, D. R., Kealy, M. P., *J. Chromatogr.* **18**, 399 (1965).
 Buttery, R. G., Black, D. R., Lewis, M. J., Ling, L., *J. Food Sci.* **32**, 414 (1967).
 Drawert, F., Tressl, R., *Tech. Q., Master Brew Assoc. Am.* **9**, 72 (1972).
 Guadagni, R., Buttery, R. G., Harris, J., *J. Sci. Food Agric.* **17**, 142 (1966).
 Krüger, E., Baron, G., *Mtschr. Brauerei* **28**, 109 (1975).
 Naya, Y., *Tetrahedron Lett.*, 1645 (1968).
 Naya, Y., Kotake, M., *Bull. Chem. Soc. Jpn.* **43**, 2956 (1970).
 Naya, Y., Kotake, M., *Bull. Chem. Soc. Jpn.* **44**, 3116 (1971).
 Naya, Y., Kotake, M., *Bull. Chem. Soc. Jpn.* **45**, 2887 (1972).
 Tressl, R., Friese, L., *International Ferm. Symposium, Berlin, 1976*, p 381.
 Tressl, R., Friese, L., Fendesack, F., Krüger, E., *Mtschr. Brauerei* **31**, 83 (1978a).
 Tressl, R., Friese, L., *Z. Lebensm.-Unters. Forsch.*, in press (1978b).

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Synthesis of Nootkatone from Valencene

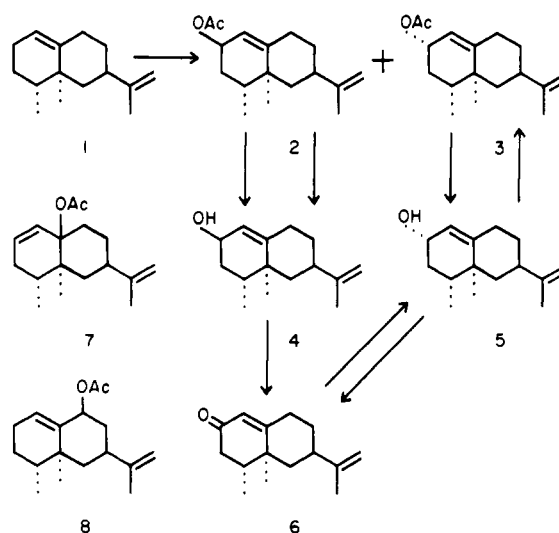
Charles W. Wilson, III* and Philip E. Shaw

Valencene (1) was converted by a three-step process to nootkatone (6) in 47% overall yield. Oxidation of 1 with *tert*-butyl peracetate afforded a mixture of esters 2 and 3 in 98:2 ratio (a/e); the esters were hydrolyzed to the corresponding alcohols 4 and 5 and the alcohols were oxidized with chromic acid to 6. The conformations of alcohol 5 and ester 3 were determined by reduction of 6 with metal hydride to 5 and acetylation of alcohol 5 with acetic anhydride/pyridine to ester 3. Thus, the *tert*-butyl peracetate oxidation of 1 to nootkatone (6) in good yield affords an alternate commercial method that provides intermediate alcohols and acetate esters with potentially valuable flavor properties and involves a less hazardous preparation of nootkatone than the presently used oxidation with chromate.

Nootkatone (6), an important flavoring constituent of grapefruit, is used commercially to flavor soft drinks and other beverages (Macleod and Buigues, 1964) and in perfumes. Nootkatone has been prepared from the sesquiterpene valencene (1) by oxidation with *tert*-butyl chromate in crude yield of 67% (Hunter and Brogden, 1965). However, this reaction is explosive if not conducted at room temperature or lower. We report a three-step synthesis of nootkatone from valencene in 47% overall yield and discuss the reaction intermediates involved. The method provides intermediate alcohols and acetate esters with potentially valuable flavoring properties and does not involve the use of *tert*-butyl chromate.

When 1 was oxidized with *tert*-butyl peracetate, the axial acetate 2 was the major reaction product (Scheme I); axial esters have been the major products of similar perester oxidation reactions (Beckwith and Phillipon, 1976; Wilson and Shaw, 1975). A small amount (ca. 2%) of the equatorial acetate 3 was also isolated by preparative thin-layer chromatography (TLC). Alternative allylic acetates 7 and 8, which are possible products of this re-

Scheme I



action, were not found, presumably because their formation was hindered sterically.

To determine the conformation of the predominant acetate 2 we converted it to alcohol 4 by treatment with lithium aluminum hydride. Comparison showed that 4

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