Detection of Terpene Compounds from Hops in American Lager Beer

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ABSTRACT

Analytical data for identification of three terpenoid compounds attributed to hops in American lager beer are presented. Glass capillary-column gas chromatography and computer analysis of mass spectrometric data were employed to facilitate identification of linalool, α-terpineol, and myrcene. Concentrations of each were estimated from gas chromatographic data, and this indicated that linalool should have the greatest influence of beer flavor since its concentration appeared to exceed that of its odor threshold of 6 ppb in water.

The flavor chemistry of beer has received much attention in recent years, the source of flavor compounds and their effect on taste continues to be an important part of this research. Hops contain abundant volatile flavor compounds, and in the past periodically have been considered substantial contributors to beer flavor. However, confusion has arisen because several reports in the literature have stated that essentially none of the many volatile flavor compounds found in hops survive the brewing process, especially wort boiling, and appear in the finished beer.

In the early 1960's Harold et al. (8) reported finding terpene compounds in Australian beer which they attributed to hops. Their identifications were based on gas chromatographic retention volumes in chromatograms showing only a few separated peaks. In light of more recent knowledge concerning the complexity of beer flavor profiles such identifications are doubtful. Subsequently, Likens and Nickerson (9) devised a distillation apparatus to specifically concentrate and isolate volatile hop flavor compounds in wort and beer. Several hop compounds were believed found in one commercial beer sample, and these were reported to be myrcene, methylbutyl isobutyrate, methyl octanoate, methyl deca-4-enoate, β-caryophyllene, methyl deca-4, 8-dienoate, humulene, and farnesene.

Later, Buttery et al. (1,2) used gas chromatography (GC) and mass spectrometry (MS) to separate and indentify more than 75 volatile hop oil components. and then followed this with a report of more than 90 compounds in the oxygenated fraction of hop oil alone (7). These investigators were unsuccessful in their attempts to isolate hop terpene compounds from finished beer. Two esters, ethyl dec-4-enoate and ethyl deca-4, 8-dienoate which were probably transesterified during brewing, were the only components found in beer which could be traced back to hops.

More recently Drawert and Tressl (5) have included a few terpene compounds as flavor components of European beer, but analytical data were not presented. Following this initial report, Tressl and Friese (15) reported that they found some natural oxygenated and oxidized terpenes in European beer, but did not observe any terpene hydrocarbons in these products. They attributed much of the loss of hop terpenes to adsorption to yeast cells rather than to losses in wort boiling. Sandra (12) and Sandra and Verzele (13) have also recently analyzed European beers, and have concluded that they could not routinely detect hop compounds. They further concluded that individual hop compounds could not be responsible for the hoppy aroma note of beer, but that collectively many trace hop compounds could have an influence on beer flavor.

This report gives analytical data from our laboratory for the isolation and indentification of linalool, α-terpineol and myrcene from an American lager which was lightly hopped beer. Linalool (3,7-methyl-6, 1-octadien-3-ol) is a tertiary alcohol while α-terpineol (p-menth-1-en-8-ol) is a monocyclic terpene alcohol. Myrcene (7-methyl-3-methylene-1,6-octadiene) is an acyclic terpene hydrocarbon which comprises the major portion of hop oil. These terpene compounds were encountered during the detailed analysis of volatile compounds in staling beer.

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2Present Address: Yeast Products Research, Anheuser-Busch, Inc., St. Louis, Missouri 63118.
MATERIALS AND METHODS

American lager beer containing hop extract was purchased locally, and was subjected to accelerated staling by holding 120 h at 40-45 C(4). The contents of a 12-oz. bottle of staled beer were poured into a separatory funnel without further treatment. Two successive 40-ml aliquots of chloroform were used to extract flavor compounds. This extract was dried over excess sodium sulfate, and the volume was brought to approximately 5 ml with a rotary evaporator under reduced pressure. Further evaporative concentration was achieved at room temperature by using a stream of nitrogen gas directed towards the surface of the extract. The sample was chromatographed on a 2.04-mm I.D. x 3.8-m packed stainless steel Carbowax 20 M column. The major portion of separated components by-passed the GC flame ionization detector through splitter arrangement, and were collected in glass capillary tubes submerged in liquid nitrogen. In this way the sample was divided into several fractions, and each sealed in a glass capillary tube.

The collected fractions containing the terpenoids were analyzed with a Varian 1740 GC equipped with 0.79-mm I.D. x 185-m glass capillary column coated with Carbowax 20 M which was interfaced to a Du Pont Model 21491 spectrometer. Mass spectra were recorded, and background was automatically subtracted by computer.

RESULTS AND DISCUSSION

The mass spectrum of linalool obtained during the gas chromatographic separation is shown in Fig. 1. This spectrum agrees closely with those for authentic linalool reported by Dieckmann and Palamand (4) and Friedel et al. (6). The characteristic features of the spectrum are peaks at m/e 71 (100), 41 (87), 93 (77), 80 (38), 69 (36), and 55 (32). Linalool is typical of alcohols in that it does not give molecular ion peak (m/e 136), but it does show a peak at m/e 136 due to loss of the hydroxyl group plus a proton (M-18). The presence of small peaks at m/e 31, 59 and 73 also support the interpretation as an oxygenated compound.

Identification of linalool from the beer extract was further verified by its retention index on Carbowax 20 M using the method of Van Den Dool and Kratz (4). This method uses ethyl esters of n-aliphatic acids and is very convenient because most of the ethyl esters are present in the volatile compound profile of beer. An authentic sample of linalool gave a retention index of 8.97 while the chromatographed peak from beer was 8.95.

The experimental mass spectrum of α-terpineol is shown in Fig. 2, and agrees with those published by Dieckmann and Palamand (4) and others (6). The spectrum contains major peaks at m/e 59 (100), 93 (67), 121 (45), 43 (38), 81 (37) and 136 (25). α-Terpineol behaves similarly to linalool in not showing a molecular ion peak (m/e 154), but does show mass fragments for the loss of one hydroxyl group (m/e 136) and for the loss of a methyl group (m/e 121). The retention index of the experimental peak was 10.50 while an authentic α-terpineol sample had a value of 10.55.

The presence of myrcene was verified by its retention index of 5.30 which matched exactly that of an authentic sample. The experimental chromatographic peak was spectral pattern even after background had been subtracted. However, major peaks at m/e 41, 93, 69 and 39 were present in addition to smaller characteristic masses at m/e 77, 79, 80, 91, 92 and 94. The spectrum matched other published data (4) well enough to assure a positive identification. Myrcene does not give a significant molecular ion peak (m/e 136) because the double bond structure decreases molecular stability and results in ready fragmentation. The prominent ion of m/e 69 is characteristic of terminal isopentenyl cleavage in acyclic monoterpenoid compounds, such as myrcene and linalool.

While these terpenoids were identified during the analysis of stale beer, it is not implied that staling had any special significance. Based on the knowledge of the composition of hop oil (7), these compounds would be expected to derive from hops, and there was no indication that they were formed during staling. Initial studies of both fresh and staled beer using stainless steel 2.04-mm I.D. packed Carbowax 20 M columns did not reveal the presence of terpene compounds. The identifications were made possible by the increased inertness and higher resolution obtained through use of a glass, open-tubular capillary column which was used during more recent studies of stale beer.
Even with the use of a glass capillary column, linalool was not always separated completely from its neighboring peaks. It was sometimes observed as a shoulder on a much larger peak attributed to isobutyric acid. The separation was also further complicated by the presence of ethyl nonanoate which eluted close to this position on the chromatograph pattern. Alpha-terpineol was also only slightly separated from a gamma-lactone which preceded it by 0.03 retention index units.

Some physical properties of the three terpenoids are listed in Table 1. Based on apparent gas chromatographic data, linalool was considered most likely to have a potential effect on beer flavor of the three terpenoid compounds identified. This compound has a distinct odor which resembles the floral woodiness of rosewood, but also contains a spicy note. Buttery and Ling (2) have found that linalool comprises less than 1% of typical hop oil, but its odor threshold is relatively low at 6 ppb in water. Since it is an unsaturated alcohol, it exhibits a much lower threshold than its saturated counterparts (11). By contrast, myrcene which has been reportedly identified in a beer sample (9) comprises about 63% of hop oil and has an odor threshold of 13 ppb (3, 7).

**Table 1. Properties of terpenoid compounds isolated from beer.**

<table>
<thead>
<tr>
<th>Property</th>
<th>Linalool</th>
<th>a-Terpineol</th>
<th>Myrcene</th>
</tr>
</thead>
<tbody>
<tr>
<td>Molecular weight (amu)</td>
<td>154</td>
<td>154</td>
<td>136</td>
</tr>
<tr>
<td>Boiling point (°C)</td>
<td>198</td>
<td>218</td>
<td>167</td>
</tr>
<tr>
<td>Odor threshold, ppb</td>
<td>6</td>
<td>350</td>
<td>13</td>
</tr>
<tr>
<td>Retention Index&lt;sup&gt;2&lt;/sup&gt;</td>
<td>8.97</td>
<td>10.55</td>
<td>5.30</td>
</tr>
<tr>
<td>Odor character</td>
<td>rosewood, terpentine, carrot tops, spicy</td>
<td>antiseptic</td>
<td>terpenoid</td>
</tr>
</tbody>
</table>

<sup>1</sup> In water.

<sup>2</sup> See text.

Superficially, it may seem unlikely that linalool, which is a component in low concentration in hop oil, should be found in beer while myrcene, the major component has not been routinely detected. Myrcene is a hydrocarbon that is less soluble in water and could be more completely distilled out of wort during boiling. On the other hand linalool is more water soluble, and coupled with its higher boiling point could be retained to a greater extent. Further, Dieckman and Palamand (4) have shown that the linalool concentration can increase through air oxidation of myrcene which is favored during storage of dried hops.

Although no specific effort was made to quantify linalool, it was estimated to be present in the low ppb range. This was based on the fact that the linalool peak was present at approximately 10% of the quantity of the 2-furfural peak (retention index, 8.22). McDougal et al. (10) reported the level of 2-furfural in beer punished in a similar manner to be about 340 ppb. From this basis, 34 ppb of linalool was estimated to be present in the current beer sample. Tressl and Friese (13) reported a level of 60 ppb of linalool in a European beer. An estimation of the amount of linalool added to wort for American lager beer through hops was calculated from the data of Guadagni et al. (7) and Buttery and Ling (2). Hops contained 1% of volatile oil which included 0.15 - 0.65% as linalool, and this would yield between 17 and 73 ppb of linalool if used at a common level of 1.12 g/L in American lager beer.

Meilgaard (11) and Sandra and Verzele (13) have reported a taste threshold of 80 and 100 ppb, respectively for linalool when added to beer. The estimated 34 ppb level found in the experimental beer would be well below these reported thresholds, but would be present at a level well above its threshold of 6 ppb in water (2). Further, its role in possible subthreshold interactions with other flavor components is not known at this time. The possibility for exceeding a threshold level of linalool in beer is real considering the amount added to wort through hop flavoring. This is especially true for European lager beers which are brewed using up to three times the hopping rate of their American counterparts, or for ales which employ about eight times more hops in brewing.

The myrcene was estimated to be present at 7 ppb or about half its odor threshold value in water (7). This concentration is dramatically below the calculated amount of 7066 ppb that is available from hops used in American lager beers. This is the first fully documented report of myrcene in lager beer as Tressl and Friese (13) and Sandra and Verzele (13) have recently stated they were unable to detect any hop-derived terpene hydrocarbons in European beer.

Very little has been reported about a-terpineol in beer and hop literature. This compound was not indentified by Sandra and Verzele (13), Tressl and Friese (13), or during the extensive hop and beer flavor studies at the USDA Western Regional Research Laboratory (1, 2, 7). However, Dieckmann and Palamand have shown that in a model system myrcene cyclizes primarily to limonene which can then be oxidized by air to a-terpineol (4). Buttery et al. (3) has reported the odor threshold of a-terpineol in water to be 350 ppb while Meilgaard (11) found a taste threshold of 2,000 ppb when it was added to beer. The current beer sample contained approximately 20 ppb. This final concentration is greater than that found for myrcene, and probably reflects lower losses because of a higher boiling point and greater solubility in wort rather than a high concentration hop oil. In this respect it is similar to linalool. However, its effect on beer flavor would be expected to be much less because of its higher flavor threshold value.

This study has shown that sensitive techniques reveal the presence of ppb levels of terpenes in American lager beers. The actual amount of terpenes obtained from hops in beer logically depends upon the variety, the amount added to wort, the length of wort boiling, adsorption on yeasts, and the methods of wort filtration. Detailed research will be required to determine the ultimate role of the ppb levels of linalool and other hop-derived volatile constituents in the interactive flavor impressions of beer.
REFERENCES