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Bitter Tasting Compounds of Beer. Chemistry and Taste Properties of Some Hop Resin Compounds

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The subject of beer flavor is presented and a description of the aroma and taste properties is given. Importance of studying the taste character of beer is stressed and a brief review is presented of the chemistry and taste properties of some nonvolatile resin constituents of hops. Some results are presented on the application of ion-exchange chromatographic and high-pressure liquid chromatographic analysis for the separation of hop resin compounds present in beer as well as in some samples representing different parts of the brewing process. The ion-exchange chromatographic procedure, while capable of resolving some beer bitter compounds, suffers from being a slow procedure with the possibilities of producing artifacts when working with the rather unstable group of hop compounds. The high-pressure liquid chromatographic procedure appears to have great potential in the analysis of hop bitter compounds. The method is rapid and produces a good resolution of hop compounds.

The flavor of beer, like that of many foods and beverages, is composed of many volatile and nonvolatile compounds present in a definite blend. Modern separation and identification techniques place the number of volatile and nonvolatile compounds in beer close to 400. It is reasonable to suppose, however, that only a small number of these compounds are "flavor active," that is to say, directly involved in producing the flavor sensation when the product is consumed.

The aroma of beer consists mainly of the sweet and pleasing note of esters, harsh tingling sensation of alcohols, characteristic aroma of aldehydes, ketones, and mercaptans, sour note of lower organic acids, and the indescribable yet pleasing note produced by hitherto unidentified compounds present in beer. Several reviews are available on the general composition of beer[11,28,32,42] but only a limited amount of work has been reported concerning the actual flavor influence of some of the beer constituents.[35-37] The complex nature of beer aroma still leaves it inadequately understood.

The taste aspect of beer has not been studied as extensively as the aroma. One of the reasons for this may be that the phenomenon of taste is considered to be much simpler than that of aroma. When applied to beer, the view of the taste phenomenon represents an oversimplification of the situation. Although generally, three basic tastes, namely sweet, sour, and bitter notes, are recognized in beer, the nature of beer constituents is such as to modify these tastes in a unique way. In addition, beer taste experience includes mouthfeel factors such as smoothness, harshness, and astringency which contribute to the overall characteristic sensation of beer taste. The object of this paper is to present a brief review of the chemistry and taste properties of some hop resin-derived compounds in beer. Results of ion-exchange and high-pressure liquid chromatographic tecnhiques for the separation of nonvolatile hop constituents in beer will also be reported.

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SOURCES OF TASTE COMPOUNDS IN BEER

In Table I are listed types of compounds believed responsible for the various taste notes in beer. Although a number of beer constituents such as polypeptides, proteins, and high molecular weight carbohydrates contribute to the bitter taste of beer, hop resins are considered to be by far the greatest contributors to this important property of beer. The bitter tasting compounds of hops are now known to be formed during the kettle boiling step in the manufacture of beer.

BITTER RESINS OF HOPS, THEIR CHEMISTRY AND DERIVATION INTO BEER

Hop resins, or the so-called bitter principles of hops, are present in the lupulin glands of the cones of female hop flowers. The hop plant (*Humulus lupulus*) is a climbing herbaceous plant belonging to the natural family of Maraceae and the natural order of urticales. The lupulin glands containing the bitter resins and essential oil are secreted at the base of the female flowers (Figure 1). The hop petals contain polyphenolic compounds. The hop polyphenols, which constitute about 4% of the hop cone, also enter into some reactions during the brewing process and are believed responsible for imparting an astringent taste to beer, but this aspect of hops will not be discussed here.

A typical composition of hop cone is shown in Table II.

Hop resins, which account for about 15% of the hop cone (dry weight), consist of several distinct compounds. Early work on the fractionation of hop resins,[14,15] which was based on the solubility of resins in various organic solvents, classified them into soft resins (α and β acids and uncharacterized soft resins) and hard resins (xanthohumol, oxidized resins). At that time α and β acids were believed to represent single compounds but subsequent

Table I. Compounds Responsible for Beer Taste

Taste description	Causative compounds
Sweet	Low molecular weight carbohydrates, ethyl alcohol, amino acids
Sour	Organic acids
Bitter	Hop resin-derived compounds, polypeptides, proteins, high molecular weight carbohydrates
Astringent	Ethyl alcohol, polyphenols
Harsh	Ethyl alcohol, minerals

Table II. Typical Composition of a Hop Cone

Constituent	Percent composition[a]
1. Water	10
2. Total resins	15.0
3. Essential oil	0.5
4. Tannins	4.0
5. Monosaccharides	2.0
6. Pectin	2.0
7. Amino acids	0.1
8. Proteins (N × 6.25)	15.0
9. Lipids and wax	3.0
10. Ash	8.0
11. Cellulose, lignin, etc.	40.4
	100.0

[a] Taken from *Malting and Brewing Science*, J. S. Hough, D. E. Briggs, and R. Stevens, Chapman and Hall, Ltd., p 324 (1971). Reprinted with permission.

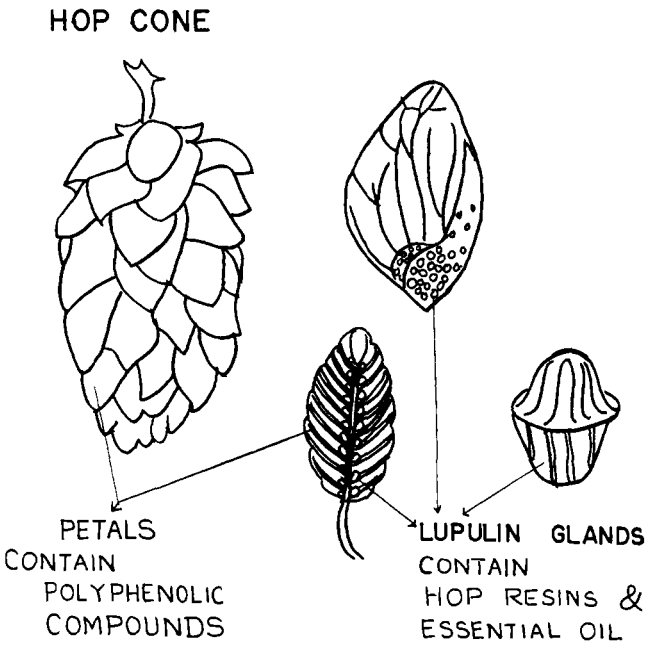


Figure 1. *Components of a hop cone showing the locations of* different hop constituents. Taken from *Malting and Brewing Science*, J. S. Hough, D. C. Briggs, and R. Stevens, Chapman and Hall, Ltd., p 303 (1971). Reprinted with permission.

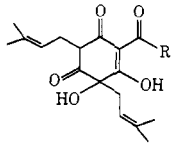
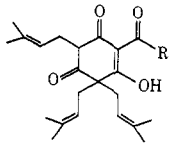
work[40,41] showed that α acids indeed consist of several analogs. Similar observations were made on β acids also.[19,20] Continued research work on hop resins, resulting in additional data, necessitated reclassification of this group of compounds.[16] Figure 2 contains the composition and the most recent nomenclature of hop resins and in Tables III and IV are shown structures of major hop resin constituents.

In the brewing of beer, hops are boiled with malt and other cereal extracts (wort) in the brew kettle for a given period of time. During this process a part of the α acids (I) of hops undergoes heat-induced isomerization into iso-α acids (II). The iso-α acids are soluble in wort and possess a very bitter taste. Unconverted α acids are very slightly soluble in wort and possess very slight bitter taste. Part of the iso-α acids survives the rest of the brewing process and persists in the beer and is responsible for part of the beer's bitter taste. The isomerization of α acids can also be brought about by the action of alkali.

The wort boiling process also extracts other compounds of hops such as tannins, sugars, proteins, essential oils, and amino acids, but isomerization of α acids and extraction of bitter principles of hops into wort is considered by far the most important outcome of this operation as far as the role of hops in brewing is concerned. Some of the other reactions that take place during kettle boiling of wort are inactivation of malt enzymes, coagulation of excess proteins, etc., but these are not relevant to the present discussion.

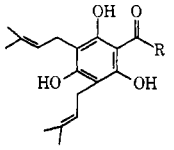
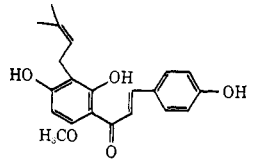
In the early days of brewing, boiling hops in the kettle was thought to derive some preservatives and put them into the beer. However, the current view is that hops contribute little to the biological stability of beer[16] and today boiling the hops is considered only to impart to beer the refreshing bitter taste well recognized by the beer consumer in all well brewed brands of beer. A secondary, nonetheless important, contribution of hops is believed to lie in the transfer into wort and beer of some essential oil-derived components which produce a subtle but charac-

Table III. Structures and Properties of Major Hop Resins[a]

Compound	Name	R	Mp, °C	Concn in hops (dry wt), %
 α acids	Humulone	$CH_2CH(CH_3)_2$	64.5	3–12
	Cohumulone	$CH(CH_3)_2$	Oil	
	Adhumulone	$CH(CH_3)CH_2CH_3$	Oil	15–20
 β acids	Lupulone	$CH_2CH(CH_3)_2$	92	2–5
	Colupulone	$CH(CH_3)_2$	93–94	
	Adlupulone	$CH(CH_3)CH_2CH_3$		

[a] Taken from *Malting and Brewing Science*, Chapman and Hall, Ltd., London, p 326 (1971).

Table IV. Structures and Properties of Some Minor Resin Constituents of Hops

Compound	Name	Mp, °C	Concn in (dry wt), %
 R = *i*-Bu	4-Deoxycohumulone, $C_{20}H_{28}O_4$	83.0	0.025
	Xanthohumol, $C_{21}H_{22}O_5$	172.0	0.2

teristic hop flavor in beer. However, Kuroiwa and Hashimoto[26] have been unable to show the presence of any hop oil constituents in beer.

Although conversion of nonbitter α acids into bitter iso-α acids (isohumulones) is regarded as the major and the most important reaction involving hops in the kettle boiling operations, considerable attention has been given to the study of other reactions of α acids,[1,2,4,49,51] as well as to the reactions involving other resin constituents of hops.[3,8] Brew kettle boiling operations involve rather harsh conditions (boiling in the presence of air) and hop resin constituents contain many vulnerable bonds. This creates opportunities for the occurrence of many reactions that could result in the production of a number of new compounds. Many investigations have been carried out with a view to studying these reactions and their products.[31,44,45]

CHEMISTRY AND PROPERTIES OF HOP RESIN CONSTITUENTS

α Acids and Their Derivatives. α Acids (I) represent a group of acyl-substituted phloroglucinols. The enolic proton in the phloroglucinol ring is responsible for the acidity of these acids. Several analogs of α acids are known, these being differentiated by the acyl side chain (R = isovaleryl, isobutyryl, and α-methylbutyryl for humulone, cohumulone, and adhumulone, respectively).

α Acids, like some of the other resins, are β-dicarbonyl compounds and as such can exist in several tautomeric forms. The very reactive nature of α acids allows them to enter into many reactions when subjected to the influence of heat, light, pH, oxidizing and reducing conditions, etc.

Heat-Induced Isomerization. One of the major reactions undergone by α acids under the conditions of brewing is the heat-induced transformation into the bitter iso-α acids (as has already been pointed out). This reaction, which can also be brought about by boiling humulones in a buffered solution (pH 5–9), produces a mixture of two pairs of stereoisomers, the first pair being termed *cis*-isohumulone (III) and *trans*-isohumulone (IV). The second pair comprises alloisohumulone A (V) and alloisohumulone B (VI).[52]

For each of these acids there is a cis and trans isomer. On the basis of the fact that there are six analogs of α acids, there can be present in wort 24 different isohumulones resulting from heat-induced transformation of α acids alone. A considerable amount of work has been reported on the heat- and alkali-induced transformation of α acids.[20,21,47,49] It is not the object of this paper to present an exhaustive review of this subject but rather to present some typical examples so as to indicate the complexity of the reaction mixture.

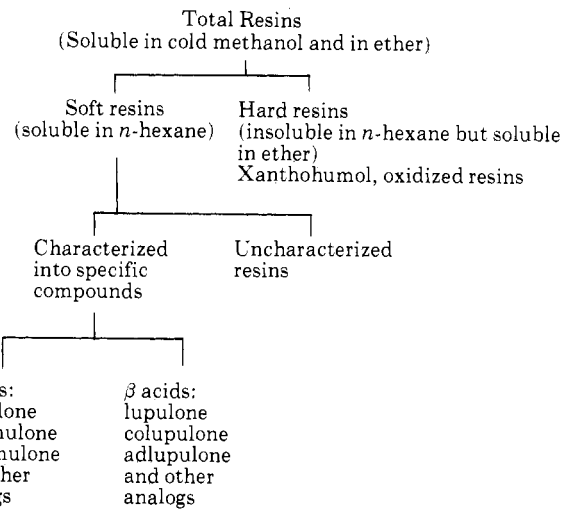
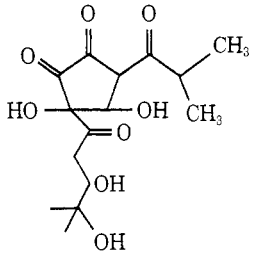


Figure 2. Classification and nomenclature of hop resins.

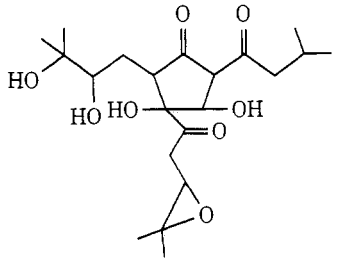
Photoisomerization. Isomerization of α acids can also be brought about by irradiating the compounds with visible light.[8,46,48] The product of reaction, designated as photoisohumulones, has been shown to be identical with one of the isohumulones produced by the action of alkali and heat.[8]

Autoxidation. Autoxidation of α acids has been studied by a number of workers.[4,6,9] Humulone undergoes rapid aerial oxidation to 3-hydroxy-3-(3,4-dihydroxy-4-methylpentanoyl)-5-isobutyrylcyclopentane-1,2,4-trione (VII), a



VII

bitter compound. This reaction is reported to take place both in hops and in solution.[5] Stronger oxidation conditions, such as oxidation with monoterephthalic acid of either humulone or isohumulone, yield 5-(2,3-dihydroxy-3-methylbutyl)-4-(3,4-epoxy-4-methylpentanoyl)-3,4-dihydroxy-2-isovalerylcyclopent-2-en-1-one (VIII). This compound is present in the hard resin fraction of hops. Oxida-



VIII

tion of humulone produces humulinone (IX), and under conditions of wort boiling, *cis*-humulinone (X) is produced.[17] cis Oxidation conditions during the wort boiling process have also been shown to convert α acids into *abeo*-iso-α acids (XI).[51] These oxidation products are generally nonbitter but possess the property of foam stabilization to a considerable degree.

Hydrolysis. Hydrolysis of humulone results in the formation of humulinic acid (XII). This product, which pos-

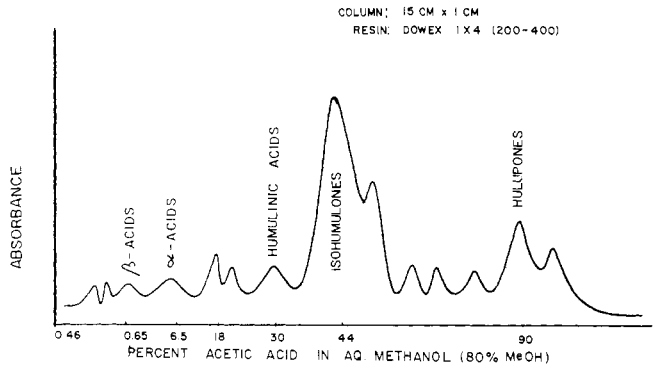


Figure 3. Ion-exchange chromatogram of isooctane extract of acidified beer (pH 2.0).

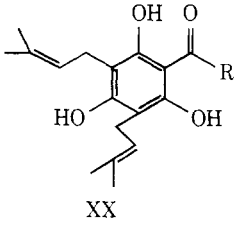
sesses a mild bitter character, is also believed formed by the hydrolysis of isohumulone. Both cis and trans isomers of humulinic acids are known.[1,2]

β Acids. Compared to α acids, β acids (XIII) have been subjected to less extensive studies. Until recently β acids were thought to be unchanged by the action of alkali and considered stable toward oxidation conditions. It has now been shown, however,[38] that oxidation of β acids yields bitter tasting hulupones (XIV). Hulupones are further oxidized to hulupinic acid (XV).[38]

Hydrolysis. β Acids (XIII) undergo pyrolysis and hydrolysis to yield luputriones (XVI).[25] Luputriones have also been detected in beer[26] and are said to possess a bitter taste character.

Autoxidation. Autoxidation of β acids has been shown to produce three distinct compounds, designated lupoxes A (XVII), lupoxes B (XVIII), and lupdoxes A (XIX).[24] These compounds are reported to be present in hops and are introduced into beer without change. They are also formed during the kettle boiling operations, and are then derived into wort and beer without change.[24] All the three compounds are claimed to contribute to the bitter taste of beer.

In addition to the study on α and β acids, a considerable amount of work has been reported on some of the other constituents of hops such as δ resins,[27] 4-deoxy-α acids (XX),[29] and xanthohumol (XXI).[7] Deoxy-α acids can be oxidized to α acids which can, in turn, be transformed into iso-α acids (bitter compounds). The importance of δ resins and of xanthohumol to beer bitter taste is not clear.



XX

The foregoing treatment, while claiming no exhaustive coverage of the literature on the chemistry of hop constituents, serves, it is hoped, to point out the reactivity of hop resins and their possible derivation into beer. Some of the reactions discussed have been found to take place in the brew kettle during the boiling of wort, resulting in derivation of their end products into wort and beer.

A list of hop resin-derived compounds and their taste properties (wherever available) is presented in Table III.

RESEARCH ON HOP BITTERING COMPOUNDS

Practical Implications. Research carried out on hop resins and their transformation products points out the fact that α acids are the main source for beer bitter compounds, followed by β acids and to some extent other uncharacterized resins. Further, the work done to date indicates that these resinous compounds of hops are highly reactive and that they participate in many reactions under the conditions such as those that prevail during the kettle boiling operations of brewing. Work referred to in this paper shows that a number of hop-derived compounds are present in beer and over 30 compounds are derived from α acids alone. Some workers[24] report the detection of about 90 bitter substance constituents in beer by the ion-exchange chromatographic method. The study of reactions that take place in the kettle boiling operations and the understanding of transformations that occur during this process are by no means complete. Nevertheless, these investigations do emphasize the complex nature of hop resin compounds. From the standpoint of the brewer, qualitative identifications of hop compounds in beer are not always sufficient for the purpose of selection and proper utilization of the raw material, hops. Results presented in this paper on hop resin-derived compounds in beer and their taste properties (Table V) point to the lack of information on the quantitative aspect of this subject. A fuller understanding of the nature and quantities of all the hop-derived compounds in beer and their actual flavor influence is necessary before a meaningful evaluation of hops is possible. Two of the methods that are being evaluated in our laboratories for the separation of hop-derived compounds for subsequent identification and estimation are ion-exchange chromatography and high-pressure liquid chromatography.

Ion-Exchange Chromatography. The ion-exchange chromatographic method used is essentially the same as the one used by Hansen and Ramus[11] except for a modification consisting of running two parallel columns, one serving as the sample column and the other as the control. This was found necessary in order that the composition of the eluent going through the reference cell more closely approximated that going through the sample cell. This ensured an accurate subtraction of the eluent blank value and thus elimination of the base line drift.

Sample Preparation. 200 grams of beer was acidified with 4 ml of hydrochloric acid (1:1) to which a few drops of silicone antifoam (HG-10) was added. The sample was extracted with 200 ml of isooctane by shaking for 10 min. The isooctane extract was evaporated to dryness and the dried sample (resinous in appearance) was taken up in a minimum volume of aqueous methanol (80% MeOH). The sample was then subjected to ion-exchange chromatographic analysis. A typical ion-exchange chromatogram of a beer extract is shown in Figure 3. Five groups of hop compounds are separated by this method. Each peak represents several individual isomers and, for purposes of estimating them as a group, this separation method was found to be adequate. In our laboratories we are using this method for the determination of hulupones in beer. This is accomplished as follows. The sample, dissolved in 80% methanol (in water), is placed on the ion-exchange column and washed with 100 ml of 44% acetic acid in methanol and the concentration of hulupones present in this eluate is determined by measuring absorption at 280 mμ in a spectrophotometer using the following relationship.

$$\text{ppm (hulupones)} = \frac{\text{absorption at 280 m}\mu \times \text{volume of eluate} \times 10^4}{E_{280\ \text{m}\mu}^{1\%,\ 1\ \text{cm}} \times \text{wt of beer (g)}}$$

Hulupone values obtained for beers ranged from 2 to 15 ppm.

Kokubo *et al.*[24] have described a silicic acid chromatographic separation of chloroform extract of beer into three

Table V. Hop Resin-Derived Compounds in Beer and Their Taste Properties

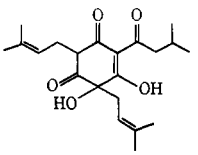
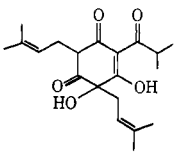
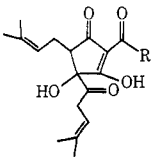
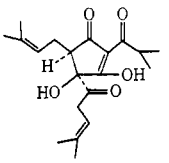
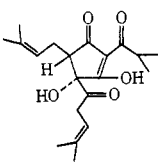
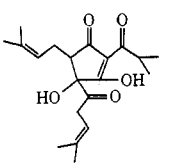
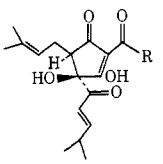
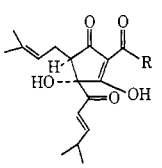
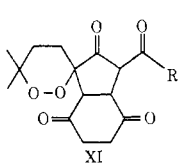
Compound	Name	Source and mode of formation	Physical properties	Concentration in beer, ppm	Taste property
 I	Humulone	α acids, unchanged	Mp, 64.5° $\alpha^{26}D$ −211° pK_a 5.5[17]		
	Cohumulone	α acids, unchanged	Oil $\alpha^{26}D$ −208.5° pK_a 4.7[17]	1–2	Very slightly bitter
 II	Isohumulones (bitter substances as measured by bitterness units)	α acids, isomerized during wort boiling operations	Oil pK_a 3.2[40,41]	15–20	Bitter, sharp and fast (bitterness rapidly disappears after each swallow)
 III	*cis*-Isohumulone	Humulone, isomerization during kettle boiling	Oil	4.4	Bitter (++)[50,51]
 IV	*trans*-Isohumulone	Humulone, isomerization during kettle boiling	Mp, 72°[17]	13.2	Bitter (+++)
	*cis*- and *trans*-isocohumulones	Cohumulone		7.5	Bitter, harsh
 V	*cis*-Alloiso-α acid	α acids, isomerization and double bond shifting in isohexenoyl chain	Soluble in water[52]		Not known
 VI	*trans*-Alloiso-α acid	α acids, isomerization and double bond shifting in the isohexenoyl chain			
 XI	*abeo*-Iso-α acids	α acids	Light yellow/powder liquefies in air λ_{max} 278 and 230 mμ in acidic methanol[53]	88–160	Practically tasteless

Table V *(Continued)*

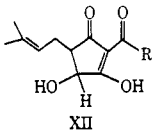
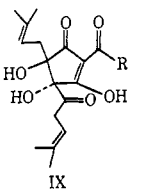
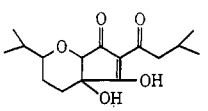
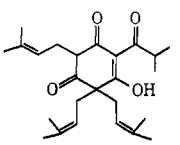
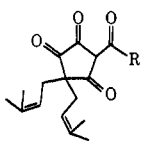
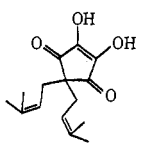
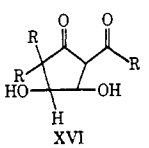
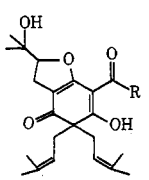
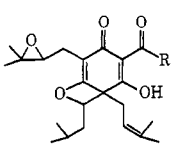
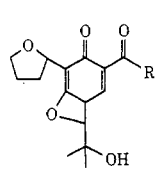
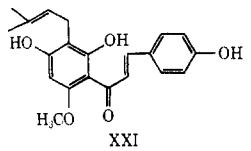
Compound	Name	Source and mode of formation	Physical properties	Concentration in beer, ppm	Taste property
 XII	Humulinic acids	α acids, hydrolysis	Trans isomer, mp, 95° Cis isomer, mp, 68° λ_{max} 270 mμ	0.1–2.5	About 30% as bitter as isohumulones. Slightly harsh bitterness
 IX	Humulinone (1) CO-Humulinone (2) AD-Humulinone (3)	α acids, oxidation	$C_{21}H_{30}O_6$ (1) mp, 74° pK_a 2.7 (2) mp, 111° (3) mp, 98°[49]		Bitter
	Isohumulinone (1) Iso-Cl-humulinone (2)	α acids	(1) mp, 195° (2) mp, 171°[49]		Bitter, bacteriostatic
 XIII	Colupulone	β acids, unchanged	$C_{26}H_{38}O_4$ crystalline mp 90°[49]	Trace	Tasteless
 XIV	Hulupone CO-Hulupone AD-Hulupone	β acids, oxidation	pK_a 3.25 uv absorption at 255 and 325 mμ[38]	2–10	About 50% as bitter as isohumulones
 XV	Hulupinic acid	Hulupones, autoxidation	$C_{15}H_{20}O_4$ mp, 168° Insoluble in light petroleum ether. Soluble in Et_2O[38]	Present	Not bitter
 XVI	Luputriones	β acids, pyrolysis–hydrolysis	uv absorption at 255 and 325 mμ in alkaline MeOH pK_a 270[25,26]		Bitter
 XVII	Lupoxes-A	β acids, autoxidation	Oil		Bitter
 XVIII	Lupoxes-B	β acids, autoxidation	$C_{25}H_{36}O_5$ oil but crystallizes at −20°. Pale yellow crystals, mp 84°[24]		Bitter
 XIX	Lupdoxes-A	β acids, autoxidation	Oil but crystallizes at −20°. $C_{25}H_{36}O_6$, mp 77°[24]		Bitter

Table V (*Continued*)

Compound	Name	Source and mode of formation	Physical properties	Concentration in beer, ppm	Taste property
 XXI	Xanthohumol	Hard resins	Mp 172° yellow crystals, max 372 mμ, min 275 mμ, in acidic methanol[7]		

fractions prior to subjecting them to ion-exchange chromatographic analysis. Ninety individual peaks were detected by this method in the beer extract. This method is being examined in our laboratories for application to our work.

High-Pressure Liquid Chromatographic Analysis. Application of a high-pressure liquid chromatographic (hplc) technique for the separation of hop resin and transformation compounds showed that this is a very useful tool. The instrument used in our laboratories for the hplc analysis was a Model 501 Chromatromix (Chromatromix, Inc., Berkeley, Calif.). The monitor used was uv detector and measurements were made at 254 mμ. A flow diagram of the hplc system is shown in Figure 4.

Preparation of the sample for the analysis consisted of extraction of acidified beer (adjusted to pH 2.0 with hydrochloric acid) with isooctane, evaporation of the solvent, and redissolution of sample in a minimum volume of chloroform prior to placing it on the column. In a typical analysis, 2.84 l. of beer were extracted with twice its volume of isooctane. The residue after evaporation of solvent was dissolved in 400 μl of chloroform. One microliter of chloroform extract was analyzed.

The column used was made of glass 50 cm long and 2 mm (i.d.) packed with Vydac packing (manufactured by Chromatromix, Inc.). Eluent used was isooctane with increasing concentrations of chloroform. A flow rate of 1 ml/min was maintained during the analysis.

Typical hplc patterns of extracts of hopped and nonhopped beers are shown in Figure 5. Analysis of nonhopped beer was found to be necessary in view of the fact that this beer contained compounds extractable by isooctane and which showed adsorption characteristics at 254 mμ. Figure 5 also contains an hplc pattern of hopped wort. This sample was analyzed in order to determine the hop compounds extracted into wort during the kettle boiling operations. These hplc charts show that under the conditions of the experiment at least 11 compounds (or groups of compounds) were present in beer, of which about six are nonhop derived. The hopped wort contains about twice as many compounds as the beer. Most of these presumably get eliminated from beer during the various steps of the process.

Figure 6 contains hplc patterns of hopped wort and beer, along with that of a water extract of yeast used in the fermentation of wort run under different elution conditions. Results show that under the modified conditions of the run, a better resolution of the eluted compounds was obtained and about 20 compounds were detected in hopped wort. Solvent peaks and other blank readings have been subtracted while preparing this chart. Hplc pattern for beer shows that a number of these hop compounds (peaks 7 through 12, and peak 15) were eliminated from this product during the manufacturing process, while a number of new compounds were made (peaks 21 and 22). The hplc pattern of the water extract of yeast indicates that a major portion of compounds, represented by peaks 13, 14, and 15, was eliminated from beer by yeast. Compounds represented by peaks 4A through 4F could be a result of reactions between non-hop and hop constituents.

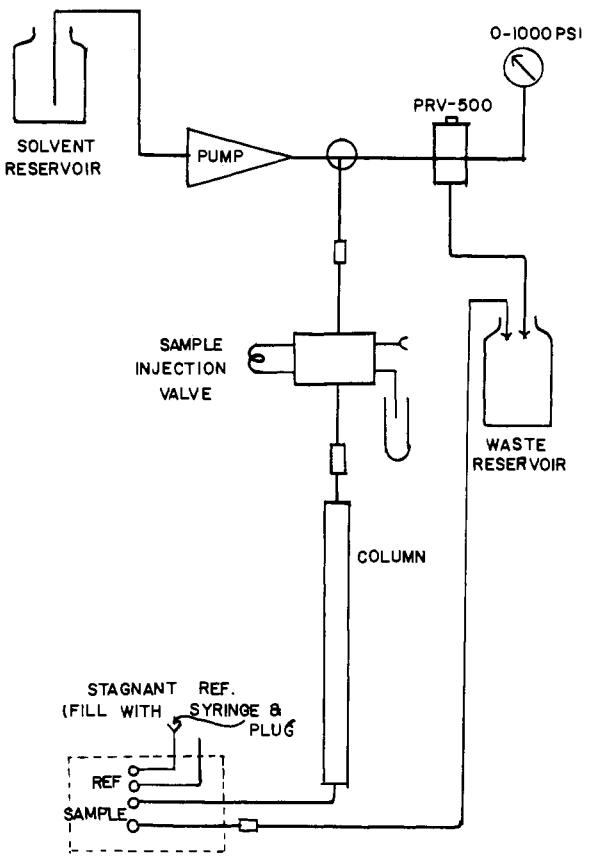


Figure 4. Flow diagram of liquid chromatograph.

More work is being carried out in order to understand the derivation of hop compounds into wort and their elimination during various stages involved in the brewing process. Also under study are methods to combine ion-exchange and hplc techniques for this study. A more general purpose detector, namely a refractive index detector, is also being evaluated in place of the uv detector.

STATE OF ISO-α ACIDS IN BEER

There appear to be several indirect evidences for the fact that iso-α acids may exist in beer in combination with other constituents. The facility with which iso-α acid compounds concentrate in the beer foam during fermentation,[10,23,25,39] as well as when beer is poured into a glass, may indicate their affinity or binding with proteinaceous substances (the main components of beer foam). The fact that iso-α acids possess different taste properties[50] when tasted in water solutions (undesirable bitterness) and in beer may indicate some evidence of interactions between isohumulones and some nonhop constituents of beer.

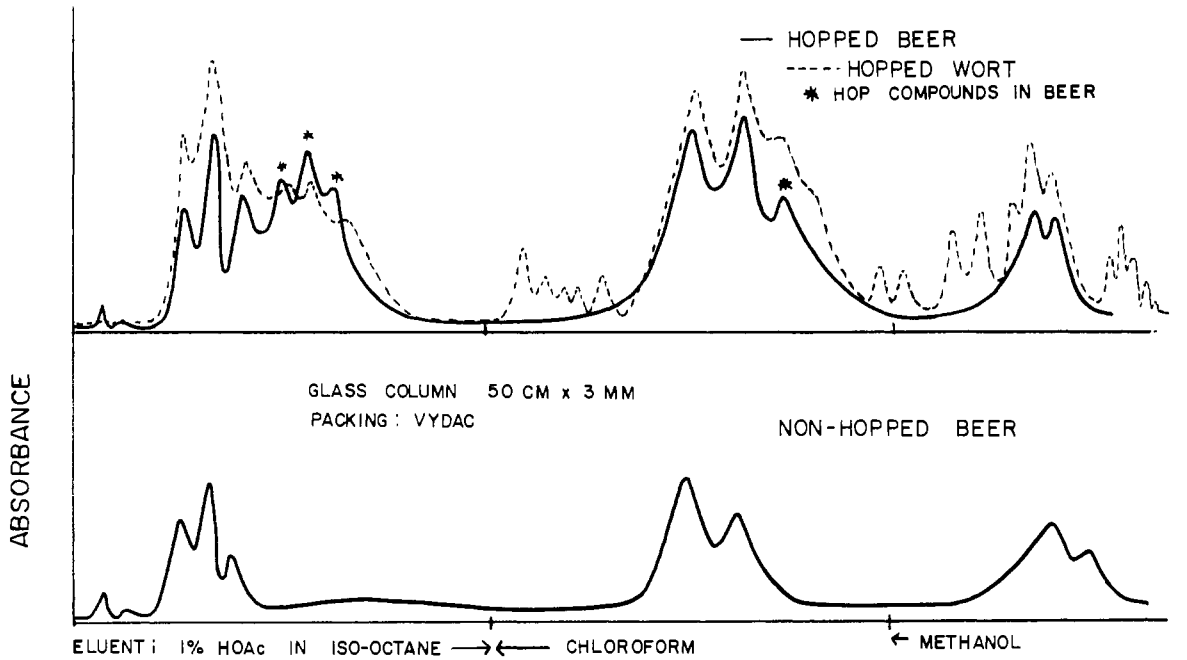


Figure 5. High-pressure liquid chromatographic patterns of isooctane extracts of hopped and nonhopped beers acidified to pH 2.0.

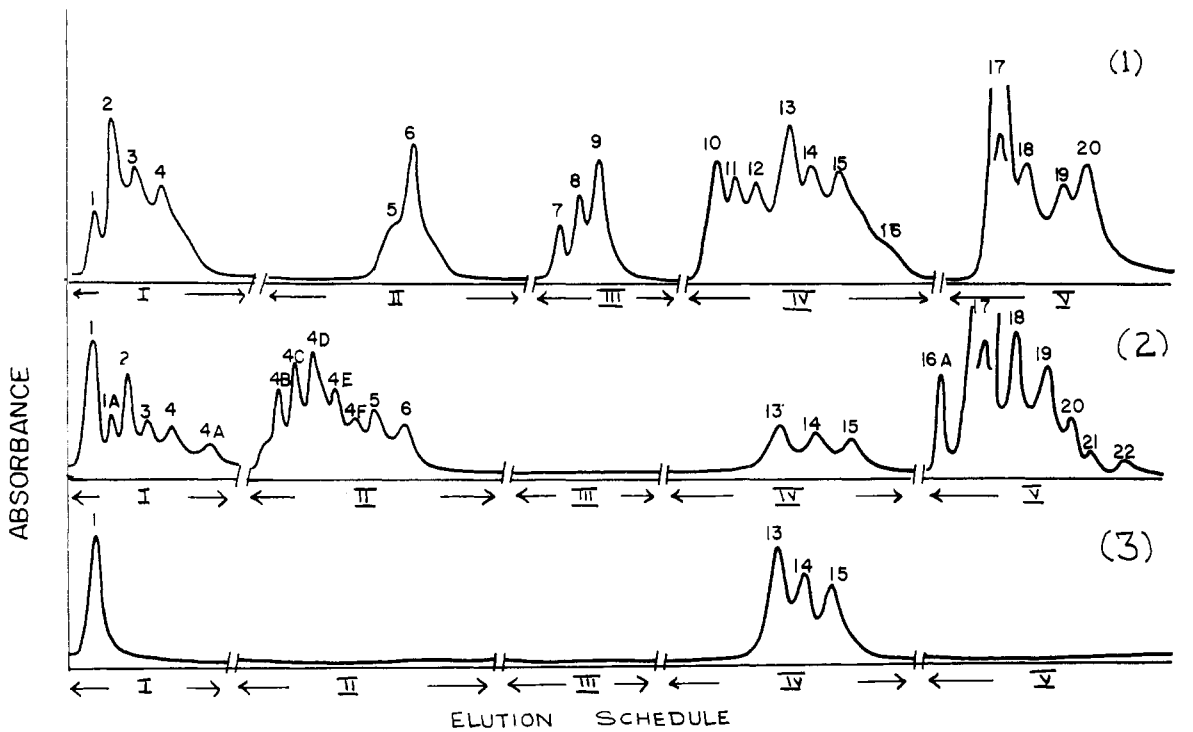


Figure 6. High-pressure liquid chromatographic patterns of isooctane extracts of (1) hopped wort, (2) beer, and (3) settled yeast. Eluting solvents: I, isooctane; II, isooctane–chloroform, 9:1; III, isooctane–chloroform 4:1; IV, isooctane–chloroform 2:1; and V, chloroform–methanol 1:1.

Studies in our laboratories involving dialysis of beer and examination of beer before and after dialysis for iso-α acids by spectrophotometric method[3] indicate that the iso-α acids are dialyzable, indicating that perhaps these compounds are not bound to proteins or other high molecular weight compounds (mol wt = 12,000).

In a typical experiment, 100 ml of beer was placed in a dialysis tubing (Item No. 8667A, capable of withholding compounds with molecular weights >12,000, supplied by Fisher Scientific Co., St. Louis, Mo.) and the tube was suspended in a beaker containing 1 l. of distilled water. Samples were prepared in replicates. The water was continuously stirred with a magnetic stirring bar and samples of dialyzed beer were taken out of the bath at intervals of 2, 6, 24, and 48 hr for analysis for iso-α acids. A nonhopped beer which was used as a control did give a blank reading of 8% of the absorbance value of that of hopped beers under the condition of analysis. In presenting results of our experiments, this value has been subtracted from the absorbance readings. Results are presented in Table VI.

While our experiments exclude proteins with molecular weights above 12,000 as part of the "bitter complex," other low molecular weight compounds could still be in-

Table VI. Percent Iso-α Acid Concentration and Protein Nitrogen in Beers after Dialysis

Analysis	After dialysis for, hr			
	2	6	24	48
Iso-α acids[a]	61	38	5	0
$N \times 6.25$	53	43	38	20

[a] Iso-α acids expressed as percent of absorbance of isooctane extract of beer at 285 mμ before dialysis. Details of analytical procedure for the determination of iso-α acids are given in ASBC method.[3]

volved in forming this bitter complex. The noniso-α acids part of the complex is probably not protein or peptide in nature, as the "complex" is apparently extractable into isooctane. Environment of beer rather than a specific reaction of iso-α acids with other beer components may be responsible for the modified taste of iso-α acids in beer. This factor is being investigated at present.

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