Characterization of Some C_{15} Constituents of Hop Oil

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A number of C_{15} components of hop oil have been isolated by conventional and capillary gas-liquid chromatography separation and characterized using infrared absorption, nuclear magnetic resonance, and mass spectrometry, combined in some cases with data from ozonolysis and other chemical methods. Beside the previously reported hop sesquiterpene hydrocarbons—humulene, caryophyllene, farnesene, and β-selinene—evidence was found for the identities of others as copaene, α-selinene, and δ-cadinene and the probable identities of more as γ-cadinene, selina-4(14), 7(11)-diene, and selina-3,7(11)-diene. A number of oxygenated sesquiterpenoids were detected. The major ones were identified from their infrared spectra as humulene epoxide II and humulenol. Evidence for the probable identities of C_{15} di- and tri-unsaturated straight-chain ketones as pentadeca-6,9-dien-2-one and pentadeca-6,9,13-trien-2-one was discovered also.

The brewing industry generally accepts that the volatile part of hops, usually called hop oil, is responsible for the hop character of beer aroma. To determine which hop oil components contribute this character, discovery of their identity is necessary first in order to follow them through the brewing process and understand what changes they might undergo.

Considerable data have been accumulated on the identities of hop oil constituents (*1*, *2*, *4*, *10*). However, knowledge of the characteristics of the C_{15} components of hops was limited because the more than 27 C_{15} compounds were difficult to separate and, when separated, required considerable study beause of their complex nature and the lack of basic analytical reference data.

Other workers had shown that the four major C_{15} constituents of hops were the sesquiterpene hydrocarbons humulene, caryophyllene, farnesene (*18*), and β-selinene (*19*). One oxygenated C_{15} component had been isolated some years ago (*6*), a sesquiterpene alcohol, luparenol, but no structural determination had been made. Two C_{15} mono epoxides had been detected more recently and identified by gas-liquid chromatography (GLC) retention time data as humulene and caryophyllene epoxides (*15*).

A study was made to separate and identify the previously unidentified C_{15} constituents of hops and to try to predict from some experiments with model systems their role in lager aroma. Some preliminary results of this work were reported (*3*).

Experimental

Materials. Hop oil was obtained by the steam distillation of hops according to the method of Wright and Connery (*23*). Oregon-grown Bullion variety hops were used for the main part of the work.

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Hop oil was separated into hydrocarbon and oxygenated fractions by selective adsorption on silica gel (*2*). Hydrocarbons were eluted with pentane, whereas oxygenated components were held on the column and later eluted with ether. Distillation of the hydrocarbon fraction under vacuum gave the sesquiterpene hydrocarbon fraction—b.p. 90° to 130° C. at 3 mm.

Authentic samples of copaene, δ-cadinene, α- and β-selinenes, and β-santalene were obtained from commercially available oils—copiaba, ylang ylang, celery seed, and sandalwood, respectively. The oils generally were first chromatographed on silica gel to obtain the hydrocarbon fraction and the above compounds separated by GLC and compared with published data.

Gas-Liquid Chromatography (GLC). Packed columns used were: 10-foot long by $^3/_8$-inch O.D. aluminum and 10-foot long by $^1/_4$-inch O.D. stainless steel both packed with 60- to 80-mesh Chromsorb P coated with silicone SF96(100) (10% by weight of Chromosorb) and Carbowax 20M (0.1% by weight of Chromosorb); 10-foot long by $^3/_8$-inch O.D. aluminum and 10-foot long by $^1/_4$-inch O.D. stainless steel packed with 60- to 80-mesh Chromosorb P coated with Carbowax 20M (10% by weight of the Chromosorb); 6-foot long by $^3/_8$-inch O.D. aluminum packed with 60- to 80-mesh Chromosorb coated with ethylene glycol succinate polyester (15% by weight of Chromosorb); 3-foot long by $^1/_4$-inch O.D. stainless steel packed with 60- to 80-mesh Chromosorb coated with Apiezon M (15% by weight of Chromosorb).

Three principal types of capillary columns were used, a 0.01-inch I.D. by 150-foot long stainless steel capillary coated with silicone SF96(100) with Igepal CO-880 (5% by weight of the silicone) as tail reducer; a 0.03-inch I.D. by 1000-foot long stainless steel capillary coated with silicone SF96(50) with Igepal CO-880 (5% by weight of the silicone); a 0.03-inch I.D. by 500-foot long stainless steel capillary coated with Apiezon M containing 5% by weight Igepal CO-880.

Injectors were of stainless steel and were heated at 200° C. A split stream injector with a split of $^1/_{300}$ was used for the 0.01-inch I.D. capillary. Detectors

were thermal conductivity in the case of the packed columns and flame ionization in the case of the capillaries.

For collection of samples from the 0.03-inch I.D. capillaries, the effluent was split leading a small percentage to a flame ionization detector and the rest to a collecting outlet or by passing the total effluent through a thermistor thermal conductivity detector.

Separation of Sesquiterpenes. The sesquiterpene hydrocarbon fraction was first resolved into six main fractions by packed column GLC separation. Usually this was done initially on the 10-foot by $^3/_8$-inch Carbowax 20M column (column temperature 160° C., injector temperature 200° C.). Fractions separated from the $^3/_8$-inch column were then purified further through the 10-foot by $^1/_4$-inch Carbowax 20M column (column temperature 160° C., injector 200° C.). Each of the packed column fractions was further resolved by GLC separation either on the 1000-foot by 0.03-inch I.D. silicone capillary or the 500-foot by 0.03-inch I.D. Apiezon capillary using column temperatures of 140° to 160° C. and injector temperature 200° C. Separated samples were sealed in small tubes and stored at −30° F.

GLC of Humulene Epoxide and Humulenol. Analytical work was carried out on the 150-foot by 0.01-inch I.D. silicone capillary programmed at 100° to 160° C. at $^1/_2$° per minute. Samples were separated for infrared absorption spectrometry using the 3-foot by $^1/_4$-inch Apiezon-packed column at 160° to 180° C. with the injector at 200° C.

Isolation of C_{15} Unsaturated Methyl Ketones. The C_{15} ketone fraction was separated from the other oxygenated components of the hop oil oxygenated fraction by gas chromatography separation on the 10-foot by $^3/_8$-inch O.D. silicone column (as a single peak). Alcohols were then removed from this fraction by rapid chromatography on neutral alumina, the ketones being eluted with ether. The ketones were then resolved into three main fractions (peaks) by gas chromatography separation on the 6-foot by $^3/_8$-inch O.D. ethylene glycol succinate polyester column.

Infrared Absorption (IR) Spectra. IR spectra were generally run as a film between two micro salt plates (laboratory constructed) with a grating infrared spectrophotometer. Infrared spectra are reported in microns with the size of maxima abbreviated as S meaning strong, M for medium, and W for weak. Spectra found for the compounds isolated from hops are listed below.

Peak 41 (copaene): S 6.75, 6.85, 7.15, 7.21, 12.7; M 6.0, 7.5, 8.6, 10.3, 11.0; W 7.6, 7.75, 7.87, 8.2, 8.8, 8.9, 9.0, 9.2, 9.4, 9.5, 9.8, 10.9, 11.3, 11.6. This was the same as that of an authentic sample of copaene isolated from copiaba oil.

Peak 54 (β-selinene): S 6.1, 6.87, 6.92, 7.2, 11.3; M 5.6, 7.0, 7.9, 8.55, 8.7, 9.6, 10.8, 11.6, 12.7; W 7.5, 7.7, 8.2, 9.1, 10.2, 10.4, 10.5, 10.75. This was the same as that of an authentic sample of β-selinine isolated from celery seed oil.

Peak 56 (α-selinene): S 6.1, 6.85, 6.95, 7.25, 11.3; M 8.2, 8.65, 8.8, 9.35, 9.7, 10.0, 10.65, 11.8, 12.6; W 7.4, 7.7, 7.9, 10.25, 10.5, 12.0, 12.2. This was the same as that of an authentic sample of α-selinene isolated from celery seed oil (*16*).

Peak 58 (γ-cadinene): S 6.05, 6.8, 6.9, 7.2, 7.3, 11.3; M 6.0, 7.25, 8.0, 8.2, 8.6, 9.3, 9.8, 12.0, 12.2, 12.6; W 5.6, 7.1, 7.6, 7.75, 7.85, 8.45, 8.9, 9.2, 10.0, 10.4, 10.6. This was consistent with a spectrum published for γ-cadinene (*14*).

Peak 60 (δ-cadinene): S 6.8, 6.9, 6.95, 7.2, 7.25, 7.3; M 8.0, 8.5, 8.8, 8.95, 9.25, 9.55, 11.3, 11.45, 12.0, 12.5, 13.2; W 6.0, 6.1, 7.6, 8.25, 8.65, 9.05, 9.4, 9.8, 10.0, 10.5, 13.6. This was the same as that of an authentic sample of δ-cadinene isolated from ylang ylang oil.

Peak 61 [selina-4(14),7(11)-diene]: S 6.1, 6.95, 7.22, 11.4; M 7.9, 8.2, 8.6, 8.7, 9.8, 10.6, 11.7, 12.4; W 5.64, 7.1, 7.8, 8.8, 9.1, 9.2, 9.4, 9.6, 10.0, 10.2.

Peak 62 [selina-3,7(11)-diene]: S 6.85, 6.92, 7.25, 12.5; M 8.15, 8.6, 8.8, 9.4, 11.75; W 6.1, 7.4, 7.5, 7.6, 7.7, 7.85, 8.05, 8.3, 8.95, 9.25, 9.7, 9.9, 10.0, 10.2, 10.7, 11.4, 13.2.

Peak 70 (humulene epoxide II): S 6.85, 6.9, 7.2, 7.3, 10.3, 12.2; M 5.85, 6.1, 8.0, 8.5, 9.1, 9.35, 11.62; W 7.8, 9.9, 10.0, 10.6, 11.0, 11.1, 11.4, 12.1, 12.9. This was the same as that of a sample prepared by perphthalic acid oxidation of humulene and purified by GLC.

Peak 73 (humulenol): S 6.9, 7.2, 7.3, 8.6, 9.2, 9.6, 10.0, 10.2, 10.3, 11.1; M 6.1, 7.8, 11.9; W 5.56, 5.8, 10.7, 11.8, 12.4. This was the same as that prepared according to Damodaran and Dev (*7*) and consistent with that published (*13*).

Proton Magnetic Resonance (PMR) Spectra. Most spectra were taken at 60 Mc.p.s. (10^6 cycles per second) in carbon tetrachloride solution in spherical cavity microcells. The CAT technique (computer of average transients) was used in most cases. Some spectra were taken at 100 Mc.p.s. All PMR data are given in parts per million (δ) referred to internal tetramethylsilane. Data obtained for C_{15} compounds isolated from hops are listed below.

Peak 41 (copaene): PMR peaks at 0.80 and 0.87 (total 9H); a group of overlapping peaks 1.3 to 1.8 with a sharp maximum at 1.67 (total 11H); a peak at 2.17 (3H); a single peak at 5.17 (approximately 1H). This was consistent with that published for copaene (*12*). A 100-Mc.p.s. spectrum of peak 41 was the same as that of an authentic sample of copaene from copaiba oil.

Peak 56 (α-selinene): Peaks at 0.82 (3H); 1.73 (6H); peaks at 0.9, 1.0, 1.4, 1.6, 1.9, 2.0; 4.68 (2H); 5.29 (1H). This spectrum was the same as that of an authentic sample of α-selinene isolated from celery seed oil.

Peak 58 (γ-cadinene): 100-Mc.p.s. spectrum peaks at 0.70, 0.78, 0.90, 0.97 (total 6H); sharp peak at 1.64 (4H) above broad absorption from 1.5 to 1.7; other peaks at 1.8, 1.82, and 1.96 (2H); 4.47 and 4.57 (total 2H); 5.48 (1H).

Peak 60 (δ-cadinene): 100-Mc.p.s. spectrum peaks at 0.76, 0.83, 0.94, 1.0 (total 6H); 1.62 (approximately 7H); 1.91 (approximately 2H); 5.32 (approximately 1H) plus a number of small overlapping peaks particularly between 1.4 and 2.4 which were barely above noise level. An authentic sample of δ-cadinene iso-

lated from ylang ylang oil showed the same PMR features in a 60 Mc.p.s. spectrum.

Peak 61 [selina-4(14),7(11)-diene]: Peaks at 0.82 with three minor peaks at 0.74, 0.86, 0.98 (total 5.7H); 1.67 (16H); 4.47, 4.70 (2H); others at 1.1, 1.41, and a broad triplet at 2.41.

Peak 62 [selina-3,7(11)-diene]: peaks at 0.87 (3.1H); 1.68 (9H); 5.27 (0.8H); a triplet at 2.62 (2H); a possible triplet at 1.33 and a broad peak at 1.97.

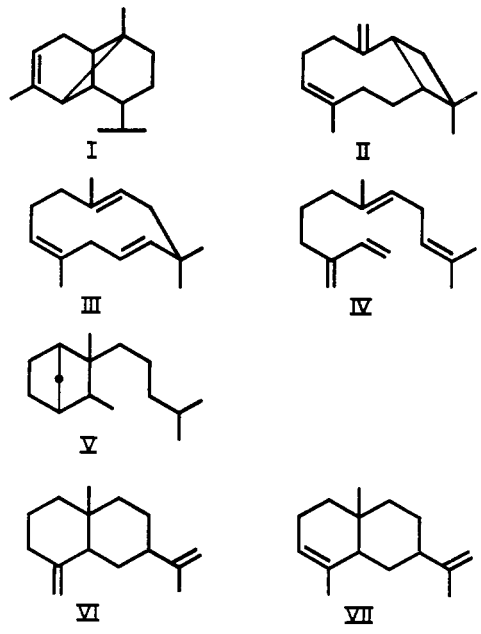
Results and Discussion

Sesquiterpene Hydrocarbons. Analysis of hop oil by the direct combination of capillary gas-liquid chromatography (GLC) with mass spectrometry (MS) detected a total of about 15 sesquiterpene hydrocarbons. At that time three of these had been previously identified (*18*) as caryophyllene (II), humulene (III), and farnesene (IV). In 1964 another sesquiterpene, β-selinene (VI), was also identified from an abnormal variety of hops by Stevens (*19*). The sesquiterpene hydrocarbon fraction was separated from hop oil by silica gel chromatography and vacuum distillation as described under Experimental.

The solid curve in Figure 1 shows a capillary GLC analysis of such a sesquiterpene fraction from a Bullion variety of hops. The two large peaks at 47 and 50 are caryophyllene and humulene. Farnesene is almost absent from this variety and is an insignificant peak between 50 and 52. Some varieties of hops, particularly those grown in Europe, have much larger quantities of farnesene. Peak 54 has the retention time of β-selinene. The authors' main interest was in the identification of peaks 41, 52, 56, 58, 60, 61, and 62. The capillary column used for this analysis was of the conventional type, $^1/_{100}$-inch I.D. and 150 feet long. The maximum load for this type of capillary is of the order of a few micrograms. This amount is sufficient for mass spectrometry but much too small for proton magnetic resonance (PMR) and infrared absorption spectrometry (IR). Packed column GLC separation gave sufficient amounts of sample, but could not resolve all the components. The top broken line in Figure 1 shows a typical $^1/_4$-inch packed column analysis of this mixture. It gave six main peaks, labeled *A*, *B*, *C*, *D*, *E*, and *F*. The authors' study began with these packed-column fractions.

Comparison of the IR and PMR spectra of fraction *A* (MS showed $C_{15}H_{24}$) with published data (*8*, *12*) showed that it was composed largely of copaene (I). An authentic sample of copaene isolated from copaiba oil had the same capillary GLC retention time and IR spectrum as *A*. Capillary GLC showed that peak *A* was composed of about 80% of one component (copaene from the above data) and about 20% of another. PMR and IR spectra did not indicate the presence of any other component besides copaene and probably the smaller peak is a closely related isomer, possibly that which has been called ylangene, a stereo isomer of copaene with the isopropyl group on the other side of the ring.

Packed column GLC peaks *B* and *C* contained principally the previously identified caryophyllene (II) and humulene (III). From a Tettnang variety of hops



the above-mentioned farnesene was also isolated and had an IR spectrum consistent with published data (*14*).

The authors' packed column peak *C*, besides humulene, contained about 5 to 10% of the capillary peak 52. One unusual property of humulene is that it forms a very strong nitrate complex (*11*). By extracting the sesquiterpene fraction three times with a 50% solution of silver nitrate, all of the humulene could be removed without removing peak 52, which could then be separated by packed column GLC. Catalytic hydrogenation of peak 52 (Pd on charcoal, 25 hours, 20 p.s.i. of H_2) gave a compound whose IR spectrum was consistent with that of β-santalane (V). IR and NMR analysis of 52 showed evidence for a $-CH(CH_3)_2$ group, a $-C{=}C-CH_3$ group, and a $-C{=}CH_2$ group. However, the authors were unable to suggest an arrangement of double bonds in the β-santalane (V) skeleton to fit the data. The reason for this may be that 52 is a mixture of components or that the ring system is different from β-santalane but rearranges to β-santalane under the catalytic hydrogenation conditions used.

The packed column peak *D* on catalytic hydrogenation gave selinane (IR identical with that of hydrogenated β-selinene from celery seed oil). The 0.01-inch I.D. capillary showed that *D* was composed of two main components, peaks 54 and 56. These could not be resolved by packed column GLC, although a number of different stationary phases were tried. However, Teranishi, Flath, and Mon (*20*) had developed a high resolution technique for separating components in amounts of the order of 0.1 mg. by using capillary columns 1000-foot long by 0.03-inch I.D. Using this technique the authors were able to separate packed column peak *D* into its two main components.

About 30 repeated injections and collections accumulated about 1 to 2 mg. of each component, enough for IR and PMR analysis using microcells and the CAT (computor of average transients) technique for

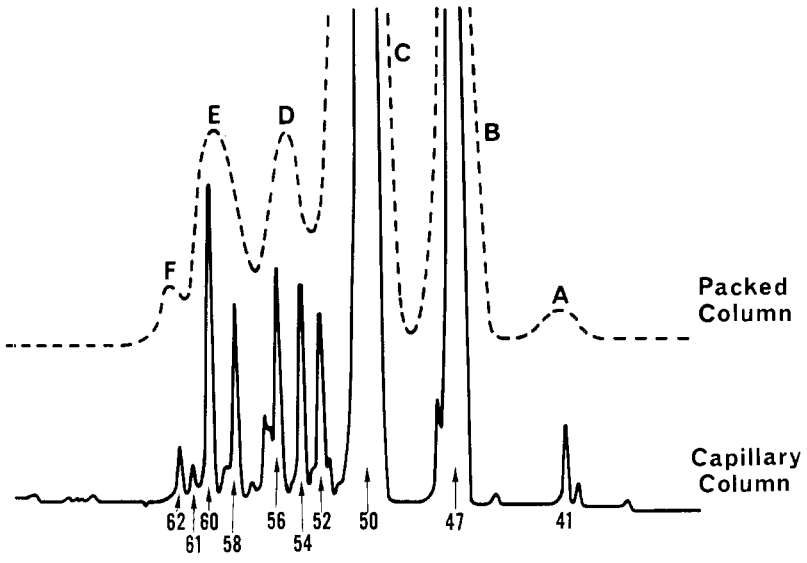


Figure 1. Separation of hop sesquiterpene hydrocarbons

- - - $^1/_4$-inch O.D. packed column gas chromatography
—— 0.01-inch I.D. capillary gas chromatography

PMR. From these data, the authors discovered that peak 54 was β-selinene (VI) and peak 56 α-selinene (VII). Their IR and PMR spectra and capillary GLC retention time were the same as those of authentic samples isolated from celery seed oil.

The study of the next packed GLC column peak *E* followed a similar pattern. Mass spectrometry showed $C_{15}H_{24}$. Hydrogenation this time gave cadinane [IR consistent with Sorm's hydrogenated δ-cadinene (*14*)]. Analytical capillary GLC analysis (0.01-inch I.D.) showed that *E* also consisted of two main components, peaks 58 and 60. These were also resolved with the long 0.03-inch I.D. capillary using about 50 repeated injections and collections to accumulate enough for IR and PMR analyses.

The authors were able to establish the identity of peak 60 as δ-cadinene (IX). PMR, IR, and capillary GLC retention time of peak 60 matched those of an authentic sample of δ-cadinene separated from ylang ylang oil and also that of published data in the case of the IR spectrum (*14*).

The infrared spectrum of the other component, peak 58, was consistent with that published for γ-cadinene (*14*). The PMR spectrum was consistent with this but did not exclude the γ'-cadinene. No authentic samples of γ- or γ'-cadinene were available. A quoted source of γ-cadinene, citronella oil, gave a packed column peak with a spectrum similar to that reported for γ-cadinene, but on resolution on a 500-foot, 0.03-inch I.D. capillary, the peak consisted of δ-cadinene and two other components, none of which had the spectrum of γ-cadinene.

Packed-column peak *F* (MS showed $C_{15}H_{24}$) is small in the Bullion variety but large in some experimental varieties, the Australian varieties, Late Grape, Golden Cluster (Figure 2), and the European Gebirg. This last variety was used in the present work to isolate reasonable quantitites of peak *F*. Catalytic reduction of *F* gave selinane (IR spectrum identical to that of hydrogenated β-selinene). The long 0.03-inch I.D. capillary column separated *F* into two main components, peaks 61 and 62. The IR spectrum of peak 61 was similar to that of β-selinene. PMR analysis (Figure 3) showed that qualitatively the main features of the spectrum were consistent with selina-4(14),7(11)-diene (X)—selinane numbering system of Theobald (*21*). The structure of X is similar to that of β-selinene (VI) except that the double bond is between the isopropyl group and the ring instead of in one arm of the isopropyl group. The PMR spectrum was not consistent quantitatively with X. The area under the peak, due to methyl groups attached to double bonds (1.67 p.p.m.), was too large for X and indicated the presence of roughly 50% of another component with at least three methyl groups attached to double bonds. No other peaks due to olefinic protons were found, and the lack of any other appreciable peaks indicated that the impurity must be very similar to X with tetrasubstituted double bonds. Structure (XII) [selina-4(5),7(11)-diene] seemed to be a possible arrangement which fitted the data. There are no other ways of arranging the two double bonds in the selinane skeleton to give three methyl groups on double bonds and have both double bonds tetrasubstituted.

The second component of peak *F* (peak 62) gave IR and PMR spectra consistent with that expected of selina-3,7(11)-diene (XI) which is related to α-selinene but having the double bond between the isopropyl group and the ring instead of in one arm of the isopropyl group. In this case the PMR data (Figure 4) fitted XI well both qualitatively and quantitatively. Most varieties studied which had unusually large amounts of α- and β-selinene also had unusually large amounts of peaks 61 and 62 and relatively smaller amounts of other sesquiterpenes. This can be seen in Figure 2 for the Tasmanian Golden Cluster variety. β- and α-selinene (peaks 54 and 56) are very large, as also are selina-4(14),7(11)-diene (X) and selina-3,7(11)-diene

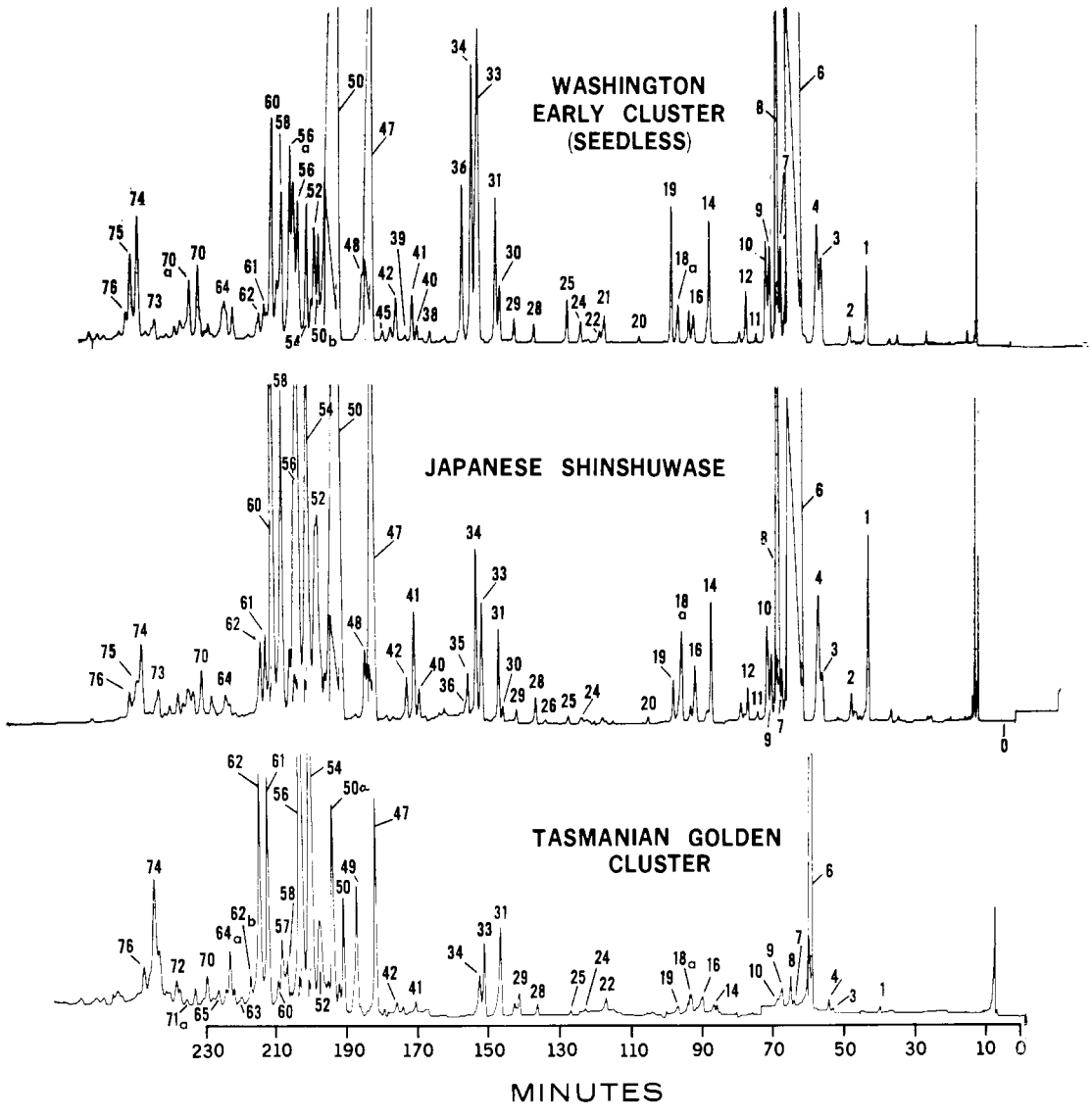


Figure 2. Capillary GLC analyses of hop oils from three varieties of hops

Capillary column was 150-foot long by 0.01-inch I.D., coated with silicone SF96(100), linearly temperature-programmed from 50° to 160° C. at ½° per minute

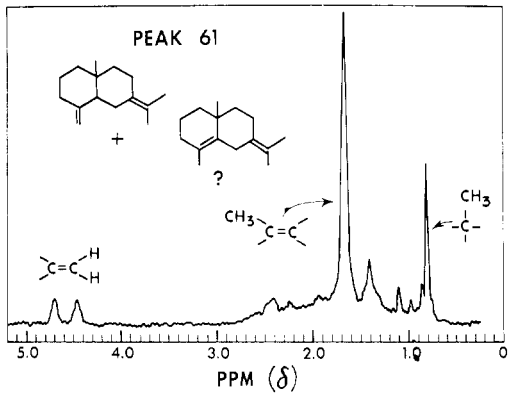


Figure 3. Proton magnetic resonance spectrum of hop sesquiterpene hydrocarbon peak 61

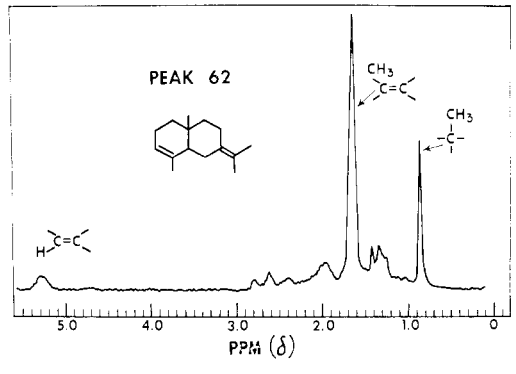
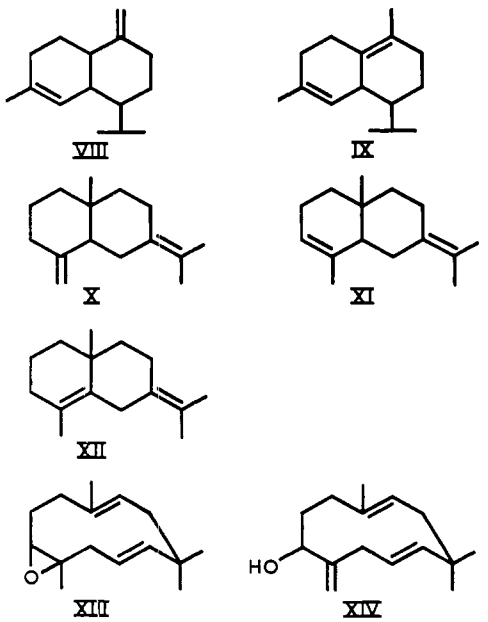


Figure 4. Proton magnetic resonance spectrum of hop sesquiterpene hydrocarbon peak 62

(XI), peaks 61 and 62. The cadinenes (peaks 58 and 60), humulene (peak 50), and caryophyllene (peak 47) are all relatively small.

The Japanese variety Shinshuwase (Figure 2) is the only exception to this rule that the authors discovered. It has large amounts of the cadinenes (peaks 58 and 60) and of β- and α-selinenes (peaks 54 and 56) but relatively small amounts of peaks 61 and 62. Shigematsu and Kitazawa (*17*) had reported finding a cadinene-type sesquiterpene in this variety but did not characterize it.



Some varieties such as Fuggle and Hallertau contain very little of the selinenes but have reasonable amounts of the cadinenes, the major sesquiterpenes, however, being still humulene and caryophyllene. In most hop varieties, humulene is the major sesquiterpene. The variety Tasmanian Golden Cluster (Figure 2) is a member of an unusual group where humulene is only a minor component.

Oxygenated Sesquiterpenes. The capillary GLC and mass spectrometry combination showed that hop oil contained about 11 oxygenated sesquiterpenoids (*5*). Most of these occurred in very small amounts. The authors were able to isolate seven of these and measure their IR spectra but could match only three of them with any known compounds: caryophyllene epoxide, humulene epoxide II (XIII), and humulenol (XIV). Damodaran and Dev (*7*) had isolated these compounds from the essential oil of wild ginger and had published details of their infrared absorption spectra. These compounds were also isolated 2 years later by Nigam and Levi (*13*) who published the infrared spectrum of humulenol and called it β-humulen-7-ol. Damodaran and Dev (*7*) had shown that humulene epoxide (XIII) rearranges to humulenol (XIV) on chromatography on alumina, and Nigam and Levi (*13*) found that this change could also be brought about by refluxing with pyridinium bromide in pyridene solution.

Some studies with model systems to parallel the brewing process indicated that the volatile hop constituent transferred most efficiently into the hopped wort was humulene epoxide II. Another component transferred relatively efficiently was humulenol. Because of their likely importance to hop flavor imparted to beer, the previous studies on the epoxidation of humulene and the formation of humulenol were extended to include them.

Perphthalic acid oxidation of humulene gave a mono-epoxide mixture which separated as three peaks in the mono-oxygenated sesquiterpene region on capillary GLC. The three peaks apparently correspond to the three possible mono-epoxides of humulene. One of the peaks was approximately four times the size of each of the other two. The infrared spectrum of a sample purified by packed column GLC showed that the epoxide formed in largest amount was humulene epoxide II (XIII).

The reasons why this isomer is formed in largest amounts may be steric. The other double bonds are both relatively close to the *gem*-dimethyl group, and the construction of models indicates that the double bond which is epoxidized for humulene epoxide II (XIII) is the least sterically hindered.

Air oxidation of humulene in the presence of light also gave XIII as the main epoxide and much lesser amounts of the others. Oxidation by air, or oxygen in light in addition to XIII, gave humulenol (XIV) in an amount about one third of that of the epoxide. The humulenol may be either formed directly or by a secondary reaction by rearrangement of the epoxide.

In the above experiment, care was taken that the humulene epoxide did not rearrange on the column. This could easily be checked by injecting pure samples of the epoxide. If the column temperature was above 200° C., the humulene epoxide could rearrange to humulenol and other products, but this depended upon the particular column and its condition.

These studies showed that humulene epoxide II and the humulenol found in hop oil are probably formed by air oxidation of humulene. This has been observed on storage of hop oil but also seems to occur to some extent in the hop cones.

Luparenol. Chapman (*6*) isolated an alcohol from hop oil which he showed had the formula $C_{15}H_{24}O$, had only one double bond, was tricyclic, and was a tertiary alcohol. Buttery *et al.* (*4*) also isolated a similar alcohol from Hallertau variety hops. Its PMR analysis indicated that it was a tertiary alcohol, which had one double bond of the type $-\overset{|}{C}=\overset{|}{C}-CH_3$ and three tertiary methyl groups. Mass spectrometry showed $C_{15}H_{24}O$ which, with the evidence of only one double bond, would indicate a tricyclic molecule. The authors have yet to establish the basic ring system for this compound. It does not appear to be related to any of the ring systems of the sesquiterpene hydrocarbons of hop oil.

C_{15} Unsaturated Ketones. Capillary GLC combined with mass spectrometry (*5*) identified the compound pentadeca-2-one in hop oil. A study of the oxygenated fraction of hops (*1*) showed, in addition, three components of hops which had the formulas $C_{15}H_{24}O$,

$C_{15}H_{26}O$, and $C_{15}H_{28}O$. These were at first thought to be sesquiterpenoids but more detailed study of the isolated samples by mass, IR, and NMR spectrometry showed that they were straight-chain methyl ketones differing in degrees of unsaturation.

Thus, there were four C_{15} straight-chain methyl ketones, pentadecan-2-one, pentadecene-2-one, pentadecadiene-2-one, and pentadecatriene-2-one. These were isolated by a sequence of separation methods; the final separation was carried out on an ethylene glycol succinate polyester column. Evidence for the structure of the three unsaturated ketones is shown in Table I.

The pentadeca-6,9-diene-2-one is the major C_{15} ketone, being two to three times that of any of the others. Assignment of the position of the two double bonds was based mainly on the ozonolysis evidence, showing that one of the double bonds was six carbon atoms from the hydrocarbon end and the PMR evidence that the two double bonds were separated by one CH_2 group. This was shown by a characteristic group of peaks at 2.8 p.p.m., which is very similar to that found for other molecules with the —CH=CH—CH_2—CH=CH— grouping—e.g., linolenic acid (*22*). The tri-unsaturated ketone showed a similar group in its PMR spectrum and, therefore, also had the arrangement —CH=CH—CH_2—CH=CH—. The other evidence for the double bond location in the tri-unsaturated compound is based on the PMR detection of CH_3CH=CH— (doublet, peaks at 1.6 and 1.7 p.p.m.) and the succinic acid obtained as a major product of oxidation of the ozonide which indicated the arrangement —CH=CH—CH_2-CH_2—CH=CH—.

The C_{15} mono-unsaturated ketone was difficult to purify and appeared to be a mixture of several isomers. The evidence for the position of the double bond was not conclusive.

Role of C_{15} Components in Aroma of Hop Beverages. The present study was primarily concerned with the identification of the major C_{15} constituents of hops. Little work has yet been carried out on their odor properties.

Previous work (*9*) had shown that humulene had an odor threshold in water solutions of 120 p.p.b. and caryophyllene of 64 p.p.b.

The pentadeca-6,9-dien-2-one was found to have an odor threshold of about 1 p.p.b. Recent work with model systems by the authors has indicated that the main sesquiterpene hydrocarbons are transferred very poorly to the wort during the brewing process, whereas the C_{15} oxygenated components, particularly humulene epoxide and humulenol, were transferred much more efficiently.

In view of the low odor strengths of the main sesquiterpene hydrocarbons and their inefficient transference to the wort, the authors would expect the hydrocarbons to have little importance to the aroma of hop beverages. On the other hand, the oxygenated C_{15} components, because of their high odor strength and efficient transference, could contribute much of the hop character imparted to hop beverages.

Table I. Evidence for the Structures of Unsaturated C_{15} Methyl Ketones of Hop Oil

Type of Evidence	C_{15} Mono-unsaturated Ketone	C_{15} Di-unsaturated Ketone	C_{15} Tri-unsaturated Ketone
Mass spectrometry	$C_{15}H_{28}O$; probably methyl ketone	$C_{15}H_{26}O$; probably methyl ketone	$C_{15}H_{24}O$; probably methyl ketone
Infrared absorption spectroscopy	Nonconjugated ketone (maximum at 5.8 μ)	Nonconjugated ketone (maximum at 5.8 μ)	Nonconjugated ketone (maximum at 5.8 μ)
	cis double bond (no maximum around 10.3)	Both double bonds *cis* (no maximum around 10.3)	At least one trans double bond (maximum at 10.35)
Proton magnetic resonance spectra	CH_3CH_2—... ...—CH_2COCH_3 ...—CH=CH—...	CH_3CH_2—... ...—CH_2COCH_3 ...—CH=CH—CH_2—CH=CH—...	CH_3—CH=CH—... ...—CH_2COCH_3 ...—CH=CH—CH_2—CH=CH—...
Ozonolysis plus Zn and AcOH	Several aldehydes	Gave $CH_3(CH_2)_4CHO$	.....
Ozonolysis plus H_2O_2 and NaOH	.....	Gave $CH_3(CH_2)_4COOH$ and some $HOOC(CH_2)_8COOH$	Gave $\begin{matrix}CH_2COOH\\ \vert\\ CH_2COOH\end{matrix}$
Probable structure	Not certain	$CH_3(CH_2)_4CH$=$CHCH_2CH$=CH $\vert$ $CH_3CO(CH_2)_3$	CH_3CH=$CHCH_2CH_2CH$=CH $\vert$ $CH_3CO(CH_2)_3CH$=$CHCH_2$ or CH_3CH=$CHCH_2CH$=$CHCH_2$ $\vert$ $CH_3CO(CH_2)_3CH$=$CHCH_2$

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