al., 1977b). The results of this report demonstrate that intestinal absorption of hesperetin derivatives can be reduced by nearly two orders of magnitude by increasing molecular weight and charge through O-sulfoalkylation and "dimerization". The findings also prove that absorption levels of approximately 1% can be obtained with nonpolymeric, water-soluble derivatives with molecular weights of about 1000 and charged functional groups. The data in Table II also demonstrate, indirectly, the nearly complete stability of the O-sulfoalkylated hesperetin derivatives toward the intestinal microflora metabolism typical of flavonoid compounds and previously demonstrated for hesperetin-*3*-$^{14}C$ (Honohan et al., 1976) and neohesperidin DHC (Gumbmann et al., 1976). In the case of the 4-*O*-sulfopropylhesperetin dihydrochalcone-$\beta$-$^{14}C$, stability was demonstrated by in vitro anaerobic incubations with rat cecal microflora (Table III) and by the absence of $^{14}$C-labeled phenylpropanoic acids in the urines of orally dosed animals (Table IV). It seems clear that the higher molecular weight DHC derivatives are also highly resistant to microbial degradation.

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# Gas Chromatographic–Mass Spectrometric Investigation of Hop Aroma Constituents in Beer

Roland Tressl,* Lothar Friese, Friedrich Fendesack, and Hans Köppler

More than 110 aroma constituents of a German beer, which delivered a desirable, fine hops aroma, were identified and semiquantified by means of liquid–liquid extraction, liquid–solid chromatography, and capillary gas chromatography–mass spectrometry. Forty-five constituents (among them: esters, ketones, alcohols, ethers, terpenoids, and sesquiterpenoids) were characterized for the first time in beer. Forty-seven of the determined volatiles had been found in Spalter hops.

Many beer consumers prefer beers with a distinct hops flavor, whereby, in addition to hops bitter, a fine hops aroma is also desirable. The bitter character of beer is essentially determined by the isomerization of humulone into isohumulone. At the present there are few analytical reference points as to what extent the hops aroma constituents are transferred to beer. To date more than 150 constituents of the essential oil of hops are known (van Straten and de Vrijer, 1973); however, there are few indices as to the extent of their contribution of beer flavor. The presence of the major sesquiterpenes of hop oil could not be proved by the use of presently available techniques. This led to the assumption that oxygenated hop oil constituents which have sensory relevance are also not present in beer.

In 1967 Buttery and Black identified ethyl dec-4-enoate, ethyl deca-4,8-dienoate, 2-heptanone, and ethyl heptanoate

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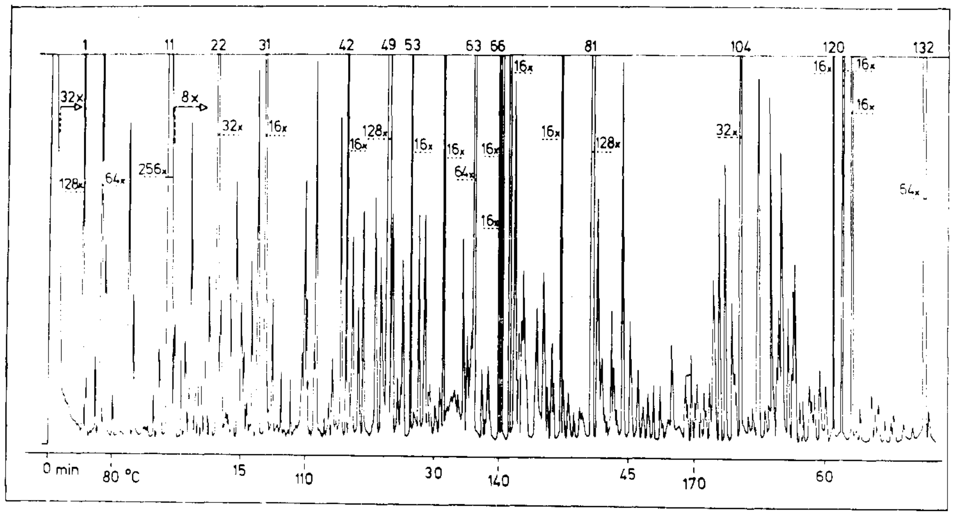


**Figure 1.** Gas chromatogram of LSC fraction IV; column CW-20M, 50 m (0.25 mm i.d.): 3-methyl-1-butanol (**11**); 7,7-dimethyl-6,8-dioxabicyclo[3.2.1]octane (**22**); hop ether (**30**); karahana ether (**31**); 2-acetylfuran (**42**); linalool (**49**); $\beta$-fenchyl alcohol (**53**); methionol acetate (**53**); 1-nonanol (**63**) (standard); $\alpha$-terpineol (**66**); 2-phenylethyl acetate (**81**); humulene epoxide I (**104**); humulol (**120**); humulenol II (**132**).

as hop-derived components in an American beer. Drawert and Tressl (1972) characterized 15 hop oil constituents (among them: linalool, $\alpha$-terpineol, nerol, caryophyllene) in a German beer. On the other hand, Sandra (1976) determined only some degradation products of humulones in a Belgium lager beer. In 1974 Shimazu et al. characterized humuladienone and the authors assumed that this constituent may be responsible for the hoppy aroma associated with beer. More than 30 terpenoids and sesquiterpenoids derived from hops were identified by Tressl and Friese (1976) in a German Pilsener beer, which possessed a distinct hoppy aroma. In the present study we investigated the aroma constituents of a Bavarian beer, brewed in Hallertau near Munich, which delivered a desirable, fine hops aroma.

## MATERIALS AND METHODS

**Sample Preparation.** The aroma components of 40 L of a German beer (Pilsener) were enriched by liquid–liquid extraction with pentane–methylene chloride (2:1). The extraction period was 24 h. The aroma extract was dried over $Na_2SO_4$ and then concentrated to a volume of 8 mL.

**Adsorption Chromatography.** Separation according to the polarity of components was carried out by liquid–solid chromatography. Extract (400 $\mu$L) was placed on cooled columns (29 × 1 cm i.d.) filled with aluminum oxide (Merck 1078, activity IV) and silica gel (Merck 7734, activity II to III) (2:1). Six fractions (100 mL each) with solvents of increasing polarity were eluted: I, pentane (P); II, pentane–methylene chloride (P/MC) (9:1); III, P/MC (3:1), IV, pentane–ether (P/E) (9:1); V P/E (1:1); VI, ether.

The fractions were concentrated to a definite volume and analyzed by gas chromatography and capillary gas chromatography–mass spectrometry. Trace components were enriched by preparative gas chromatography.

**Gas Chromatography.** Gas chromatography conditions were as described by Tressl et al. (1978). One additional column was used for preparative separations. Column 3: 4 m (4 mm i.d.) glass, 10% FFAP on Chromosorb WAW/DMCS, 60–80 mesh; temperature program, 80–250 °C, 4 °C/min.

**Capillary Gas Chromatography–Mass Spectrometry.** See Tressl et al. (1978). Additional columns: 50-m glass capillary column (0.32 mm i.d.), coated with SF-96; temperature program, 70–190 °C, 2 °C/min.

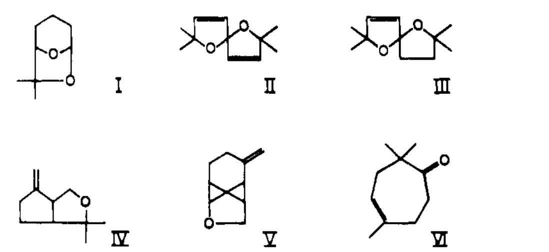


**Figure 2.** Cyclic ethers identified in beer: (I) 7,7-dimethyl-6,8-dioxabicyclo[3.2.1]octane; (II) 2,2,7,7-tetramethyl-1,6-dioxaspiro[4.4]nona-3,8-diene; (III) 2,2,7,7-tetramethyl-1,6-dioxaspiro[4.4]nona-3-ene; (IV) hop ether; (V) Karahana ether; (VI) karahanaenone.

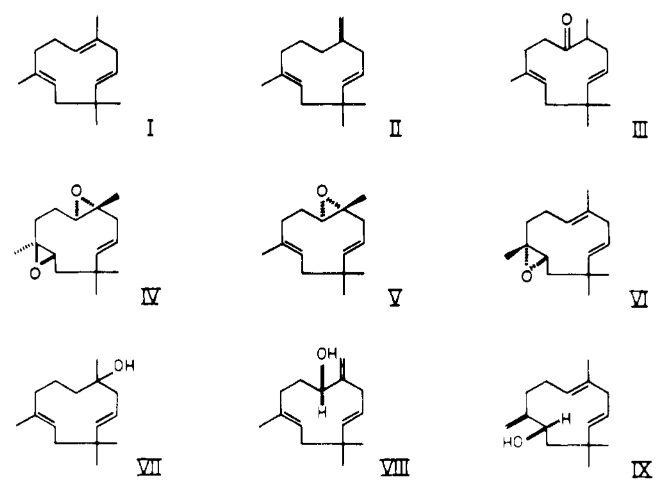


**Figure 3.** Sesquiterpenoids identified in beer: (I) $\alpha$-humulene; (II) $\beta$-humulene; (III) humuladienone; (IV) humulene dioxide; (V) humulene epoxide I; (VI) humulene epoxide II; (VII) humulol; (VIII) humulenol I; (IX) humulenol II.

## RESULTS AND DISCUSSION

The aroma components of 40 L of a German draft beer (type: Pilsener) were enriched by liquid–liquid extraction with pentane–methylene chloride, yielding an aroma concentrate of 8 mL. The extract was separated on a mixture of silica gel–aluminum oxide (2:1) into six fractions. In our opinion, the combination of LSC and GLC is most effective in tracing essential constituents in a complex mixture. In beer the hop aroma constituents are

Table I. Components Characterized in Beer

| | compd | LSC | $I_K$ (CW-20M) | $M_r$ | approx concn, ppb |
|---|---|---|---|---|---|
| esters | | | | | |
| 1 | ethyl acetate | III | 917 | 88 | 15000 |
| 2 | ethyl propionate | II | 1028 | 102 | 80 |
| 3 | ethyl 2-methyl propionate | II | 1047 | 116 | 480 |
| 4 | 3-methylbutyl acetate[b] | II, III | 1126 | 130 | 6300 |
| 5 | 3-methylbutyl propionate | II | 1184 | 144 | 15 |
| 6 | 3-methylbutyl 2-methylpropionate | I, II | 1190 | 158 | 140 |
| 7 | ethyl hexanoate | I, II | 1232 | 144 | 950 |
| 8 | hexyl acetate | II, III | 1266 | 144 | 120 |
| 9 | 3-methylbutyl 3-methylbutanoate | II | 1292 | 172 | 40 |
| 10 | 2-methylpropyl hexanoate[a] | I | 1347 | 172 | 25 |
| 11 | heptyl acetate | II | 1366 | 158 | 25 |
| 12 | ethyl octanoate | I, II | 1431 | 172 | 1500 |
| 13 | 3-methylbutyl hexanoate | I | 1453 | 186 | 420 |
| 14 | octyl acetate | II | 1469 | 172 | 30 |
| 15 | furfuryl acetate | II, III | 1524 | 140 | 60 |
| 16 | 2-methylbutyl octanoate[a] | II | 1548 | 214 | 15 |
| 17 | 3-methylbutyl heptanoate | I | 1552 | 200 | 20 |
| 18 | nonyl acetate[a] | II | 1570 | 186 | 5 |
| 19 | ethyl decanoate | I, II | 1634 | 200 | 190 |
| 20 | 3-methylbutyl octanoate | I | 1658 | 214 | 830 |
| 21 | ethyl benzoate | II | 1645 | 150 | 10 |
| 22 | 2-methylbutyl decanoate[a] | II | 1752 | 242 | 5 |
| 23 | ethyl dodecanoate | II | 1833 | 228 | 30 |
| 24 | 3-methylbutyl decanoate | I | 1851 | 242 | 95 |
| 25 | methionol acetate | IV | 1613 | 148 | 25 |
| 26 | 3-methylbutyl dodecanoate[a] | I | 2056 | 270 | 10 |
| 27 | 2-phenylethyl acetate | III, IV | 1805 | 164 | 1625 |
| 28 | 2-phenylethyl propionate[a] | III | 1890 | 178 | 10 |
| 29 | 2-phenylethyl 2-methylpropionate[a] | III | 1935 | 192 | 15 |
| 30 | 2-phenylethyl 3-methylbutanoate[a] | III | 1961 | 206 | 15 |
| 31 | 2-phenylethyl hexanoate | II, III | 2136 | 220 | 75 |
| 32 | 2-phenylethyl octanoate | II, III | 2335 | 192 | 150 |
| 33 | ethyl cinnamate | III | 2086 | 176 | 5 |
| 34 | ethyl nicotinoate | V | 1782 | 151 | 1400 |
| 35 | ethyl dec-4-enoate[c] | II, III | 1682 | 198 | 35 |
| 36 | ethyl deca-4,8-dienoate[c] | III | 1721 | 196 | 15 |
| 37 | 3-methylbutyl dec-4-enoate[a,c] | II | 1908 | 240 | 20 |
| 38 | ethyl nonenoate[a,c] | II | 1524 | 186 | 45 |
| 39 | methyl 4-methyl-hex-2-enoate[a,c] | II | 1331 | 142 | 60 |
| ketones | | | | | |
| 40 | 2-pentanone | IV | 990 | 86 | 20 |
| 41 | 4-methyl-2-pentanone[c] | III, IV | 1015 | 100 | 120 |
| 42 | 3-methyl-2-pentanone[c] | III, IV | 1023 | 100 | 60 |
| 43 | 2-heptanone[a,c] | III | 1190 | 114 | 110 |
| 44 | 2-octanone[a,c] | III | 1283 | 128 | 10 |
| 45 | 6-methyl-5-hepten-2-one[a,c] | III, IV | 1332 | 126 | 50 |
| 46 | 2-nonanone[a,c] | III | 1385 | 142 | 30 |
| 47 | 2-undecanone[a,c] | III | 1592 | 170 | 1 |
| 48 | 3-hydroxy-2-butanone | V, VI | 1270 | 88 | 420 |
| 49 | 3-hydroxy-2-pentanone | VI | 1345 | 102 | 50 |
| alcohols, phenols | | | | | |
| 50 | 2-methylpropanol | V | 1085 | 74 | 4800 |
| 51<br>52 | 3-methylbutanol<br>2-methylbutanol | V | 1197 | 88 | 84000 |
| 53 | pentanol | V | 1236 | 88 | 150 |
| 54 | hexanol | V | 1339 | 102 | 330 |
| 55 | 3-hexenol[a] | V | 1345 | 100 | 25 |
| 56 | 2-hexenol[a] | V | 1368 | 100 | 20 |
| 57 | 1-octen-3-ol | IV | 1437 | 128 | 30 |
| 58 | furfuryl alcohol | V | 1640 | 98 | 1200 |
| 59 | methionol | V | | 106 | 320 |
| 60 | 2-phenylethanol | V | 1888 | 122 | 1800 |
| 61 | heptanol-2[c] | IV | 1307 | 116 | 15 |
| 62 | octan-2-ol[c] | IV | 1405 | 133 | 5 |
| 63 | nonan-2-ol[c] | IV | 1508 | 144 | 10 |
| 64 | decan-2-ol[c] | IV | 1606 | 158 | 5 |
| 65 | 4-vinylguaiacol | V | 2145 | 150 | 100 |
| 66 | 4-vinylphenol | V | 2317 | 120 | 75 |
| lactones | | | | | |
| 67 | 4-butanolide | V, VI | 1595 | 86 | 1300 |
| 68 | 4-hexanolide | V | 1669 | 114 | 20 |
| 69 | 4,4-dimethylbutan-4-olide[c] | V | 1558 | 114 | 160 |
| 70 | 4,4-dimethyl-2-buten-4-olide[c] | V | 1583 | 112 | 1750 |
| 71 | 4-heptanolide | V | 1767 | 128 | 15 |
| 72 | 4-octanolide | V | 1881 | 142 | 20 |

Table I (*Continued*)

| | | | | | |
|---|---|---|---|---|---|
| 73 | 4-nonanolide | V | 1993 | 156 | 320 |
| 74 | 4-decanolide | V | 2096 | 170 | 20 |
| 75 | dihydroactindiolide[a,c] | V | 2258 | 180 | 30 |
| | other constituents | | | | |
| 76 | *o*-xylene | I | 1136 | 106 | 20 |
| 77 | styrene | I | 1248 | 104 | 70 |
| 78 | methylethylbenzene | I | 1275 | 120 | 20 |
| 79 | naphthalene | I | 1709 | 128 | 15 |
| 80 | benzaldehyde | III | 1505 | 106 | 10 |
| 81 | 2-phenylacetaldehyde | IV | 1624 | 120 | 5 |
| 82 | furfural | IV | 1445 | 96 | 25 |
| 83 | 2-acetylfuran | IV | 1482 | 110 | 40 |
| 84 | 2-methyltetrahydrofuran-3-one[a] | IV | 1251 | 100 | 25 |
| 85 | 2-methyltetrahydrothiophen-3-one | III | 1510 | 116 | 5 |

[a] Components identified for the first time in beer. [b] 3-Methylbutyl acetate and 2-methylbutyl acetate. [c] Components found in hops.

Table II. Terpenoids and Sesquiterpenoids Characterized in Beer

| | component | LSC | $I_K$ (CW-20M) | $M_r$ | mass spectral data | approx concn, ppb |
|---|---|---|---|---|---|---|
| 86 | 2,2,7,7-tetramethyl-1,6-dioxaspiro[4.4]nona-3-ene[a] | IV | 1236 | 182 | 109, 113, 167, 43, 69, 95 | 5 |
| 87 | 2,2,7,7-tetramethyl-1,6-dioxaspiro[4.4]nona-3,8-diene[a] | IV | 1288 | 180 | 165, 43, 122, 96, 41, 123 | 10 |
| 88 | 7,7-dimethyl-6,8-dioxabicyclo[3.2.1]octane[a] | IV | 1302 | 142 | 43, 84, 71, 41, 55, 27 | 50 |
| 89 | hop ether[a] | III, IV | 1360 | 152 | 79, 107, 43, 122, 39, 41 | 35 |
| 90 | karahana ether[a] | III, IV | 1368 | 152 | 107, 41, 79, 121, 122, 43 | 60 |
| 91 | *trans*-linalool oxide[a] | IV | 1432 | 154 | 59, 43, 55, 68, 41, 94 | 20 |
| 92 | humuladienone | III | 1952 | 220 | 96, 138, 41, 109, 67, 82 | 10 |
| 93 | caryophyllene epoxide[a] | IV | 1966 | 220 | 41, 69, 81, 93, 107, 95 | 18 |
| 94 | humulene epoxide I[a] | III, IV | 1972 | 220 | 93, 43, 41, 80, 121, 55 | 125 |
| 95 | humulene epoxide II[a] | III, IV | 2011 | 220 | 109, 43, 41, 138, 67, 55 | 40 |
| 96 | linalool | IV | 1541 | 154 | 41, 71, 43, 93, 69, 55 | 470 |
| 97 | β-fenchyl alcohol[a] | IV | 1569 | 154 | 81, 80, 43, 69, 41 | 40 |
| 98 | terpinen-4-ol | IV | 1587 | 154 | 71, 43, 55, 41, 93, 111 | 15 |
| 99 | α-terpineol | IV | 1677 | 154 | 59, 93, 43, 121, 81, 41 | 40 |
| 100 | citronellol[a] | IV | 1755 | 156 | 41, 69, 55, 67, 95, 81 | 10 |
| 101 | geraniol[a] | IV | 1833 | 154 | 41, 69, 93, 55, 84, 111 | 5 |
| 102 | caryolan-1-ol[a] | V | 2019 | 222 | 111, 55, 41, 123, 81, 95 | 25 |
| 103 | nerolidol[a] | IV | 2021 | 222 | 41, 69, 43, 55, 93, 71 | 25 |
| 104 | junenol[a] | III | 2028 | 222 | 109, 41, 161, 81, 149, 55 | 5 |
| 105 | epicubenol[a] | IV | 2039 | 220 | 119, 161, 204, 105, 41, 43 | 20 |
| 106 | caryophyllenol[a] | III | 2033 | 220 | 43, 41, 93, 91, 135, 95 | 5 |
| 107 | *T*-cadinol[a] | IV | 2136 | 222 | 161, 204, 43, 81, 95, 105 | 45 |
| 108 | humulol[a] | IV, V | 2124 | 222 | 82, 43, 81, 41, 67, 93 | 220 |
| 109 | δ-cadinol[a] | IV | 2150 | 222 | 161, 43, 119, 204, 105 | 35 |
| 110 | humulenol II[a] | IV, V | 2234 | 222 | 41, 69, 82, 67, 43 | 1150 |
| 111 | β-ionone[a] | IV | 1912 | 192 | 43, 177, 41, 91, 93, 136, 77 | 1 |
| 112 | damascenone[a] | IV | 1801 | 190 | 69, 121, 41, 105, 91, 190 | tr |
| 113 | *cis*-jasmone[a] | IV | | 164 | 81, 149, 164, 135, 95 | 10 |

[a] Components identified for the first time in beer. All components presented in Table II were found in Spalter hops.

masked by the strongly concentrated compounds derived from the yeast metabolism as well as from Maillard reactions. It may be one of the reasons why most investigators failed to prove the existence of hop aroma constituents in beer. The six LSC fractions were concentrated to a definitive volume and examined by GLC and capillary gas chromatography–mass spectrometry. Trace constituents were isolated and enriched by preparative gas chromatography. The results are summarized in Tables I and II. It can be seen that the esters are cleanly separated into fractions I–III and the alcohols and lactones are transferred into fraction V. The free fatty acids are retained on the aluminum oxide. The fraction IV contained terpenoids and sesquiterpenoids and possessed an intensive, pleasant, hoppy aroma. Neither terpene and sesquiterpene hydrocarbons, nor methyl esters, characterized in hop oil, were detected in the aroma concentrate. Some of the methyl esters identified in hops like methyl dec-4-enoate, methyl deca-4,8-dienoate, and methyl nonenoate are obviously transesterified to the ethyl esters by the yeast. This has been proved with model systems. Besides transesterification we observed reduction of ketones to secondary alcohols and adsorption of lipophilic aroma constituents by the yeast (Friese, 1977).

The LSC fraction IV contained most of the aroma constituents derived from hops. Figure 1 shows a gas chromatogram of the strongly concentrated sample. It can be seen that many oxygenated terpenes and sesquiterpenes are strongly concentrated compounds which have been found in hops. Some of the identified constituents are presented in Table II. Five cyclic ethers (compounds 86–90), first identified by Naya and Kotake (1967, 1968) in Japanese hops, are summarized in Figure 2. Some of them possess flowery notes and are hops aroma contributing constituents. Sesquiterpenoids derived from humulene are shown in Figure 3. Humuladienone (92) was 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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# 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.

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## 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 $Na_2SO_4$, 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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