THE CHEMISTRY OF HOP CONSTITUENTS

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I. INTRODUCTION

Hops, the cones of the female plant of the species *Humulus lupulus* L., are grown throughout the temperate regions of the world to meet the demands of the brewing industry. The estimated world production of dried cones in 1964 was 205,053,485 lb of which 28,259,445 lb was grown in England, 53,377,996 lb in the United States, and 112,345,756 lb in continental Europe (35).

The genus *Humulus* is represented by two species, the common hop, *Humulus lupulus* L., and the Japanese hop, *H. japonicus* Sieb. et Zucc., and belongs to the natural family *Cannabinaceae*. The wild hops of North America, *H. americanus* and *H. neomexicanus*, and the Asian *H. cordifolius* are generally considered to be varieties of *H. lupulus*. *Humulus scandens* (Lour.) Merr. is synonymous with *H. japonicus* which is an an-

(1) Eyre & Spottiswoode (Publishers) Ltd., London, EC4.

nual plant from Japan, sometimes grown as an ornamental climbing plant. Its cones have no resin glands, and it is therefore devoid of the normal resins and oils which make up the brewing value of *H. lupulus*. The only other genus in the natural family *Cannabinaceae* is *Cannabis* represented solely by *C. sativa*, Indian hemp, marihuana, or hashish. Chemical similarities between the two genera have been discussed by Hegnauer (166) and will be indicated here. A short section on the *Cannabis* resins is appended.

Burgess (64) has recently written a textbook dealing with the botany, cultivation, and utilization of hops which replaces his earlier pamphlet on the subject (63).

The hop cone consists of stipular bracts and bracteoles around a central axis or strig. At the base of the bracteoles, the resin glands are formed as the hop ripens, and it is in these lupulin glands that the main brewing principles, the resins and the essential oils, are formed. The lupulin glands can be separated from the hop by mechanical means (49, 268, 271) and can be used to replace hops in the brewing process, with particular advantage in the English practice of dry hopping (see later). Hops may be deliberately fertilized by planting male plants in the hop gardens, as in English practice; the hops will then contain 18–30% w/w of seeds, or grown "seedless" according to continental practice where the planting of male hop plants is controlled by law. In America the majority of hops are grown seedless but some varieties are fertilized. Seedless hops are generally richer in essential oil and resins than seeded ones (157).

At harvest hops contain about 80% of moisture which is reduced to about 10% by drying them in a kiln or oast with a current of air at 140–155°F (60–68.5°) when it is normal practice to burn rock sulfur in the fire to bleach the hops. After cooling the hops are compressed into bales or pockets which are usually stored at 0° until required for use. Hops will normally be used between 4 and 20 months after harvest.

In the brewing process sweet wort is prepared by mashing a mixture of ground malt, with or without other cereal adjuncts, with water so that enzymatic degradation of starch to the soluble sugars, glucose, maltose, and maltotriose occurs. This sweet wort is then boiled with hops at a rate of 0.5–1 lb/36-gal barrel (139–278 g/hectolite) for 0.5–2 hr in a copper kettle vented to the atmosphere. The hops may all be added at the start of the boil, in which case the bulk of the essential oil will be lost, or a portion may be added toward the end of the boil. This latter technique is used in America and on the European continent, whereas British brewers tend to the former. In order to add hop aroma to their beers British brewers add a proportion of "dry hops" to their finished beers, either in casks or conditioning tanks. After the wort has been boiled with hops, it is filtered and cooled, and yeast is added to ferment the sugars to ethanol. When the fermentation is complete, the yeast is removed and the beer conditioned before sale either in casks or bottles.

II. Chemical Composition of Hops

Howard (183) gives the following approximate composition of air-dried hop cones.

		%
1.	Water	10
2.	Total resins	15
3.	Essential oil	0.5
4.	Tannins	4
5.	Monosaccharides	2
6.	Pectin	2
7.	Amino acids	0.1
8.	Proteins (N × 6.25)	15
9.	Ash	8
10.	Cellulose, etc.	43.4
		100.0%

In this review the chemistry of items 2–7, namely, the resins, essential oils, tannins, monosaccharides, pectin, and amino acids will be discussed together with two groups of compounds not included in the above list: hop wax (1.5%) and the seed fat (5% in fertilized hops). The literature has been surveyed up to December 1965 through *Chemical Abstracts*.

III. Hop Resins

A. Introduction and Nomenclature

The chemistry of the hop resins has been reviewed earlier, *inter alia*, by David (107), Heilbron (167), Howard (183), and Stocker (370). The resins are derivatives of 2-acylcyclohexane-1,3-dione and are included, together with usnic acid, fern, kousso, and essential oil constituents, in a review of this group of compounds by Hassall (162) and also in a review of naturally occurring phloroacylphenones by Riedl (291). Numerous technical reviews of the composition and brewing behavior of hops have also appeared (*inter alia* 101, 170, 216, 262). Hayduck (165) separated the hop resins into various fractions on the basis of their solubility in different solvents and their ability to form a precipitate with lead acetate. Later workers have adopted different systems, but the situation was clarified by joint proposals of the European Brewery Convention (EBC) and the American Society of Brewing Chemists (ASBC) (121) who gave the following definitions.

1. *Total Resins.* These consist of the hard resins, uncharacterized soft resins, the α acids, and the β acids. In terms of solubilities the total resins are defined as that portion of the ether extract which is soluble in cold methanol, or that portion of the cold methanol extract which is soluble in ether.

2. *Hard Resins.* These consist of that portion of

the total resins which is insoluble in low-boiling paraffinic hydrocarbons.

3. *Total Soft Resins.* These consist of the fraction of the total resins which is soluble in low-boiling-point paraffinic hydrocarbons. The total soft resins consist principally of (a) the α acids, (b) the β acids, (c) the uncharacterized soft resins.

4. *The β Fraction.* This consists of the β acids plus the uncharacterized soft resins.

5. *The Uncharacterized Soft Resins.* These consist of that portion of the total soft resins which has not been characterized as any specific compound.

6. The α acids are humulone, cohumulone, and adhumulone, etc.

7. The β acids are lupulone, colupulone, and adlupulone, etc.

8–15. Chemical definitions of the above terms are included here.

16. *α Soft Resin.* This term is reversed for those substances which may later be identified as having arisen from the α acids and which, under this system of nomenclature, would constitute part of the uncharacterized soft resins.

17. *β Soft Resin.* This term is reserved for those substances which may later be identified as having arisen from the β acids and which, under this system of nomenclature, would constitute part of the uncharacterized soft resins.

Such a classification, based on solubilities, cannot be absolute, and other constituents which are not strictly resins will be included in the various fractions. The limitation that total resin should be soluble in cold methanol is designed to eliminate hop wax (see later), but the difficulties of achieving this separation in practice have been well illustrated (95). The essential oils, being soluble in cold methanol and light petroleum, will be found among the uncharacterized soft resin. The fat present in undamaged hop seed is resistant to solvent extraction, but as soon as the seed is damaged it is readily extracted and will also form part of the uncharacterized soft resin.

Before considering the constituents of these fractions, it should be mentioned that some workers, following Hayduck, still call the hard resin the γ fraction. Walker (403) proposed that the water-soluble portion of the hard resin, which had a bitter character, should be called the δ resin. Burton and Stevens (71) found that a very small proportion of the hard resin was capable of forming a lead salt and called this fraction the α hard resin and the remainder, not forming a lead salt, the β hard resin.

Many techniques have been used in order to resolve the natural mixtures of hop resins, including chromatography on silica gel (336), perlon (156), paper (335), ion-exchange resins (71, 194, 342), and papers modified with ion-exchange resins (322). Countercurrent distribution (303, 304, 390), steady-state distribution (9, 12), partition chromatography (387, 388), and reversed-phase chromatography, both in columns (30, 355, 356, 360, 363) and on paper (408), have been fruitfully employed as have paper electrophoresis (256) and thin film chromatography (160, 161, 184, 238, 239, 240).

The analysis of hop resins has been reviewed by Hudson (212, 213), and subsequent workers (29, 47, 216, 409) have discussed the significance of such analyses. Methods of hop analysis recommended by the Institute of Brewing Analysis Committee have been published (218). The preservative value of hops with regard to the biologically stability of beer has been reviewed recently and the conclusion drawn that this property of hops has been over-emphasized (252).

Most of the hop resin constituents so far examined contain an enolizable β-triketone system and exist as mono- or dienols. However, except in a few instances the direction of enolization is either not fixed or known with any certainty. Accordingly, in this review except in a few cases when discussing fine structure, only one tautomeric form will be shown. The systematic names assigned to the hop resins are often based on the unenolized ketonic structure in preference to an arbitrary enolic form.

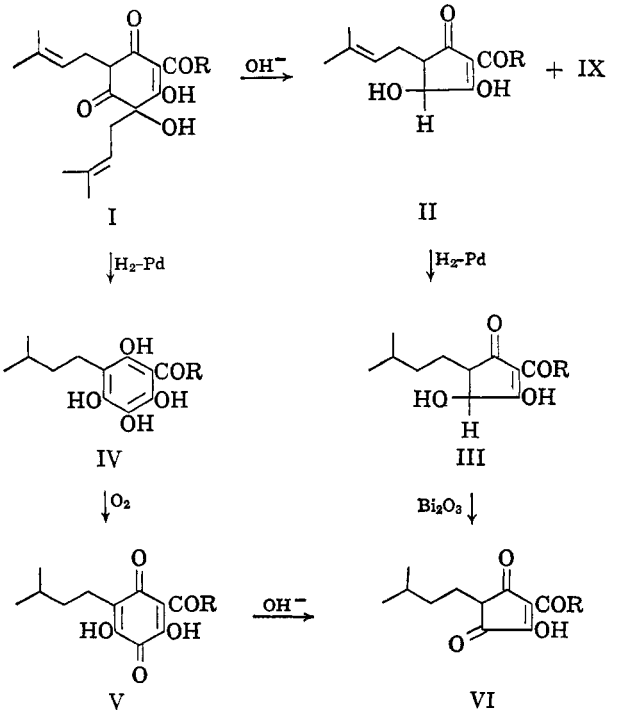
B. α ACIDS (HUMULONES)

The α acids are readily separated from the soft resin as their lead salts which are insoluble in methanol.

Humulone was the first member of the α-acid fraction to be obtained crystalline and characterized. In 1904 Lintner and Schnell (249) isolated humulone (I, R = *i*-Bu), $C_{21}H_{30}O_5$, as a pale yellow, bitter-tasting, levorotating enolic acid, mp 63°, which reduced Fehling's solution and ammoniacal silver nitrate, and made the first investigation of its alkaline hydrolysis. They were not able to prepare any derivatives of humulone, other than the lead salt, and it was left to Wöllmer (414) to prepare the first organic crystalline derivative with 1,2-diaminobenzene (*o*-phenylenediamine). This has since been widely used for the purification of humulone. The complex, mp 117°, shown by analysis to be a 1:1 compound of humulone and the amine, is completely dissociated in solution (18). The ultraviolet, infrared, and nuclear magnetic resonance spectra in solution are all the sum of those given by the two individual components. The infrared spectrum of the solid complex, however, is not the sum of the spectra of the two components in the solid state, and the differences suggest that the hydrogen bridge of the tertiary hydroxyl in humulone is replaced by some stronger interaction in the complex and that the environment of the adjacent carbonyl group is also completely changed (18). It is to be expected that the hydroxyl group in humulone is involved in this com-

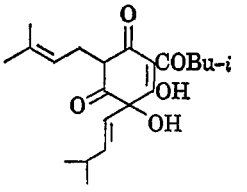
plex formation as lupulone fails to form such a complex. Complexes have also been made with substituted *o*-phenylenediamines, *e.g.*, 4-methyl-, 4,5-dimethyl-, and 4-methoxy-1,2-diaminobenzene, and more surprisingly with 2,6-diaminopyridine (109). Humulone forms a phenylurethan, mp 161° dec (150), but no crystalline derivatives of the carbonyl functions have been obtained.

Alkaline hydrolysis of humulone (249, 414) afforded isobutyraldehyde, an unsaturated aliphatic acid, $C_6H_{10}O_2$, and a crystalline acid, mp 93°, $C_{15}H_{22}O_4$, called humulinic acid (now humulinic acid A) (II, R = *i*-Bu). In contrast to humulone, humulinic acid A gave a crystalline oxime (414), mp 152–153°. In the presence of colloidal palladium it took up 1 mole of hydrogen to give a dihydrohumulinic acid (A) (III, R = *i*-Bu), mp 126°, which also formed an oxime, mp 125°. The hydrogenation of humulone is more complex; using palladium chloride as catalyst, Wöllmer (414) found that a fragment of five carbon atoms was split off as isopentane to leave a product, $C_{16}H_{24}O_5$, aerial oxidation of which gave a purple quinone, $C_{16}H_{22}O_5$. The product, $C_{16}H_{24}O_5$, mp 128°, called humuloquinol (IV, R = *i*-Bu) afforded a crystalline tetrabenzoyl derivate, mp 167°, an oily dimethyl ether, and a monophenylurethan, mp 131° dec. The oxidation product, humuloquinone (V, R = *i*-Bu), $C_{16}H_{22}O_5$,



mp 84°, was characterized as a mono-2,4-dinitrophenylhydrazone, mp 215°, and an azine, mp 110°. Mild alkaline hydrolysis of humuloquinone gave rise to a yellow crystalline product, mp 144°, isomeric with humulinic acid and therefore called isohumulinic acid (VI, R = *i*-Bu).

On the basis of the above evidence Wieland (410, 411) proposed structure VII for humulone. He as-

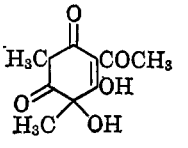


VII

sumed, in view of the simultaneous production of isobutyraldehyde, that the unsaturated acid produced by hydrolysis of humulone was 4-methylpent-2-enoic acid (VIII), but it was later shown to be 4-methylpent-3-enoic acid (IX) (102, 110) which required correction of Wieland's formula to I (R = *i*-Bu).

$(CH_3)_2CHCH{=}CHCOOH$ VIII $(CH_3)_2C{=}CHCH_2COOH$ IX

Confirmation of structure I, as opposed to VII, was provided by Carson (83) who showed that ozonolysis of humulone gave 2 moles of acetone and no isobutyraldehyde. Meanwhile Campbell and Coppinger (80) synthesized the model structure 1-acetyl-3-hydroxy-3,5-dimethylcyclohexane-2,4,6-trione (X) and showed



X

it to have similar ultraviolet light absorption to humulone. This work was rapidly extended by Riedl (284–286, 290) to the synthesis of (±)-humulone itself. Alkylation of phloroisovalerophenone with 3-methylbut-2-enyl bromide gave *inter alia* the 3,5-diisopentenyl derivative (see later) which when shaken in air or oxygen in the presence of a methanolic solution of lead acetate gave the insoluble lead salt of (±)-humulone (I, R = *i*-Bu). It should be noted that this synthesis is not an unambiguous proof of the proposed structure, and up until the time it was achieved there was no analogy for the behavior of humulone (and lupulone) upon hydrogenolysis. To explain this, cyclic ether structures were proposed for humulone, lupulone, and humulinic acid (150), but these were withdrawn (202) when an analogous hydrogenolysis was observed in the sennoside series (372).

Racemic (±)-humulone (I, R = *i*-Bu) has not been resolved but naturally occurring (−)-humulone is partially racemized by distillation at bp 150–160° (bath) at 0.2 mm (195). Racemic humulone is also obtained when (−)-humulone is heated under reflux in toluene for 18 hr or in dioxane for 48 hr (195). In boiling

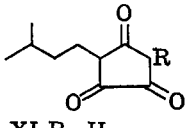
isooctane[2] (−)-humulone is half racemized in 11 hr, complete conversion to (±)-humulone taking 6 days (24).

The optical rotation of humulone is dependent on the solvent employed and the pH of the solution. The following values for the optical rotation of humulone in different solvents have been reported: −211° in methanol, −212.5° in ethanol, −245° in isooctane, −226° in benzene, −91° in pyridine, and +53° in piperidine (21). When sodium hydroxide is added to a methanolic solution of humulone the specific rotation changes from −211 to +32° with the addition of 1 equiv. Acidification restores the original rotation so that the changes are due to the formation of the humulinate ion (21). Accordingly, naturally occurring (−)-humulone was assigned the D configuration by Howard (178) since D-α-hydroxy acids also give salts which are more dextrorotary than the parent compound.

The structure of humulone, as proposed by Wieland, depended on those proposed for humulinic acid A (II, R = *i*-Bu) and isohumulinic acid (VI, R = *i*-Bu), and early attempts to relate these two structures failed. Thus, hydrogenation (150) of isohumulinic acid gave a product, mp 88°, not identical with dihydrohumulinic acid (A) and which in the light of later work (66) was most likely the epimeric dihydrohumulinic acid B. This latter acid, mp 92°, is readily obtained from isohumulinic acid by reduction with sodium borohydride (66), and treatment with alkali causes it to revert to the more stable A isomer, mp 126°.

Early attempts (150) to oxidize dihydrohumulinic acid (A) to isohumulinic acid were also unsuccessful, but this was successfully achieved later using bismuth oxide (187).

Hydrolysis of isohumulinic acid with strong alkali gave isovaleric acid and 3-(3-methylbutyl)cyclopentane-1,2,4-trione (XI) (150). The synthesis of this latter compound was achieved by condensing 6-methylheptan-2-one with 2 moles of diethyl oxalate to give the glyoxylic ester (XII) which on hydrolysis gave XI.

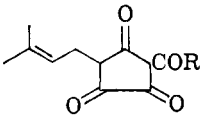


XI, R = H
XII, R = $COCOOC_2H_5$

Early attempts (150) to acylate this compound to give isohumulinic acid were unsuccessful, but it has recently been achieved by Leucht and Riedl (247) who obtained isohumulinic acid in 60% yield using isovaleric anhydride in the presence of boron trifluoride. Since isohumulinic acid has been converted to dihydrohumulinic acid (66), this reaction also represents a synthesis of the latter acid.

Leucht and Riedl's (247) synthesis can be extended to other 3-acylcyclopentane-1,2,4-triones. An alternative synthesis (382) in which the enol ether of a 1,3-diketone was condensed with ethyl oxalate was claimed to lead to these compounds. None of the products isolated corresponded to known compounds, and they were later shown to be 3-methyl-4-pyrone-2-carboxylic acids (120, 383). Repetition of the synthesis, however, gave, as well as these compounds, the required 3-acylcyclopentane-1,2,4-trione, as shown by comparison with an authentic sample prepared by the acylation route (120).

The synthesis of humulinic acid, other than from humulone, has not been reported, although considerable advances in our knowledge of its chemistry have been made. Anteunis and Verzele (22) in 1959 were the first investigators to report the occurrence of a second form of humulinic acid isolated from the mother liquors of the known isomer. This second isomer, humulinic acid B, mp 74°, was obtained pure in about 5% yields after a 510 transfer countercurrent distribution of the alkali equilibration mixture from humulinic acid A (20). The equilibrium between humulinic acids A and B is so much in favor of the former that the latter could not be detected by reversed-phase chromatography (357). Humulinic acid B was also obtained by a chemical route (66, 70). Oxidation (189) of humulinic acid A with bismuth oxide afforded 3-isovaleryl-5-(3-methylbut-2-enyl)-cyclopentane-1,2,4-trione (XIII,



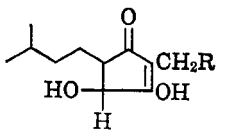
XIII

R = *i*-Bu) which has been given (131) the trivial name dehydrohumulinic acid. Reduction of this acid with sodium borohydride gave humulinic acid B in 30% yield (66) which was readily oxidized back to dehydrohumulinic acid with bismuth oxide (66). This evidence strongly suggested that humulinic acids A and B were C_4 epimers, and this was confirmed by nuclear magnetic resonance spectroscopy (10, 66) which showed the more stable A isomer has the *trans* configuration (see section VIC).

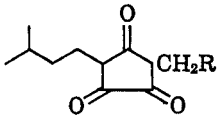
Hydrogenation of humulinic acid A affords a dihydro derivative as mentioned above, but using more active catalysts more than 1 mole of hydrogen is taken up to give a product, $C_{15}H_{26}O_3$, mp 171°, which shows the simpler ultraviolet spectrum of a β-diketone and is formulated as XIV (R = *i*-Bu) (66, 245). Hydrogenation of humulinic acid B under similar conditions gives a product different from the above, mp 172–174°. Although the melting point of a mixture of the two products is not depressed, the infrared and proton magnetic

(2) In hop chemistry isooctane refers to the petroleum fraction (bp 101°) which is 2,2,4-trimethylpentane and not to 2-methylheptane as given in ref 59.

resonance spectra show clear differences in agreement with their formulation *cis–trans* isomers. Oxidation of the two dihydrodeoxohumulinic acids (66, 245) gave the same trione (XV, R = *i*-Bu) which was cleaved with sodium hydroxide or sodium periodate to diisohexyl ketone.

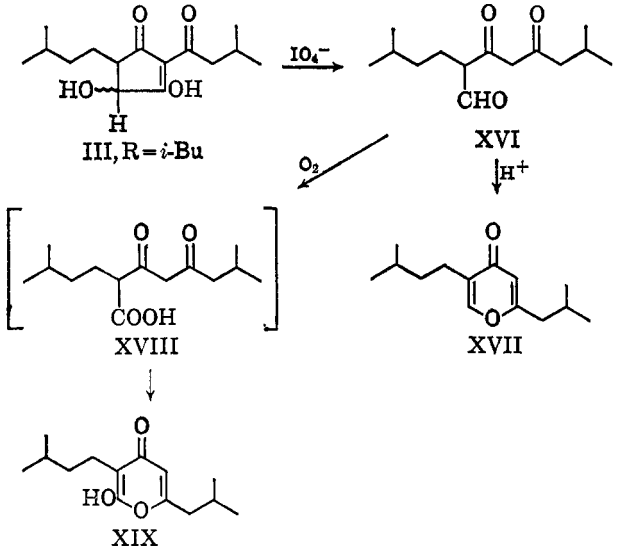


XIV

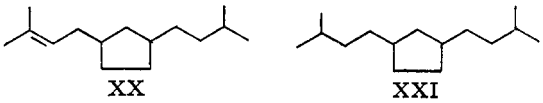


XV

Cleavage of dihydrohumulinic acids A and B with periodates gave rise to the same series of products, namely, 1 mole of carbon dioxide, an aldehyde, 2-isopentyl-7-methyl-3,5-dioxooctanal (XVI), which cyclizes in acid to the pyrone (XVII), and the corresponding acid (XVIII), which spontaneously gives the hydroxy pyrone (XIX) (245).

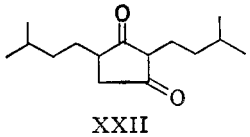


Clemmensen reduction of humulinic and dihydrohumulinic acids afforded respectively the hydrocarbons XX and XXI (412). In the case of dihydrohumulinic acid an intermediate product, called deoxydihydrohumulinic acid, was isolated and assigned structure XXII (412). The same product was obtained by



XX

XXI



XXII

dehydration of dihydrohumulinic acid followed by reduction (see section VD2) and by hydrogenating of isohumulinic acid in glacial acetic acid containing perchloric acid and using Adams catalyst (68). Examination of the proton magnetic resonance spectrum of the product confirmed the structure (XXII) assigned earlier (68).

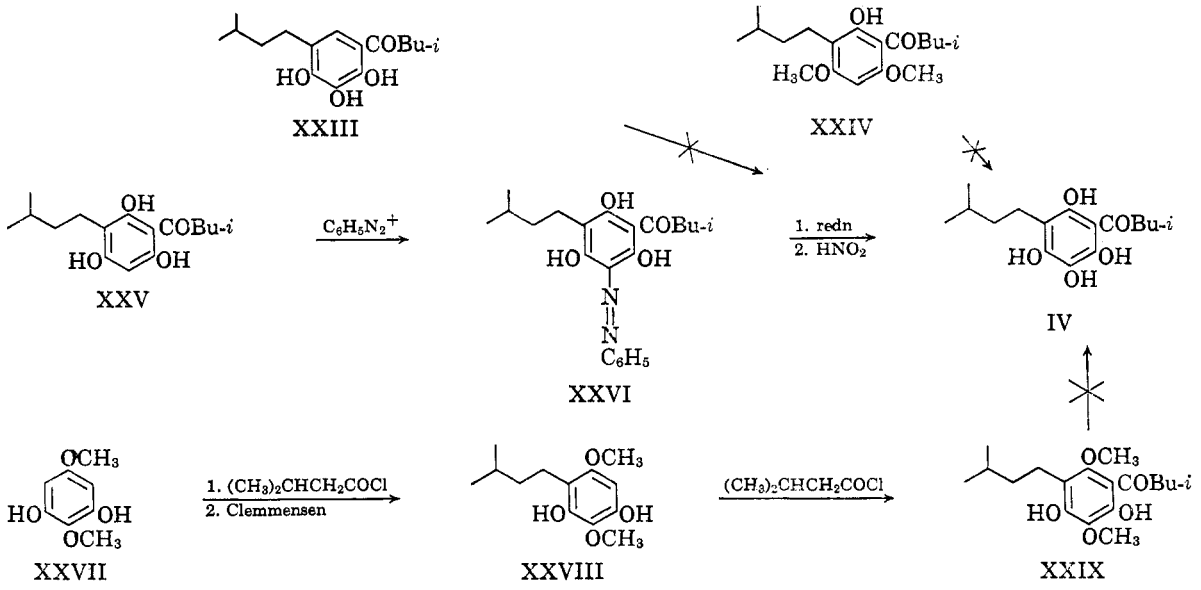
The hydrogenation of humulone has also been the subject of further study, but this is best considered together with that of lupulone (see later), but it may be mentioned here that the hydrogenolysis product, humuloquinol (IV, R = *i*-Bu), and the related quinone (V, R = *i*-Bu) have been synthesized.

In earlier attempts to prepare these compounds, David and Imer (110) had synthesized 4-isopentyl-6-isovalerylpyrogallol (XXIII) but were unable to oxidize it to humuloquinone (V, R = *i*-Bu). Riedl and Leucht (294) similarly subjected 2-isopentyl-6-isovaleryl-3,5-dimethoxyphenol (XXIV) to Elb's persulfate oxidation but were unable to obtain any characterizable product. They were able, however, to condense 2-isopentyl-6-isovalerylphloroglucinol (XXV) with benzenediazonium chloride and cleave the resultant azo compound (XXVI) with zinc chloride and hydrolyze the amine formed to humuloquinone (IV, R = *i*-Bu).

The same authors (294) synthesized humuloquinol dimethyl ether (XXIX). Acylation of 2,5-dimethoxyresorcinol (XXVII) with isovaleryl chloride followed by Clemmensen reduction gave the 4-isopentyl compound (XXVIII) which on further acylation gave humuloquinone dimethyl ether (XXIX), but attempts to demethylate this product were unsuccessful (294).

Cohumulone, Adhumulone, and Other Analogs.—Rigby and Bethune (303, 305) in 1952 were able to show by countercurrent distribution the heterogenous nature of the bittering substances in beer which were derived from the α acids. Following up this observation, they demonstrated by countercurrent distribution that the α acids themselves were heterogenous. In the system 2,2,4-trimethylpentane (isooctane) and a buffer solution of pH 8.0, a new compound, named cohumulone, was separated from humulone after a hundred transfers. Whereas the distribution pattern given by cohumulone agreed with that predicted by theory, that given by humulone did not, but after 300 transfers this material was resolved into pure humulone and a further component adhumulone.

Cohumulone, $C_{20}H_{28}O_6$, $[\alpha]_D^{27}$ $-208.5°$, failed to crystallize at room temperature but closely resembled humulone in light absorption and formed a complex with 1,2-diaminobenzene (304) and with 2,6-diaminopyridine, mp 113° (56). With phenyl isocyanate it gave an oily urethan, but the α-naphthyl derivative was crystalline, mp 157° (56). The chemistry of cohumulone closely follows that of humulone; hydrolysis gave cohumulinic acid (II, R = *i*-Pr) and 4-methyl-pent-3-enoic acid (IX), while hydrogenolysis gave cohumuloquinol (IV, R = *i*-Pr) and cohumuloquinone (V, R = *i*-Pr) (198, 202). The last by mild hydrolysis afforded



isocohumulinic acid, Mp 71–79°, which on more drastic treatment gave isobutyric acid and 3-(3-methylbutyl)-cyclopentane-1,2,4-trione (XI), identical with a sample from isohumulinic acid. Cohumulone was therefore the analog (I, R = *i*-Pr) as was proved by synthesis from phlorisobutyrophenone (200).

Adhumulone, also an oil, $[\alpha]_D^{26}$ −187° (methanol), is isomeric with humulone. It gives a crystalline derivative with 1,2-diaminobenzene, mp 98°, and on hydrolysis affords adhumulinic acid, mp 83° (307). The structure was shown by synthesis to be that of the 2-methylbutyryl analog (I, R = *sec*-Bu) and not that of the *n*-valeryl and 4-methylvaleryl analogs which were also synthesized (201). Humulone, cohumulone, and adhumulone, together make up over 95% of the α-acid fraction of hop resin, and it has been shown that, whereas the proportion of adhumulone is fairly constant (10–15%), that of humulone and cohumulone show wider variance (20–65%) and the proportions seem to be a varietal characteristic. The original estimates were made by countercurrent distribution (199, 204) and later by partition chromatography (387, 388). Oxidation of α and β acids by alkaline hydrogen peroxide cleaves the side chain as a volatile aliphatic acid characteristic of the parent. Such mixtures may be readily resolved by gas–liquid chromatography (205, 207). Pyrolysis of the lead salts of the α acids also yields the corresponding aliphatic acids (311) which can be examined by gas chromatography directly (318) or after esterification (311). The proportions of humulone, cohumulone, and adhumulone in a mixture of α acids can also be estimated from the proton magnetic resonance spectrum (13).

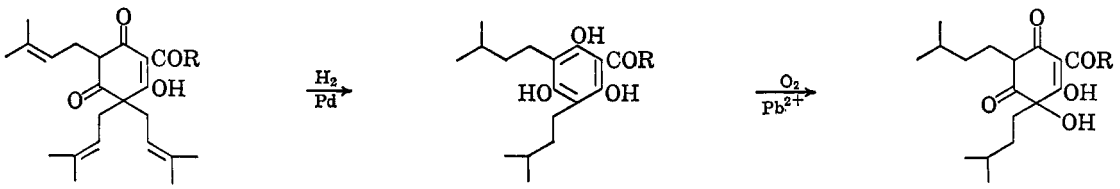
The partition chromatogram of the hop α acids showed small peaks before and after the three major constituents. Posthumulone has been characterized as the propionyl analog (I, R = Et) (389), but there is some dispute as to the nature of prehumulone. Rigby, Sihto, and Bars (312) report it to be a mixture of the *n*-butyryl, *n*-valeryl, and 4-methylvaleryl (isocaproyl) analogs, while Rillaers and Verzele (314) report it to be the last-named analog by comparison with synthetic material.

In addition to countercurrent distribution and partition chromatography mentioned above, the mixture of analogs present in the α-acid fraction has been resolved by reversed-phase column chromatography (30, 355, 356, 360, 361) and on paper (408), by steady-state distribution (9, 12), by chromatography on polyamides (Perlon) (156), and by ion-exchange chromatography using frontal analysis (342), but not by ordinary elution (194).

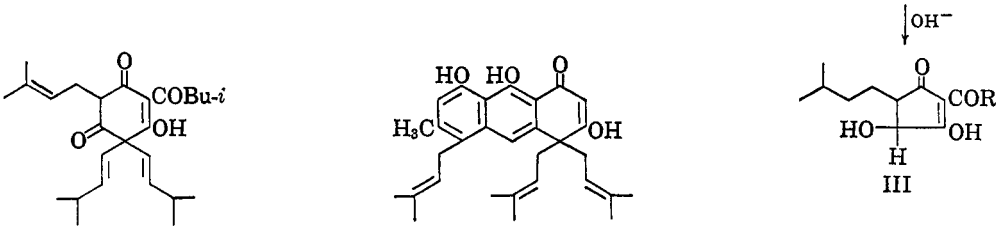
C. β ACIDS (LUPULONE, COLUPULONE)

Lupulone, $C_{26}H_{38}O_4$, a colorless crystalline compound, mp 90°, $[\alpha]_D$ 0°, was first isolated from hops by Lermer in 1863 (246). Unlike humulone it was stable to alkaline hydrolysis in the absence of air, but upon hydrogenolysis it lost an isopentane fragment in a similar manner to humulone (415). Following Wieland's structure for humulone (411), Wöllmer (416) suggested that lupulone had structure XXXI. He correctly formulated the major hydrogenolysis product as 4,6-di(3-methylbutyl)phlorisovalerophenone (XXXII, R = *i*-Bu) and characterized it as the crystalline tribenzoate, mp 165°. When this hydrogenolysis product (XXXII, R = *i*-Bu) was shaken in air or oxygen in the presence of lead acetate, the insoluble lead salt of (±)-tetrahydrohumulone was formed as shown by alkaline hydrolysis of the parent acid (XXXIII, R = *i*-Bu), mp 84°, to dihydrohumulinic acid (III, R, *i*-Bu). By alkaline fusion Verzele and Govaert (395) obtained from lupulone a mixture of acids and ketones which caused them to amend Wöllmer's struc-

ture to XXX (R = *i*-Bu) (386), a formulation supported by ozonolysis (83). This structure was confirmed synthetically by alkylation of the trisodio derivative of phloroisovalerophenone with 3 moles of 3-methylbut-2-enyl bromide (287–290).



XXX XXXII XXXIII



XXXI XXXIV III

As with the α acids, the β acids occur as a mixture of analogs, and it is noteworthy that, while lupulone crystallizes from the soft resin of continental European hops, British and American hops yield cohumulone, $C_{25}H_{36}O_4$, mp 93°, although this was not realized at first. Both Walker (400, 402) and Carson (82) thought that they were dealing with lupulone, but subsequent work (186, 187, 203, 295, 297) has shown that it was actually colupulone which they had.

Hydrogenolysis of colupulone afforded 4,6-di(3-methylbutyl)phlorisobutyrophenone (XXXII, R = *i*-Pr) (tribenzoate, mp 134°) which was oxidized to (±)-tetrahydrocohumulone (XXXIII, R = *i*-Pr) which failed to crystallize. This on hydrolysis gave crystalline dihydrocohumulinic acid (III, R = *i*-Pr), identical with a sample prepared from cohumulone. Colupulone had been synthesized by Riedl (299) as one of a series of β acids prepared to investigate the influence of the length of the acyl side on the bacteriostatic activity, and was found to be identical with natural colupulone.

The β acids are more sensitive to oxidation than the α acids and cannot therefore be separated by countercurrent distribution methods. Howard and Tatchell (204) investigated the composition of the β acid fraction by subjecting it to hydrogenolysis, and after oxidation of the product analyzed the resultant tetrahydrohumulones by countercurrent distribution. Later, they examined the β acids of many hops by oxidation and separation of the volatile acids formed (205, 207). As a result of these studies they proposed the regression equation

$$\%\ \text{colupulone in } \beta \text{ acid} = 20.2 + 0.943(\%\ \text{of cohumulone in } \alpha \text{ acid})$$

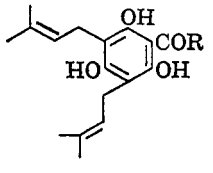
to relate the proportion of the co-component in the α and β acids.

By hydrogenolysis and countercurrent distribution of related tetrahydrohumulones and by examination of the volatile acids formed on oxidation, the third major constituent of the β-acid fraction was shown to be the 2-methylbutyryl analog, adlupulone (III, R = *sec*-Bu) (187, 204), and evidence for a further analog with a 3-methylpentanoyl side chain was obtained by oxidation (187). Both these analogs have been synthesized (297).

It is noteworthy that the only other natural product known to contain the *gem*-diisopentenyl residue is Harunganin (XXXIV) isolated from the bark of *Harungana madagascariensis* Poir. (316, 317, 373).

D. 4-DESOXYHUMULONE

This product (XXXV, R = *i*-Bu) was first encountered as an intermediate in the synthesis of (±)-humulone, but it was not isolated at that time (285, 288). Five years later 4-desoxyhumulone was obtained crystalline, mp 83°, and characterized as the tribenzoate, mp 139° (292). Subsequently, it was isolated from Hallertau hops (1957 crop) in 0.02% yield (293). In the following years the yields were: 1958, 0.038%; 1959, 0.05% (210). The natural product was identical with synthetic 4-desoxyhumulone but differed from 4-desoxycohumulone (XXXV, R = *i*-Pr) and 4-desoxyadhumulone (XXXV, R = *sec*-Bu) which were also



XXXV

synthesized (210). Nevertheless, evidence has been presented for the occurrence of 4-desoxycohumulone and 4-desoxyadhumulone in hops (312), and preliminary

results from ion-exchange chromatography suggest a higher concentration of 4-desoxyhumulones (*ca.* 0.35%) in immature Bullion hops (366).

E. UNCHARACTERIZED SOFT RESIN

This fraction is that portion of the soft resin remaining after the α acids have been precipitated with lead acetate and the β acids allowed to crystallize out. Even if the former process is quantitative, the latter is not; thus this fraction will contain β acids which have failed to crystallize. Whether or not the desoxyhumulones are found in this fraction will depend on the degree of aeration when the α acids are precipitated. By reversed-phase chromatography (363) of the soft resin, three new analogs were discovered and called hulupones. These were later shown to be oxidation products of the β acids and are considered later (see section VC2b).

The essential oil components of hops are soluble in ether and light petroleum and will accordingly be found in the uncharacterized soft-resin fraction from which they can be separated by steam distillation. The separation of hop wax from cold methanolic solution is slow and normally incomplete so that certain wax components will also accumulate in this fraction.

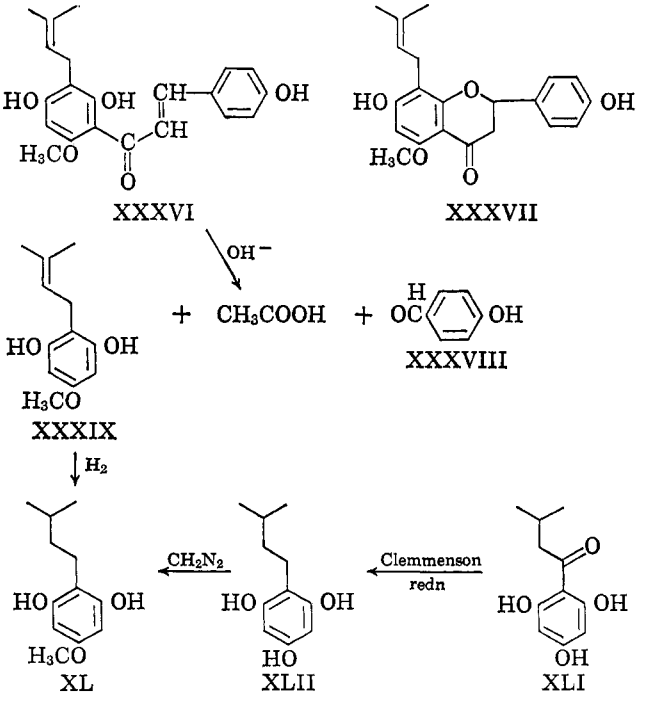
The size of the uncharacterized soft-resin fraction is the subject of some speculation. No approved analytical methods are laid down for the estimation of the total soft resin, and the removal of wax, by crystallization from cold methanolic solution, cannot be considered either quantitative or completely specific. Having arrived at a satisfactory figure for the total soft resin and the α-acid fraction, the size of uncharacterized soft resin can be obtained only by difference from a knowledge of the amount of β acid present, and methods for estimation of this are somewhat imprecise. Until these analytical problems are solved, it is impossible to estimate the size of the uncharacterized soft-resin fraction, but evidence from ion exchange and other chromatographic techniques suggests that, although many other components may be present in small amounts, no major group of hop resins remains undetected.

F. THE HARD RESIN (XANTHOHUMOL)

The hard-resin fraction is soluble in ether and cold methanol but insoluble in light petroleum. Hayduck (165) concluded that it had little brewing value. It was found to increase during the storage of hops, so that Kolbach (233) suggested that the ratio between the hard and soft resin present in a hop provides an index of deterioration and that hops in which the hard resin constituted more than 15% of the total resin should be classified as "old hops." It was therefore generally accepted that the hard resins arise by oxidation of the soft resins, but this can only be partially true as Schild and Raum (335) found hard resins in hops at the earliest stage of development. We should therefore differentiate between the native hard resin of hops and that which arises by autoxidation of the soft resin during kilning and storage.

The complex nature of the hard resin has been shown by ion-exchange chromatography (71, 194). It was also demonstrated that a small part of this fraction was capable of forming an insoluble lead salt (the α hard resin) (71) and by ion-exchange chromatography of this fraction, hulupinic acid, an oxidation product of hulupone (see section VC3) was isolated as 0.0002–0.042% of the hop. It seems likely that fraction 2 of the α hard resin (71) is α acid not completely precipitated earlier in the fractionation. The major portion of the hard resin, the β hard resin, when examined by ion-exchange chromatography, gave a complex pattern dominated by the large peak due to xanthohumol (71).

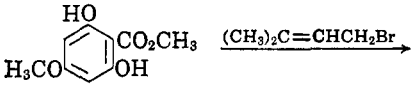
Xanthohumol (XXXVI) was first isolated from hops by Power, Tutin, and Rogerson in 1913 (280) and reisolated by Verzele and his co-workers (139, 397) in 1957. Xanthohumol, $C_{21}H_{22}O_5$, is a yellow crystalline product, mp 172°, which contains one methoxyl group and as such is the only known naturally occurring methylated hop resin. It forms a dimethyl ether, mp 127°, a 2,4-dinitrophenylhydrazone, mp 190°, and a tetrahydro derivative, mp 157°. With dilute alkaline reagents it is isomerized to a colorless product, isoxanthohumol (XXXVII), mp 198°, which is identical with



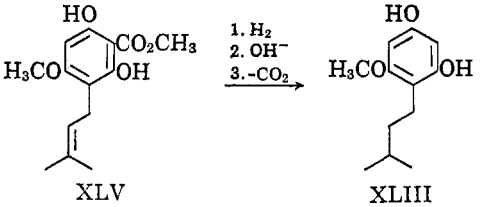
Power, Tutin, and Rogerson's humulol (280). Isoxanthohumol is reconverted to xanthohumol with strong alkali or with hydrogen fluoride in methanol

(80 hr at reflux temperature). Hydrolysis of either xanthohumol or isoxanthohumol with 20% sodium hydroxide afforded *p*-hydroxybenzaldehyde (XXXVIII), acetic acid, and a degradation product (XXXIX), $C_{12}H_{16}O_3$, mp 55°, which was crystallized only with difficulty. In contrast, the dihydro derivative (XL) of this degradation product, which retains the methoxyl group present in xanthohumol, readily crystallized, mp 100°. Ozonolysis of the original degradation product affords 0.65 mole of acetone, while alkaline fusion gave isovaleric and acetic acids. The degradation product, $C_{12}H_{16}O_3$, is therefore a methyl ether of isoprenylphloroglucinol, and xanthohumol is regarded as the chalcone (XXXVI) and isoxanthohumol the corresponding flavanone (XXXVII) (397).

The fine structure of the methyl ether of isoprenylphloroglucinol was the subject of some controversy before it was finally resolved. By Clemmensen reduction of isovalerophenone (XLI), Vandewalle and Verzele (384) obtained isopentylphloroglucinol (XLII), mp 126°, which with diazomethane gave a monomethyl ether, mp 99°, identical with the dihydro derivative of the degradation product. The methylation they claimed gave 1,3-dihydroxy-2-isopentyl-5-methoxybenzene (XL). The alternative methyl ether, 1,3-dihydroxy-4-isopentyl-5-methoxybenzene (XLIII), was prepared from methyl 2,6-dihydroxy-4-methoxybenzoate (XLIV) by alkylation with 1-bromo-3-methylbut-2-ene, followed by hydrogenation, hydrolysis, and de-

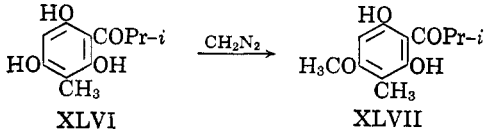


XLIV



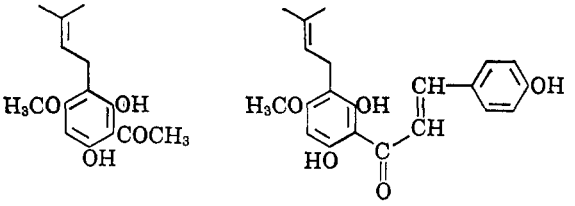
XLV XLIII

carboxylation of XLV. This had mp 91° and was not identical with the dihydrogenation product (XL). Hübner and Riedl (211), on the other hand, preferred the alternative assignment of structures. Methylation of 2,4,6-trihydroxy-1-isobutyryl-3-methylbenzene (XLVI) with diazomethane had been shown to lead to



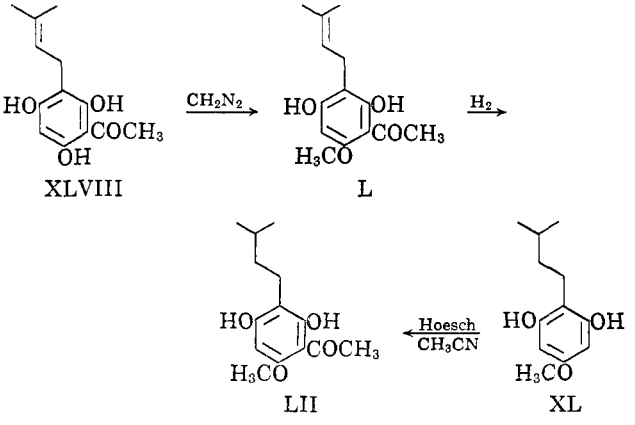
XLVI XLVII

the 4-methoxy product (XLVII) (329), and they argued by analogy that 1-acetyl-2,4,6-trihydroxy-3-(3-methylbut-2-enyl)benzene (XLVIII) should similarly give the 4-methoxy derivative (XLIX). Hydrolysis of their product afforded the degradation product of xanthohumol (XL) which they therefore regarded as LI (211). Vandewalle and Verzele later showed (385)



XLIX LI

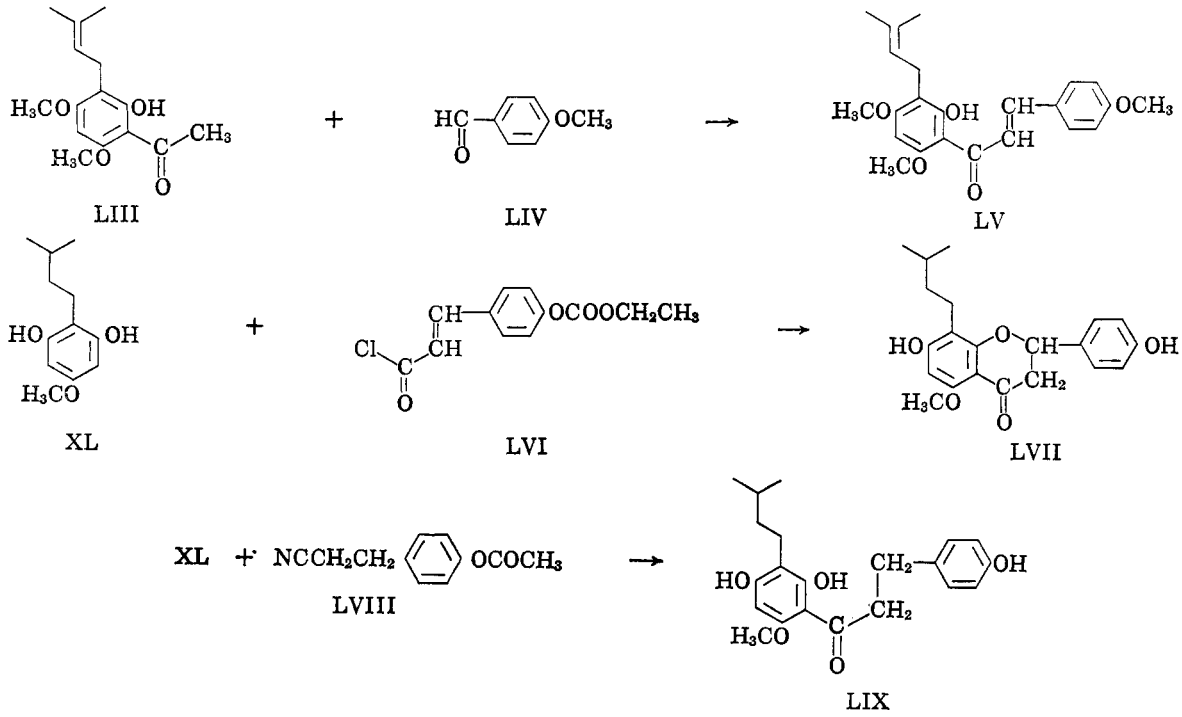
that Hübner and Riedl's product was the 6-methoxy compound (L). Hydrogenation of this product gave a dihydro derivative (LII) which could also be obtained by Hoesch synthesis from acetonitrile and 1,3-dihydroxy-2-isopentyl-5-methoxybenzene (XL). This, together with additional light absorption data, supported



XLVIII L

LII XL

structure XXXVI for xanthohumol (380), which was also favored by Seshadri (275) on the basis that 2′,6′-dihydroxychalcones (*e.g.*, LI) are unstable and not found in nature. Attempts to synthesize xanthohumol by condensation of 1-acetyl-2,4-dihydroxy-6-methoxy-3-(3-methylbut-2-enyl)benzene (L) with *p*-hydroxybenzaldehyde (XXXVIII) were unsuccessful (380). In contrast, by condensation of 1-acetyl-2-hydroxy-4,6-dimethoxy-3-(3-methylbut-2-enyl)benzene (LIII) with *p*-methoxybenzaldehyde (LIV), both Hübner and Riedl and Vandewalle obtained the dimethyl ether of xanthohumol (LV) (211, 380). Vandewalle (380) also prepared dihydroisoxanthohumol (LVII) by Friedel–Craft acylation of 1,3-dihydroxy-2-isopentyl-5-methoxybenzene (XL) with *p*-carboxyethoxycinnamyl chloride (LVI), followed by alkaline hydrolysis. Orth and Riedl (274) reinvestigated the structure of their degradation product and obtained the dihydro product (XL) by methylation of phlorisovalerophenone to give the 4-methyl ether, followed by Clemmensen reduction. By Hoesch condensation of this product with *p*-acetoxyphenylpropionitrile (LVIII), followed by hydrolysis, tetrahydroxanthohumol (LIX) was obtained.



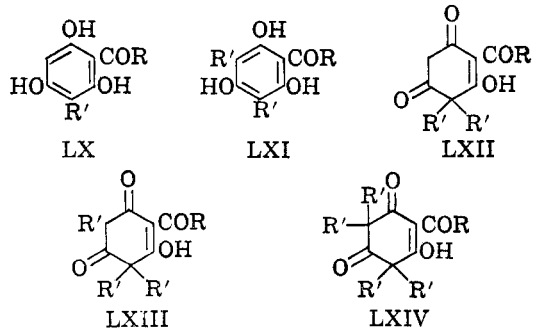
Evidence from ion-exchange and paper chromatography suggests that other analogs of xanthohumol may be present in hops (28, 71, 194). Verzele, *et al.* (397), claimed that only xanthohumol was present in hops and isoxanthohumol was an artifact, but it has been characterized as one of the fractions of the β hard resin by ion-exchange chromatography under conditions which did not cause isomerization of xanthohumol into isoxanthohumol (28). The xanthohumol content of fresh green hops (0.86% dry weight) is dramatically reduced (to 0.31% dry weight) during kilning, but the corresponding increase in the isoxanthohumol content is not observed (28).

G. δ RESIN

Walker, Zakomorny, and Blakebrough (403) observed that a portion of the hard resin was water soluble and bitter and had bacteriostatic properties. They called it the δ resin, and it was observed that this fraction accumulated during the storage of hops (1), but despite an extensive fractionation no pure components could be characterized (223, 341). Hulupinic acid (see later) is a hard-resin component with appreciable solubility in water and may therefore be classed as a δ resin (72).

IV. SYNTHESIS OF HOP RESINS

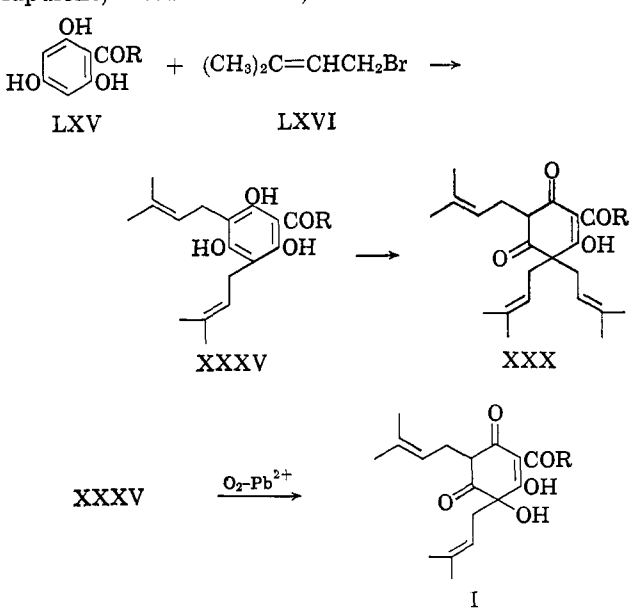
The hop resins, humulone, lupulone, and 4-desoxyhumulone, have been synthesized by Riedl, *et al.*, using nonspecific alkylation of a phloracylphenone (284–290). Phloracylphenones are readily available from phloroglucinol by condensation with acid chlorides (Friedel–Crafts synthesis) (138, 289), nitriles (Hoesch) (332, 364), or acids using boron trifloride as catalyst (138). C-Alkylation of a phloracylphenone can then produce one monoalkyl derivative (LX), two dialkyl derivatives (LXI and LXII), and one tri- (LXIII) and one tetraalkyl derivative (LXIV). The possibility of O-alkylation cannot be excluded.



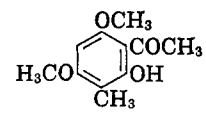
The original synthesis (285) of (±)-humulone consisted of treating a suspension of the disodium salt of phlorisovalerophenone (LXV, R = *i*-Bu) in benzene with 2 moles of 3-methylbut-2-enyl bromide (isoprenyl bromide) (LXVI) at 0° to give 4-desoxyhumulone (XXXV, R = *i*-Bu). This was not isolated in the first synthesis but, after removal of the precipitated sodium bromide and solvent, the residue was taken up in a methanolic solution of lead acetate and shaken in an atmosphere of oxygen when the lead salt of (±)-humulone was slowly formed. Decomposition of the lead salt followed by chromatography on silica gel afforded (±)-humulone (I, R = *i*-Bu) in 5.7% yield, characterized as the complex with 1,2-diaminobenzene, mp 109°, and by hydrolysis to humulinic acid (285). An improvement to this synthesis consisted of adding a

palladium catalyst to the methanolic solution of lead acetate and 4-desoxyhumulone when the oxygenation was complete in 70 min instead of several days, but the over-all yield was not improved (286, 288). If a solution of 4-desoxyhumulone in methanol with a palladium catalyst is shaken in an atmosphere of oxygen, the gas is rapidly absorbed, but oxidation appears to take place at other sites, *e.g.*, the isoprenyl side chains, as no pure *o*-phenylenediamine complex could be isolated from the product (286). Following this synthesis (±)-cohumulone (I, R = *i*-Pr) was prepared in 2.6% over-all yield from phlorisobutyrophenone, the product being isolated by countercurrent distribution (200). Similarly, adhumulone (I, R = *sec*-Bu) was synthesized in 1.8% yield and shown to be the 2-methylbutyryl analog and not the *n*-valeryl or the 4-methylvaleryl analogs which were also synthesized in unspecified yields (201). This last analog, 4-methylvaleryl (I, R = $CH_2CH_2CH(CH_3)_2$), was later synthesized in 4% yield and shown to be identical with prehumulone (314). The propionyl analog (I, R = Et), posthumulone, has also been synthesized in unspecified yield (389). Although these syntheses add considerable support to the structures assigned to the α acids, the low yields do not recommend them for preparative purposes.

Lupulone (XXX, R = *i*-Bu) was first synthesized by treating a suspension of the trisodium salt of phlorisovalerophenone in ether with 3 moles of 3-methylbut-2-enyl bromide (LXVI); after chromatography on neutral silica gel, lupulone was obtained in 21% yield (287–289). Using this technique several analogs of lupulone, listed in Table I, have been obtained.

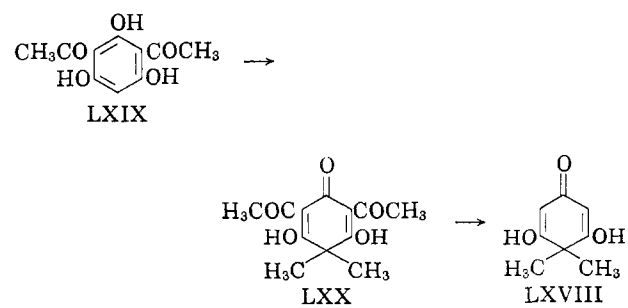


These syntheses had been carried out in nonpolar solvents in view of the sensitivity to hydrolysis of known triacylmethanes, but it was found that *trans*-fixed β-triketones, such as lupulone, were much more resistant to hydrolysis and so alkylations could be attempted in polar solvents. Preliminary experiments were made on the methylation of phloracetophenone (294) in which, in addition to the C-alkylated products (LX–LXIV, R = CH_3), 3-methylphloracetophenone 4,6-dimethyl ether (LXVII) (328) was obtained, par-



LXVII

ticularly when the alkylation was carried out in acetone–methanol (8:1). In a more detailed study the trimethylation of phloracetophenone in various media and using various bases was investigated (298). Thus, the yield of the lupulone analog (LXIII, R = CH_3), using sodium methoxide as condensing agent, was 40.8% in methanol, 41.2% in ethanol, 20% in 2-methyl-2-propanol, 26% in dioxane, and 1% or less in benzene or ether. In methanol the yield, using sodium methoxide (40.8%), was increased by using potassium methoxide (57.0%) but was less using the methoxides of barium (29.9%), calcium (9.8%), and magnesium (12.4%) (298). Finally, the yields of the different analogs, using different proportions of phloracetophenone, sodium methoxide, and methyl iodide, were investigated. The yields given in Table II illustrate the difficulty of preparing any specific analog in good yield. It may be mentioned that these methylated phloracetophenones are also related to the constituents of the male fern (291). Hydrolysis of the geminal dimethylphloracetophenone (LXII) gives filicinic acid (LXVIII) (330) in 5% over-all yield (299), but a more convenient synthesis of this compound consists of dimethylation of 2,4-diacetylphloroglucinol (LXIX) followed by hydrolysis of LXX which affords a 25% yield of filicinic acid from phloroglucinol (175).



To revert to consideration of the hop resins, after preliminary experiments in which 3,5-dimethylphloracetophenone (LXI, R = R′ = CH_3) and 3,5-di(3-methylbut-2-enyl)phloracetophenone (XXXV, R = CH_3) were synthesized (292), 4-desoxyhumulone (XXXV, R = *i*-Bu) was isolated in a crystalline form, mp 83°, and characterized as the tribenzoate, mp 139°, and

TABLE I
ANALOGS OF LUPULONE (LXIII)

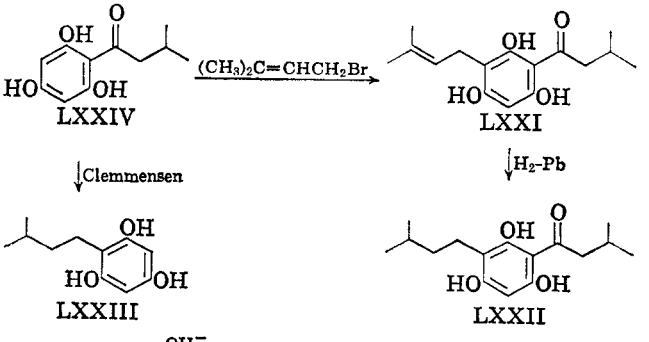
		R	R′	Mp, °C	Yield, %	Ref
1	Lupulal	CHO	$CH_2CH{=}C(CH_3)_2$	101	10	289
2	Lupucarboxylic acid, methyl ester	CO_2CH_3		124	7.3	289
3	Acetolupuphenone	$COCH_3$		120	26.2	288, 289
					32	298
4	Propiolupuphenone	$COCH_2CH_3$		101	30	298
5	Butyrolupuphenone	$COCH_2CH_2CH_3$		107	21.6	288, 289
6	Colupulone	$COCH(CH_3)_2$		93	19	289
7	Lupulone	$COCH_2CH(CH_3)_2$		93	20.8	287, 288, 289
8	Adlupulone	$COCH(CH_3)CH_2CH_3$		90	5.7	297
9	Caprolupuphenone	$CO(CH_2)_4CH_3$		90	21.4	289
10	Isocaprolupuphenone	$CO(CH_2)_2CH(CH_3)_2$		91	13.5	289
11	3-Methylvaeryllupuphenone	$COCH_2CH(CH_3)CH_2CH_3$		91	8.1	297
12	Benzolupuphenone	COC_6H_5		152	27	288, 289
13	Phenacetolupuphenone	$COCH_2C_6H_5$		109	30	289
					40	298
14		$COCH_3$	CH_3	158	40.8	299
15		$COCH_3$	CH_2CH_3	150	43	298
16		$COCH_3$	$CH_2CH{=}CH_2$	79	31	298
17		$COCH_3$	$CH_2CH_2CH_3$	108	54	298
18		$COCH_3$	$CH_2CH{=}CHCH_3$	111	46	298
19		$COCH_3$	$(CH_2)_3CH_3$	122	43	298
20		$COCH_3$	$CH_2CH(CH_3)_2$	157	42	298
21		$COCH_3$	$(CH_2)_4CH_3$	101	30	298
22		$COCH_3$	$CH_2CH_2CH(CH_3)_2$	163	39	298
23		$COCH_3$	$(CH_2)_5CH_3$	84	41	298
24		$COCH_3$	$CH_2C_6H_5$	124	35	298
25		$COCH_3$	$(CH_2)_{15}CH_3$	73	28	298
26		$COCH_3$	$(CH_2)_{17}CH_3$	58	22	298
27		$COCH_2CH_3$	$CH_2CH_2CH(CH_3)_2$	145	26	298
28		$COCH_2CH_2CH_3$	CH_3	87	49	298
29		$COCH_2C_6H_5$	CH_3	93	21	298
30		$COCH_2C_6H_5$	CH_2CH_3	100	21	298
31		$COCH_2C_6H_5$	$CH_2C_6H_5$	64	27	298
32	Hexahydrocolupulone	$COCH(CH_3)_2$	$CH_2CH_2CH(CH_3)_2$	141	...	297
33	Hexahydrolupulone	$COCH_2CH(CH_3)_2$	$CH_2CH_2CH(CH_3)_2$	110	23.4	297
34	Hexahydroadlupulone	$COCH(CH_3)CH_2CH_3$	$CH_2CH_2CH(CH_3)_2$	103	11.5	297
35		$COCH_3$	$CH_2CH(CH_3)CH_2CH_3$	113	17	297

TABLE II
METHYLATION OF PHLORACETOPHENONE (a) USING DIFFERENT MOLECULAR PROPERTIES OF SODIUM METHOXIDE (b) AND METHYL IODIDE (c) (298)

Molecular proportion a:b:c	Yield, % (R = Me) LX and LXI	LXII	LXIII	LXIV	Other products	Total
1:1:3	64.0	Trace	4.0	...	9.0	77.0
1:2:6	44.0	5.3	25.2	5.4	9.2	89.1
1:3:6	24.6	4.3	40.8	1.1	2.4	73.2
1:4:8	6.0	Trace	30.8	35.8	2.5	75.1

by conversion to (±)-humulone (292). The alkylation was carried out in water using a molecular ratio of phlorisovalerophenone:potassium hydroxide:3-methylbut-2-enyl bromide of 1:1.5:1.5, but the yield of 4-desoxyhumulone was still only 9.75% (292). By similar methods 4-desoxycohumulone, mp 89° (tribenzoate, mp 153°), and 4-desoxyadhumulone as an oil (tribenzoate, mp 127°) were obtained, the former in 2.7% yield (210).

Mono-3-(3-methylbut-2-enyl)phloracylphenones, other than the chalcone xanthohumol, have not been characterized as hop constituents but have been obtained by synthesis. Thus, 3-(3-methylbut-2-enyl)-phlorisovalerophenone (LXXI), mp 140°, was obtained in 9% yield as a by-product of the synthesis of humulone (288). Its structure was confirmed by hydrogenation to the dihydro derivative (LXXII), mp 170°,

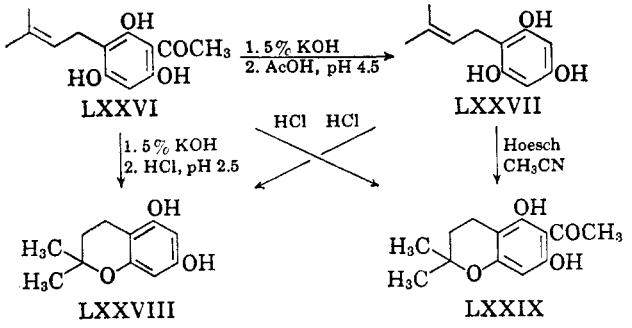


$$\text{LXXII} \overset{OH^-}{\rightleftharpoons} \text{LXXIII} + (CH_3)_2CHCH_2COOH$$

LXXV

which on hydrolysis gave 2-isopentylphloroglucinol (LXXIII) and isovaleric acid (LXXV). 2-Isopentylphloroglucinol was known earlier and is readily obtained by Clemmensen reduction of phlorisovalerophenone (LXXIV) (128, 228, 352). 3-Isopentylphlorisovalerophenone (LXXII) has also been prepared in 22% yield by Friedel–Crafts acylation of LXXIII (128).

3-(3-Methylbut-2-enyl)phloracetophenone (LXXVI), mp 172°, was prepared in 4.7% yield (298); hydrolysis of this product with 5% potassium hydroxide followed by acidification to pH 4.5 with acetic acid afforded 2-(3-methylbut-2-enyl)phloroglucinol (LXXVII), mp 97°, but acidification to pH 2.5 with hydrochloric acid gave the benzpyran (LXXVIII), mp 157° (265). Acid treatment of 3-(3-methylbut-2-enyl)-

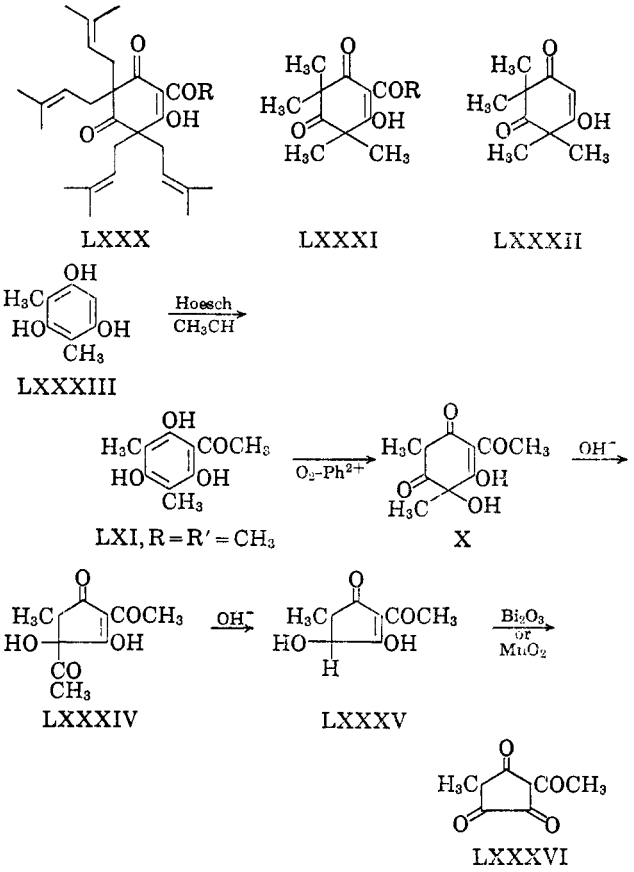


phloracetophenone (LXXVI) gave the acetylbenzpyran (LXXIX) also obtained from 2-(3-methylbut-2-enyl)-phloroglucinol by Hoesch synthesis (265).

Riedl also claimed (288) to have prepared 2-isovaleryl-4,4,6,6-tetra(3-methylbut-2-enyl)cyclohexane-1,3,5-trione (LXXX, R = *i*-Bu) but the melting point (93°) and analysis suggest that this product was contaminated with lupulone. Repetition gave the tetrasubstituted compound, mp 44°, for which the trivial name lupone was proposed (242). Colupone (LXXX, R = *i*-Pr), mp 42°, and adlupone, mp 45° (LXXXL, R = *sec*-Bu), were also prepared, but their presence in hops could not be confirmed (242). 2-Isovaleryl-4,4,6,6-tetramethylcyclohexane-1,3,5-trione (LXXXI, R = *i*-Bu), leptospermone, is a constituent of the essential oil of *Leptospermum* spp. (53), which has been synthesized by alkylation of phlorisovalerophenone (52) and from acyclic precursors (270). Recently, the corresponding 2-isobutyryl analog, flavesone (LXXXI, R' = *i*-Pr), has been found to accompany leptospermone in the essential oil of *Leptospermum flavescens* Sm. and *Eucalyptus decorticans* Maiden (43), and it is probable that the 2-methylbutyryl analog (LXXXI, R = *sec*-Bu) will be found.

2,2,5,5 - Tetramethylcyclohexane - 1,3,5 - trione (LXXXII) has also been isolated from *Syncarpia larrifolia* (174) and synthesized (270). Several other tetrasubstituted phloracylphenones have been prepared for hydrogenolysis studies and will be mentioned later (269).

Model compounds of the hop resins in which the acyl group is acetyl and the alkenyl side chain is replaced by methyl have been prepared. Thus, Riedl and Risse (299) prepared models of deoxyhumulone (LXI, R = R' = CH_3) and lupulone (LXIII, R = R' = CH_3).



Compound LXI, (R = R' = CH_3) had been prepared earlier by acylation of dimethylphloroglucinol (LXXXIII), and Campbell and Coppinger (80) oxidized it to the model humulone (X). By isomerization of this product Brown and Howard obtained crystalline models of isohumulone (LXXIV), humulinic acid (LXXXV), mp 112°, and dehydrohumulinic acid (LXXXVI) (58).

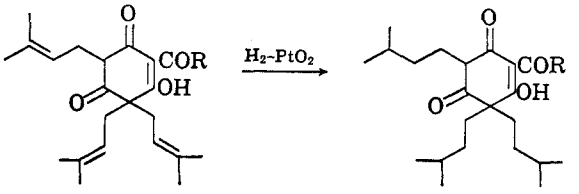
As with dehydrohumulinic acid reduction of LXXXVI with sodium borohydride gave what appeared to be a second isomer of the model humulinic acid (LXXXV), mp 81°, but proton magnetic spectroscopy established that both these model humulinic acids were mixtures of *cis–trans* isomers (27).

V. Reactions of Hop Resins

A. Hydrogenation

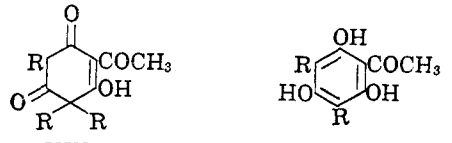
As mentioned earlier, when humulone and lupulone are hydrogenated in the presence of palladium chloride, they lose a molecule of isopentane leaving respectively the aromatic compounds humuloquinol (IV, R = *i*-Bu) and tetrahydrodesoxyhumulone (XXXIII). In con-

trast, hydrogenation of 3-(3-methylbut-2-enyl)phlorisovalerophenone (LXXI) and desoxyhumulone (XXXV, R = *i*-Bu) give respectively the di- (LXXII) and tetrahydro (XXXIII) derivatives. By use of Adams platinum oxide or palladium–charcoal catalyst, Carson (82, 85) was able to prepare the hexahydro derivative of colupulone (LXXXVII, R = *i*-Pr), which he thought



XXX LXXXVII

was hexahydrolupulone (*cf.* 186, 187, 203, 245, 297) but not the tetrahydro derivative of humulone. No hydrogenolysis of colupulone occurred using a palladium–charcoal catalyst even when hydrochloric acid was added to the reaction mixture (82). Also, using palladium chloride as catalyst, neither hexahydro(co)lupulone nor the analog (LXXXVIII, R = Me) underwent hydrogenolysis.



LXXXVIII LXXXIX

In order to investigate the influence of the alkyl side chain, Riedl and Nickl (296) synthesized the analogs LXXXVIII (R = $CH_2CH{=}CH_2$, $CH_2CH{=}CH{-}CH_3$, $CH_2CH{=}CMe_2$, and $C_6H_5CH_2$). Hydrogenation using palladium chloride under standardized conditions gave either the hexahydro derivative (LXXXVIII, R = Pr, Bu, or isopentyl) and/or the hydrogenolysis product (LXXXIX, R = Pr, Bu, isopentyl, or $C_6H_5CH_2$). The yields of hydrogenolysis products (291, 296)

	Allyl	Crotyl	Dimethylallyl	Benzyl
% hydrogenolysis product	7.2	36.9	20.2	92.0

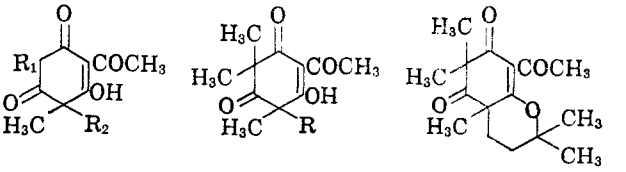
demonstrate the influence of the side chain on the course of the reaction.

The hydrogenolysis is acid catalyzed so that the addition of acid to the methanolic palladium chloride in the hydrogenation of the analog (LXXXVIII, R = $CH_2CH{=}CH_2$) increased the yield of the hydrogenolysis product (291)

% H_2SO_4 (in CH_3OH)	0	0.5	10	20
% hydrogenolysis	7.2	8.4	25.5	29.4

but again no hydrogenolysis occurred using a catalyst made from palladium oxide.

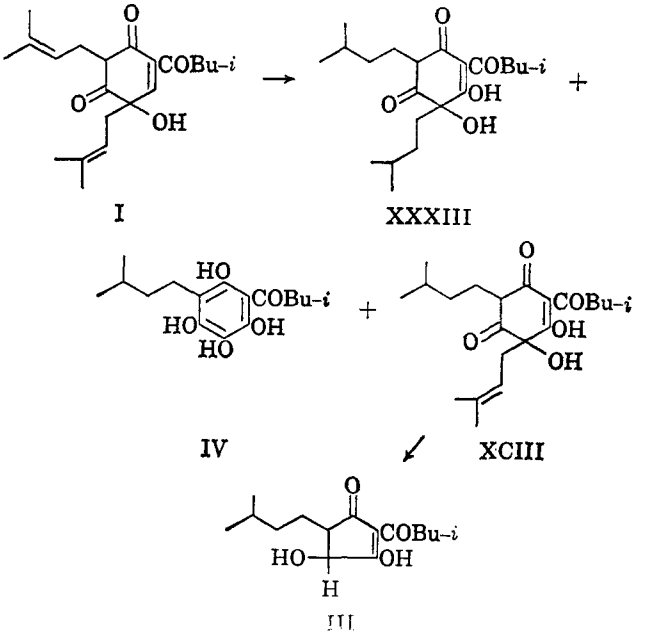
In a further experiment, the analog XC (R_1 = R_2 = $C_6H_5CH_2$), mp 141°, underwent hydrogenolysis



XC XCI XCII

to give XC (R_1 = $C_6H_5CH_2$; R_2 = H), mp 115°, in 72% yield showing that it is not essential for hydrogenolysis that both the geminal groups should be reactive (291). Murin and Riedl (269) also synthesized the tetrasubstituted analog XCI (R = $C_6H_5CH_2$, $CH_2{=}CHCH_2$, $CH_3CH{=}CHCH_2$, and $(CH_3)_2C{=}CH{-}CH_2$), but under standard conditions using palladium chloride only the benzyl analog underwent hydrogenolysis, in 82% yield; the other analogs afforded the dihydro derivatives. An attempt to induce the hydrogenolysis of XCI (R = $(CH_3)_2C{=}CHCH_2$) by addition of acid only led to the pyran XCII (291). The lupone analog (LXXX, R = *sec*-Bu) gave only an octahydro derivative and did not undergo hydrogenolysis (242).

To return to the hydrogenation of humulone, Verzele and Anteunis (391), using platinum oxide in acetic acid, obtained a complex mixture consisting of tetrahydrohumulone (XXXIII), humuloquinol (IV), an un-



I XXXIII

IV XCIII

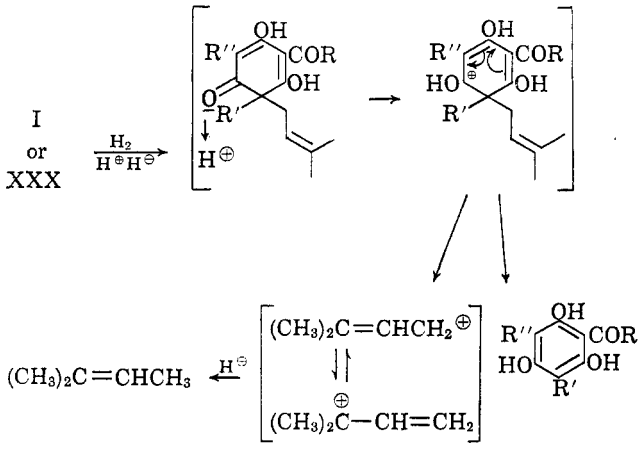
III

characterized hexahydrohumulone, and a dihydrohumulone shown to be XCIII, as hydrolysis gave exclusively dihydrohumulinic acid. In glacial acetic acid, the optimum yield of tetrahydrohumulone (54%) was obtained when 2.40 moles of hydrogen had been absorbed, but a higher yield was obtained (84%) in a methanolic solution adjusted to pH 5.1 (24). (−)-Tetrahydrohumulone is a pale yellow oil, $[\alpha]_D^{26}$ −116° (MeOH), which forms a complex with 1,2-diaminobenzene, mp 96°. In a similar manner, hydrogenation of

racemic humulone using a platinum catalyst at pH 5.1 affords (±)-tetrahydrohumulone, identical with the product obtained from lupulone by hydrogenolysis and oxygenation (24).

Riedl and Nickl (296) proposed the (krypto) ionic mechanism for the hydrogenolysis (of lupulone), as shown in Scheme I, requiring that the hydrogenolysis

SCHEME I



should be pH dependent and also effected by the ease of H^- ion formation on the catalyst. Experimental proof that this was indeed the case was provided by Anteunis and Verzele (23), who carried out a series of hydrogenations of humulone using platinum, palladium, and rhodium catalysts at different pH's and obtained a linear relationship between the pH and log [% hydrogenolysis]. As judged by the per cent hydrogenolysis at a given pH or by the slope of the graph, palladium catalysts are more active than platinum catalysts which, in turn, are slightly more active than rhodium catalysts. This is in agreement with the theoretical requirements for the intervention of the hydride ion (23). With the hydrogenolysis of humulone, Anteunis and Verzele found, in contrast to the results of Carson (82) and Riedl (291) with lupulone, that with palladium catalysts, irrespective of whether prepared from palladium chloride solutions, palladium or charcoal, or palladium on strontium carbonate, the same results were obtained at a given pH (23). Unfortunately they did not repeat the hydrogenolysis of lupulone under their conditions, and the discrepancy in these results could be due to the quality of the catalyst employed by the different investigators.

B. ISOMERIZATION OF HUMULONE–ISOHUMULONE

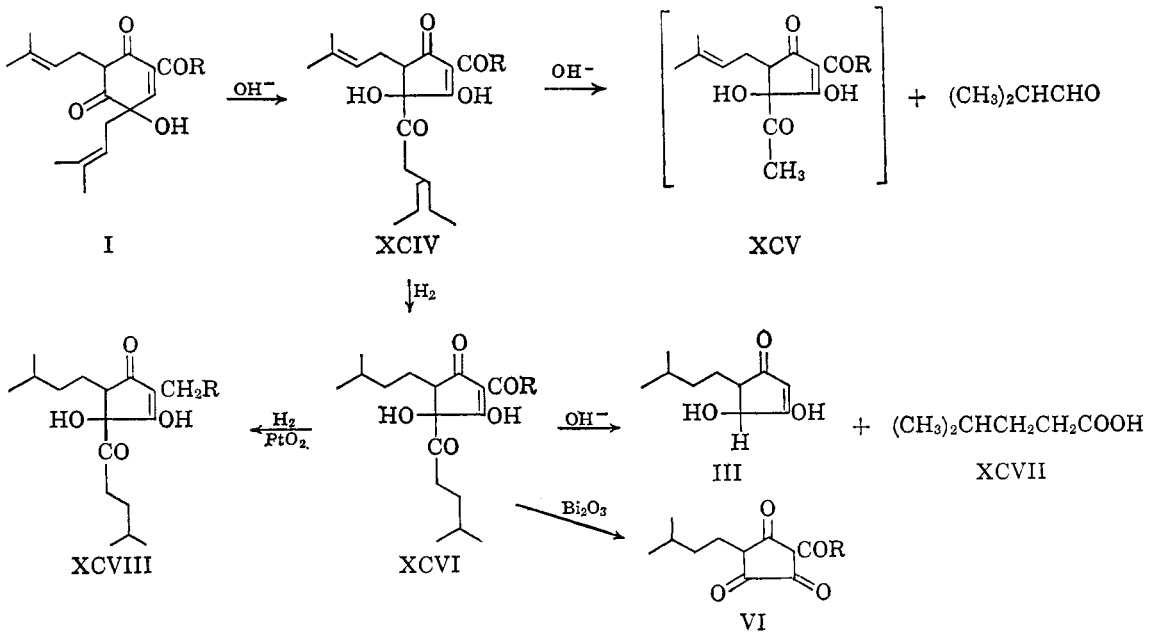
In the brewing process, hops are boiled with wort at pH 5.0 to extract the bittering principles. Under these conditions humulone and its analogs are only slightly soluble (353) and the humulone which goes into solution is largely isomerized into isohumulone, which is much more soluble (331). Thus, although wort leaving the copper kettle may contain some humulone, little or none of the unisomerized product finds its way into finished beer (301). Isohumulone is, therefore, the most important hop bittering principle in beer and the nature of this product has attracted the attention of brewing chemists for the last 40 years.

1. *In Aqueous Solution*

Wieland (411) in 1925 suggested that the hydrolysis of humulone to humulinic acid, isohexenoic acid, and isobutyraldehyde proceeded *via* an intermediate which he did not isolate but formulated as XCIV (R = *i*-Bu).

Later, Windisch, Kolbach, and Schleicher (413) investigated the products obtained by boiling humulone in various buffer and dilute alkaline solutions. In particular, by boiling humulone for 3 min with 0.067 *N* sodium hydroxide solution, they obtained a resinous oil which was more bitter than humulone and for which, on the basis of titratable acidity and degree of unsaturation, they adopted Wieland's structure (XCIV, R = *i*-Bu) and the name "resin A." They also suggested further that hydrolysis either gave humulinic acid and isohexenoic acid directly or gave isobutyraldehyde and a "resin B," formulated as XCV (R = *i*-Bu), which was hydrolyzed to humulinic and acetic acids. Subsequent work has failed to confirm the presence of resin B although isobutyraldehyde is regularly encountered. When Verzele and Govaert (394) obtained two crystalline products by hydrogenation of isomerized humulone, they thought that these products were the corresponding saturated derivatives of resins A and B, but both were later shown to be substantially the same tetrahydroisohumulone (XCVI, R = *i*-Bu) (59). Verzele and Govaert (394) also suggested that resin A should be called isohumulone and this has been generally accepted.

Investigation of the bittering principles of beer by countercurrent distribution showed them to consist of three major analogs corresponding to the hop α acids (303). The distribution patterns shown by the bittering principle and by the isohumulone obtained by alkaline isomerization were identical, but about 15% broader than the calculated distribution curve (195). No other evidence could then be obtained to suggest that each isohumulone was heterogeneous, but it was later shown that two theoretical distribution curves could be fitted into the pattern obtained (409). Meanwhile, Spetsig (354) had shown that the product of boiling humulone with a phosphate buffer solution at pH 5.0 could be resolved into two peaks by reversed-phase column chromatography (356), while the bittering substances from beer gave six peaks, two from each α acid. It was, however, another 6 years before he was able to separate the two isohumulones on a preparative scale (359). Similar analytical separations have been achieved by paper chromatography (277, 409).



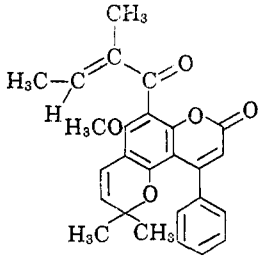
At pH 5.0 only small amounts of humulone can be isomerized in aqueous solution owing to its low solubility, but the solubility increases at higher pH's. Thus, the method of Windisch (413), boiling for 3 min with 0.067 *N* sodium hydroxide solution, was preferred for the laboratory preparation of isohumulone, but if the critical time was exceeded the isohumulone was hydrolyzed to humulinic acid. Howard (181) later showed that if humulone was boiled with 0.1 *N* sodium carbonate solution for 15 min it was converted into isohumulone and that longer periods of boiling and stronger solutions (*e.g.*, 0.2 *N*) of sodium carbonate did not hydrolyze the product to humulinic acid, and this is probably the most convenient route to the mixed isomers of isohumulone. Spetsig has shown that the same reversed-phase chromatogram is given by these two preparations and by the product obtained by boiling humulone in a phosphate buffer at pH 5.0 (359). He also showed that, within the range pH 5–9, the proportion of the two isohumulone isomers remained constant and the rate of reaction, which followed first-order kinetics, was independent of pH but dependent on the nature of the buffer solution (358). Before describing the separation of the two isomers of isohumulone, the more important work carried out with the mixed isomers will be discussed.

Although it has not been possible to prepare any characteristic organic derivative from the mixed isomers of isohumulone, a series of metal salts which had characteristic melting ranges have been prepared (215). Of particular interest were the nickel compound, the formation of which formed the basis for a conductometric method of analysis of isohumulone (214), and the lead complex. From the mixed isomers of isohumulone the lead salt had mp 80°. The derivative isolated from beer brewed with pure humulone, however, had mp 85–90°, whereas lead derivatives from beer extracts had a melting point in the range of 125–150°, while in a few cases beers brewed from old hops gave rise to lead salts with mp >200°. Admixture of the lead salt from isohumulone (mixed isomers), mp 80°, with these latter products raised the melting point (409). The metal complexes of isohumulone are not without practical importance as they have been implicated in the phenomenon of gushing and stability of beer foam (215). It was also observed that passage of ammonia through a methanolic solution of isohumulone (mixed isomers) gave a noncrystalline yellow substance which liquified fairly sharply at 46° (215).

The hydrogenation of the mixed isomers of isohumulone was mentioned briefly above when Govaert and Verzele claimed to have obtained the saturated derivatives of resins A and B. Repetition of this work gave what appeared on the basis of solubility to benzene to be two products, corresponding to those obtained by Govaert and Verzele, but they were shown by countercurrent distribution to be basically the same product and further purification by silica gel chromatography and vacuum distillation gave a crystalline tetrahydroisohumulone (XCVI, R = *i*-Bu), mp 32–34°, $[\alpha]_D$ + 24°, claimed to be homogeneous by countercurrent distribution (59). In the light of later work it seems likely that this product is also a mixture of two stereoisomers. This product, which was most conveniently obtained by the use of Adams catalyst in glacial acetic acid, was hydrolyzed to dihydrohumulinic

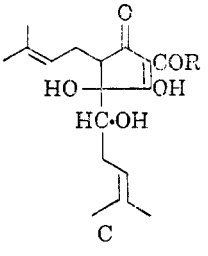
acid (A) (III, R = *i*-Bu) and 4-methylvaleric acid (XCVII). Racemic tetrahydroisohumulone, mp 53°, was obtained by isomerization of racemic tetrahydrohumulone, in turn prepared by hydrogenolysis of lupulone. (+)- and (±)-tetrahydroisocohumulone (XCVI, R = *i*-Pr) were similarly prepared (59). Oxidation of tetrahydroisohumulone with bismuth oxide afforded isohumulinic acid (VI, R = *i*-Bu) and under similar conditions isohumulone gave dehydrohumulinic acid (XIII, R = *i*-Bu), albeit in very small yield.

Hydrogenation of isohumulone isomers using Adams catalyst in methanol gave an oily product, $C_{21}H_{36}O_4$ (XCVIII, R = *i*-Bu), in which the acyl side chain is reduced to alkyl; this product was called neohydroisohumulone (59). This type of reduction has now been observed with all the 2-acylcyclopentane-1,3-dione systems encountered from hop resins (59, 66, 69), but not with the corresponding cyclohexane derivatives. Polonsky and Rondest (279), however, observed such a reduction with callophyllolide (XCIX).



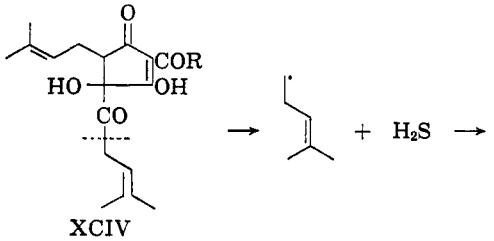
XCIX

It is claimed (177) that reduction of isohumulone with sodium borohydride gives C and that this product is less susceptible to photolysis than isohumulone.



C

Thus, when beer is exposed to sunlight it develops an unpleasant "sunstruck" flavor due to the formation of isopentenylthiol (CI). This substance is thought to be



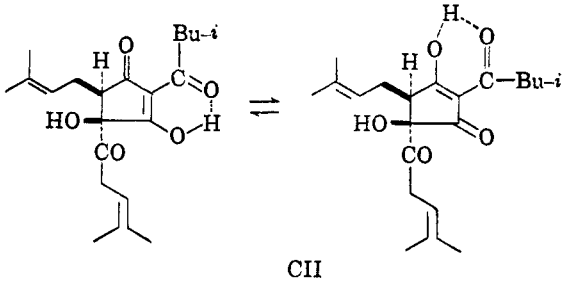
XCIV

$(CH_3)_2C{=}CHCH_2SH$

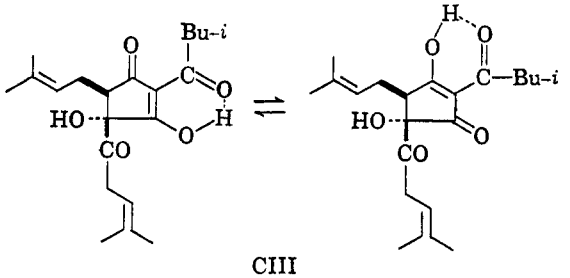
CI

formed by condensation of an isopentenyl radical, produced by photolysis of the isohexenoyl side chain of isohumulone (XCIV), with a suitable thiol donor present in beer (241). In order to prevent this unpleasant flavor some American beers are brewed with an extract of hops, isomerized with dilute alkali, and reduced with sodium borohydride (16, 177).

Before isohumulone was resolved into its two isomers it was possible to predict from its proton magnetic resonance spectrum and from a study of the spectra of humulinic acids A and B that it was a mixture of two stereoisomers. The major isomer (about 60% of the mixture) had humulinic acid B stereochemistry (CII) and the other that of humulinic acid A (CIII) (73). These estimates of the proportions of the isomers were in good agreement with those obtained from countercurrent distribution (409) and reversed-phase chromatography (356, 358, 359).



CII



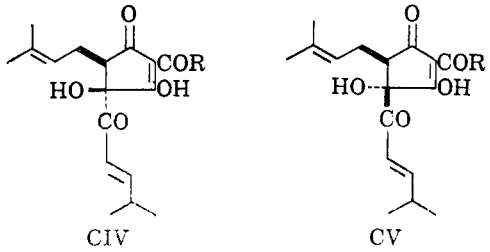
CIII

The resolution of the two isomers of isohumulone was first accomplished by Spetsig (359). The minor isomer, eluted from his column at pH 5.8, solidified to a mass of white needles which had, by mass spectrometry, a molecular weight of 362. He reported them to be optically inactive (see later, however) but did not record the melting point. The major isomer, eluted at pH 6.0, failed to solidify, had $[\alpha]_D$ +44° (methanol), and was somewhat more bitter than the crystalline product (359).

The same products were also obtained by the Belgian team (14) by extensive countercurrent distribution studies. After 400 transfers in the system, phosphate buffer at pH 5.0–isooctane, two broad peaks with $K \simeq 0.41$ and 0.87 were present. The first peak was removed from the machine and all the tubes, except those with the band $K \simeq 0.87$, were emptied and refilled with fresh solvent and buffer solution. The distribution was then continued until 2000 transfers had been

completed by which time two bands with $K = 0.61$ and $K = 0.784$ were well separated ($\beta = 1.28$). The first component ($K = 0.61$) (26%) crystallized and after recrystallization from isooctane had mp 62°, $[\alpha]_D$ −7.8° in neutral methanol and +40.4° in alkaline methanol. The second component ($K = 0.784$) (74%) did not crystallize and had $[\alpha]_D$ +47.6° in neutral methanol (in agreement with Spetsig (359)) and +73.0° in alkaline methanol. From the proton resonance spectrum they were able to allocate the structure CIII with humulinic acid A stereochemistry to the solid isomer (isohumulone A) and the structure CII with humulinic acid B stereochemistry to the liquid isomer (isohumulone B) (14).

The broad peak ($K \doteq 0.41$) obtained after 400 transfers was not completely resolved into two compounds during 1000 transfers, but the proton magnetic spectrum of the mixture showed them to have a conjugated olefinic system in agreement with structures CIV and CV. They were called alloisohumulones. It was es-



CIV CV

timated that when the isomerization of humulone was carried out at pH 9.0 the mixed isomers contained 8% of alloisohumulones, while in wort they only accounted for 4.5% of the mixture. They appear to be more bitter than the isohumulones (14).

Clarke and Hildebrand (96) resolved the product of isomerizing humulone with carbonate into two fractions by chromatography upon silica gel, using elution with isooctane–ethyl acetate (15:1). The first fraction was a yellow oil (isohumulone A1), $[\alpha]_D^{20}$ +39.2° (6% in methanol), which would appear to correspond to the liquid isohumulone obtained by Spetsig and the Belgian workers. The second fraction (isohumulone A2) crystallized and had mp 61–62°, $[\alpha]_D^{20}$ −7.2° (6% in methanol), in good agreement with the Belgian data. Clarke and Hildebrand drew attention to the similarity of this crystalline product and a crystalline product with mp 55°, $[\alpha]_D$ −7.4°, which Hashimoto and Kuroiwa (159, 160) isolated from beer.

To recapitulate, the isohumulone complex in beer will consist of (using Alderweireldt, *et al.*'s, nomenclature) isohumulone A and B, isocohumulone A and B, isoadhumulone A and B, alloisohumulone A and B, alloisocohumulone A and B, and alloisoadhumulone A and B. Other bittering substances in beer, hulupones, are derived from the β acids (see later). As hops age during storage the α acids undergo oxidation but the oxidation products are still capable of bittering beer. These isomerized oxidation products have similar chromophores to isohumulone, and it is probable that they have undergone oxidation in the dimethylalkenyl side chains.

2. *Photoisomerization*

Preliminary observations showed that the ultraviolet spectrum of a methanolic solution of humulone exposed to diffuse daylight changed over 28 days to one resembling isohumulone (95). In a more detailed study (96) a 0.2% methanolic solution of humulone was irradiated with a 500-w lamp for 36 hr. The product from the reaction partially crystallized; recrystallization from isooctane afforded photoisohumulone, mp 63°, $[\alpha]_D^{20}$ −7.6° (6% in methanol), which was shown to be identical with isohumulone A2 (isohumulone A: Alderweireldt, *et al.*) (CIII) (96).

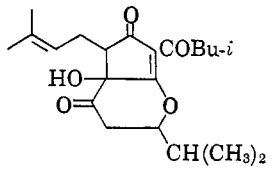
3. *In Alcoholic Solution*

Although not comparable with the brewing process, the isomerization of humulone in alcoholic solution has been investigated (84, 237). Perhaps the most significant contribution was made by Carson (84) who, after heating humulone for 3–6 hr with methanolic potassium hydroxide or sodium methoxide, obtained an isomeric oil from which 15–18% of crystalline material could be separated. By fractional crystallization this material gave three fractions: (i) mp 133–134°, $[\alpha]_D$ +113° (ethyl acetate); (ii) mp 134–135°, $[\alpha]_D$ −60.6° (ethyl acetate); and (iii) mp 145–146°, $[\alpha]_D$ 0°. It was shown further that iii was not a racemate of i and ii. None of the crystalline isomers were bitter and all gave humulinic acid, acetone, and isobutyraldehyde on hydrolysis, but only acetone on ozonolysis. Hydrogenation of fractions i and ii gave, as oils, optically active dihydro derivatives which on hydrolysis afforded dihydrohumulinic acid (A), acetone, and isobutyraldehyde. No crystalline tetrahydro derivatives could be obtained. By column chromatography of the oily isomerization product two oily isomers, $[\alpha]_D$ +17.7 and 50.3° (both in ethyl acetate), and a yellow crystalline product, mp 133°, were obtained (84). The latter was later characterized as dehydrohumulinic acid (XIII, R = *i*-Bu) (191, 195). Hydrolysis of the oil gave a 50–60% yield of humulinic acid (A), acetone, and isobutyraldehyde, while ozonolysis also gave acetone and small amounts of isobutyraldehyde.

Carson's isomerization was repeated and the products examined further by Howard, Slater, and Tatchell (195), who by fractional crystallization obtained products which had optical activities between +34 and −42°. The most levorotatory product, mp 130°, was still not homogeneous and was resolved into three components by countercurrent distribution. A sample of the dextrorotatory product, $[\alpha]_D$ +110° (supplied by

Dr. Carson), was shown to be essentially one component but contaminated by 15% of the coanalog (195). As Carson's work was carried out before the mixture of analogs present in the hop α acid had been resolved, it seems probable that many of his fractions were similarly contaminated.

On the basis that hydrogenation of the crystalline products gave only a dihydro derivative, Anteunis (17) suggested that they had the structure CVI.



CVI

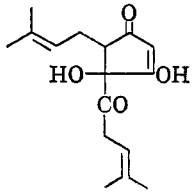
4. *Nomenclature of Isohumulone*

Howard, Slater, and Tatchell (195) proposed a scheme of nomenclature in which "the bitter-tasting bacteriostatic principle which is derived from humulone and is present in beer was called isohumulone A." They further named Carson's products (84) as follows: isohumulone B (mp 133–134°, $[\alpha]_D$ +113°), isohumulone C (mp 134–135°, $[\alpha]_D$ −60.6°), isohumulone D (mp 145–146°, $[\alpha]_D$ 0°), isohumulone E (oil, $[\alpha]_D$ +17.7°), and isohumulone F (oil, $[\alpha]_D$ +50.3°).

With regard to isohumulone A, since the product of aqueous alkaline isomerization of humulone gave the same broadened distribution pattern as isohumulone from beer, the two were assumed identical and the term isohumulone A had been also applied, perhaps more often, since it is more readily available, to the product of alkaline isomerization. In fact, Whitear and Hudson (409) use the term isohumulone A only for the product of alkaline isomerization in order to distinguish it from beer isohumulone.

When a second form of humulinic acid was found, it was called humulinic acid B and the previously known isomer, humulinic acid A (22). These were later shown to have respectively *cis* and *trans* stereochemistry (10, 66, 245) with regard to the alkenyl side chain and the hydroxyl group. With the resolution of isohumulone into two components, with similar differences in stereochemistry, the Belgian workers (14) suggest that the suffixes A and B should be reserved for these stereochemical differences and earlier meanings of the suffixes should be discontinued. This can hardly be objected to as Howard, Slater, and Tatchell themselves (195) showed that it is doubtful if any of Carson's products were homogeneous. Nevertheless, the term isohumulone A is now used for three different preparations although only that of Alderweireldt, *et al.*, is claimed to be a pure compound. On the other hand, Clarke and Hildebrand (96) called the two fractions which they separated from isohumulone, which are almost certainly identical with those separated by Spetsig and the Belgian workers, isohumulone A1 and A2, so that isohumulone A and B of Alderweireldt, *et al.* (14), are the same as isohumulone A2 and A1, respectively, of Clarke and Hildebrand (96). Furthermore, the photoisohumulone of Clarke and Hildebrand is identical with their isohumulone A2 (isohumulone A of Alderweireldt, *et al.*). An authoritative statement by the combined ASBC–EBC Committee on nomenclature would be timely.

After the above was written a similar plea was issued by the Belgian school (392) who, at the same time, gave their own suggestions that the A, B, C, D suffixes used with the humulinic acids (see sections IIIA and VD2) should be retained and applied as appropriate to the isohumulones. The term *allo*-isohumulone should be reserved for compounds with an isohex-2-enoyl side chain. Such compounds can also exist in both *cis*- and *trans* conformations. They suggested further that resin B (XCV) should be called apoisohumulone and that the deacylated product (CVII) should be known as norisohumulone.



CVII

C. OXIDATION

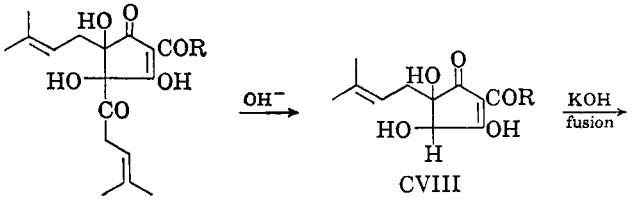
1. *Humulone*

a. Humulinone

By storing ethereal solutions of either the α acid or soft-resin fraction of hops with a saturated solution of sodium hydrogen carbonate, Cook and Harris (102) obtained, as the insoluble sodium salt, mp 277° dec, a new compound which they called humulinone. The parent compound, $C_{21}H_{30}O_6$, mp 74°, was optically inactive, a stronger acid (pK_a = 2.7) than humulone (pK_a = 5.5) or isohumulone (pK_a = 3.4), and had light absorption properties very similar to the latter compound. In addition, humulinone gave a characteristic cherry-red color with concentrated sulfuric acid. Whereas Cook and Harris (102) reported hops contained up to 1.7% of humulinone, Verzele and Govaert (396) were unable to confirm this even with old hops, and claimed that humulinone was an artifact. In support of their view they showed that treatment of an ethereal solution of humulone containing either perhydrol or natural peroxides with sodium hydrogen carbonate readily afforded the sodium salt of humulinone in up to 70% yield, and in the absence of air or peroxides no humulinone was formed. In a survey of methods of oxidation of humulone, Cook, Howard, and

Slater (103) found ethereal perhydrol gave only a 30% yield of humulinone, the best results (50–60% yield) being obtained with cumene hydroperoxide. By this means, cohumulinone, mp 111° (103), (±)-adhumulinone, mp 98° (104), and (+)-adhumulinone, mp 115°, $[\alpha]_D$ +11° (191), were obtained, the first having been encountered earlier by chromatography of mixed humulinones on silica gel (396).

Hydrolysis of humulinone afforded 4-methylpent-4-enoic acid (IX) (102), oxyhumulinic acid, mp 72°, and a deacylated product, mp 62° (103). Cohumulinone similarly gave IX, oxycohumulinic acid, mp 102°, and the same deacylated product, mp 62° (103). The mixture of analogs of humulinone, obtained by oxidation of the α acids, could not, on account of the low solubility of their sodium salts, be separated by countercurrent distribution, but the corresponding oxyhumulinic acids were satisfactorily resolved (11). In view of the similarity of the light absorption properties of oxyhumulinic acid with humulinic acid (III, R = *i*-Bu), the former compound was formulated as CVIII,

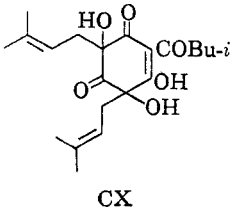


CXI CVIII

$(CH_3)_2C{=}CHCH_2COCH_3$
CIX

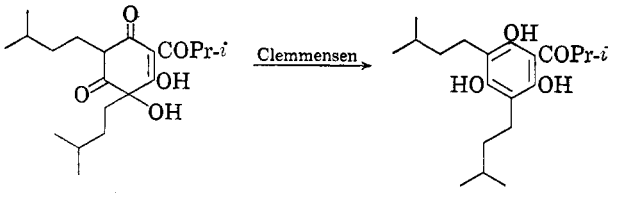
(R = *i*-Bu) which was accepted by both the English and Belgian investigators (11, 103). This structure was supported further by alkaline fusion of oxyhumulinic acid to give methylheptenone (CIX) and by dehydration to dehydrohumulinic acid (XIII, R = *i*-Bu), identical with a sample obtained by oxidation of humulinic acid with bismuth oxide (189). Dehydrohumulinic acid was also obtained by treatment of humulinone with methanolic sodium hydroxide (189).

On the basis of these experiments, Cook, Howard, and Slater (103, 189) proposed the six-membered ring structure CX for humulinone, whereas Alderweireldt



CX

and Verzele (11) preferred, on the basis of ultraviolet and infrared spectra and p*K* values, the five-membered ring system (CXI). Howard and Slater (191) thought they had confirmed the six-membered ring structure (CX) for humulinone when, having previous obtained 4,6-di(3′-methylbutyl)phlorisobutyrophenone (CXIII) by Clemmensen reduction of tetrahydrocohumulone (CXII), they obtained the same product, albeit

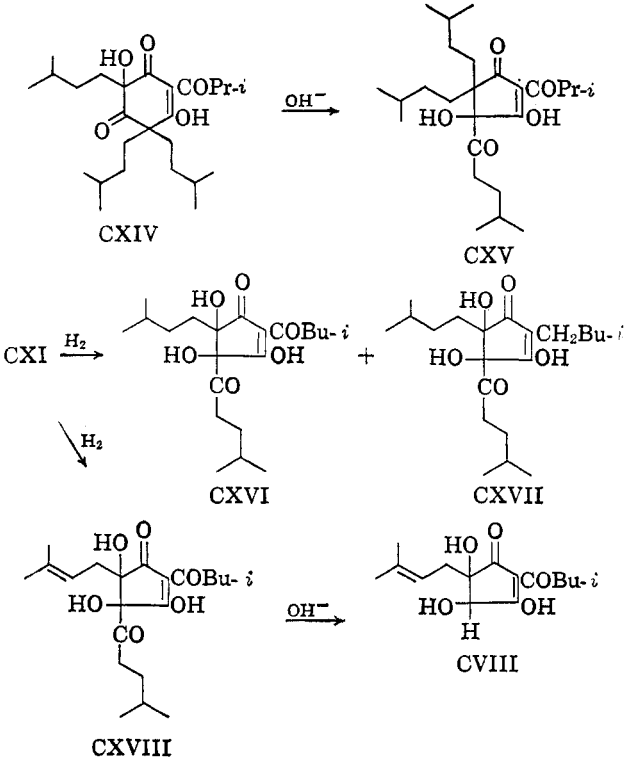


CXII CXIII

only in 1% yield by Clemmensen reduction of tetrahydrocohumulinone. It was later shown by proton magnetic resonance spectroscopy that humulinone had the five-membered ring structure CXI, Howard and Slater's results being explained by the fact that at high concentrations humulone can form an insoluble sodium salt and so contaminates the more insoluble sodium salt of humulinone (340).

Further evidence for the structure (CXI, R = *i*-Bu) for humulinone came from analogy with the lupulone series (369). As mentioned above, humulinone has ultraviolet light absorption very similar to isohumulone (XCIV) and different from the tetrasubstituted acylphloroglucinol (CXIV) obtained from hexahydrocolupulone. With sodium hydrogen carbonate, CXIV readily rearranges to the five-membered ring structures CXV, analogous to humulinone (369).

Hydrogenation of humulinone (CXI), using palladium chloride as catalyst, affords only the tetrahydro derivative and no hydrogenolysis is observed as with



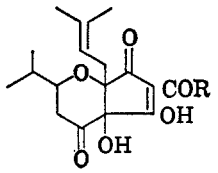
humulone and lupulone (261, 369). Using platinum oxide as catalyst, tetrahydrodeoxohumulinone (CXVII), mp 101°, is obtained (261) paralleling the behavior of other five-membered β-triketones on hydrogenation (59, 66, 69, 245).

Final chemical proof for the five-membered ring structure for humulinone (CXI) came from the fact that it was possible to prepare two dihydro derivatives (the six-membered ring structure could only give one). Only one of these (CXVIII) was obtained pure, mp 76°, and on hydrolysis it gave only oxyhumulinic acid (CVIII), whereas the dihydro derivative of the six-membered ring structure should lead to a mixture of oxyhumulinic acid and dihydrooxyhumulinic acid (261).

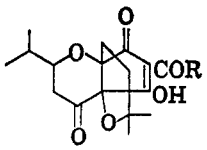
Despite more recent claims (250) that humulinone is an important bittering substance in old hops, other workers have failed to detect it in either hops or beer by countercurrent distribution (304) and thin film chromatography (184, 409), and it would appear only to be formed at unnatural alkaline pH's and thus to be an artifact.

b. Isohumulinone

By chromatography of mixed humulinones on silica gel, Verzele and Govaert obtained three products (396), two of which were identified as humulinone and cohumulinone (103). Oxidation of the third product, mp 184°, showed it to be a mixture of analogs and by further recrystallization, isohumulinone, mp 195°, was isolated (104). Both this compound and isocohumulinone, mp 171°, were formed when the respective analogs were treated with silica gel (104). The structure of the isohumulinones has recently been reexamined (30). In contrast to the yields of 4–5% obtained using silica gel, acidic reagents such as hydrogen chloride in methanol, formic acid in benzene, or perchloric acid in acetic acid gave the isohumulinones in 50–60% yields. The previously isolated isohumulinone, mp 195°, now called isohumulinone A, had one less active hydrogen atom than humulinone, and on hydrogenation gave a dihydro derivative. On alkaline hydrolysis isohumulinone A, in contrast to humulinone, gave no 4-methylpent-3-enoic acid and was partially converted into a further isomer, isohumulinone B, mp 152°. This isomer was also obtained from the mother liquors when humulinone was treated with benzene in formic acid. Isohumulinone B failed to take up hydrogen under normal conditions. In the light of these results isohumulinone A was formulated as the bicyclic derivative (CXIX) and isohumulinone B as the tricyclic derivative (CXX). Proton magnetic resonance measurements supported a cyclized structure for isohumulinone A but otherwise were of limited value owing to the low solubility of the substances in the normal solvents employed (30). The isocohumulinone isolated



CXIX



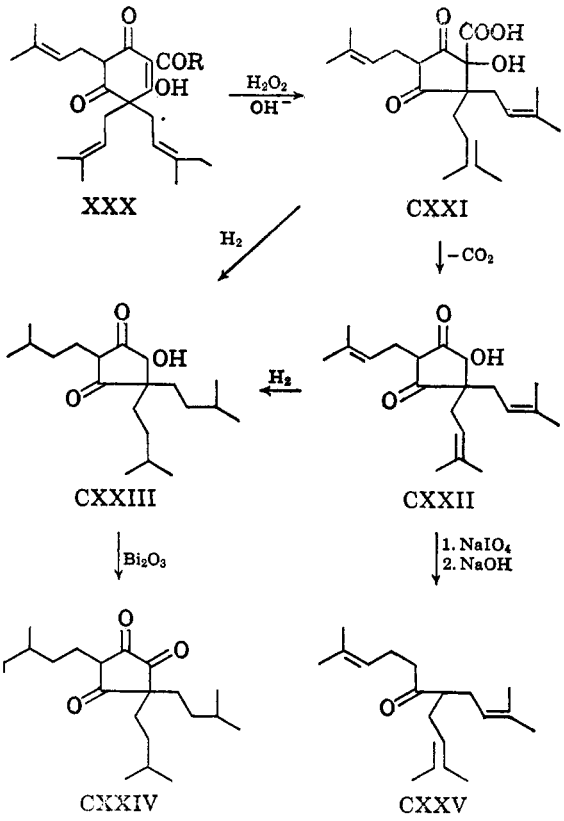
CXX

earlier, mp 171° (85), was shown to be isocohumulinone B; isocohumulinone A has mp 232° (30).

2. *Lupulone*

a. Lupuloxinic Acid and Lupulenol

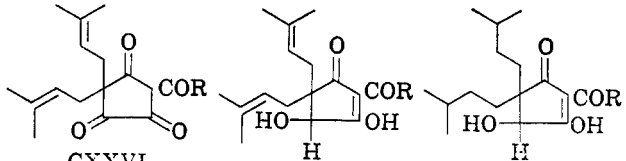
By oxidation of the crystalline β acid from English hops, Howard and Pollock (185) obtained isobutyric acid and a crystalline acid, lupuloxinic acid, which was readily decarboxylated to an alcohol, lupulenol. The formation of isobutyric acid was difficult to explain when they thought they were dealing with lupulone, but readily explainable when they realized the β acid was actually colupulone (187). Lupuloxinic acid, $C_{21}H_{30}O_5$, mp 108° dec, is a strong dibasic acid (pK_1 = 3.9, pK_2 = 9.7) which hydrogenated in the presence of palladium chloride to give a hexahydro derivative, mp 156–157° dec. Using platinum oxide in dioxane–glacial acetic acid, the hydrogenation was accompanied by decarboxylation to give hexahydrolupulenol (CXXIII), mp 178°, which could be characterized as a phenylurethan, mp 148°. Lupulenol, $C_{20}H_{30}O_{31}$, mp 118°, with periodic acid gave an enolic acid which by



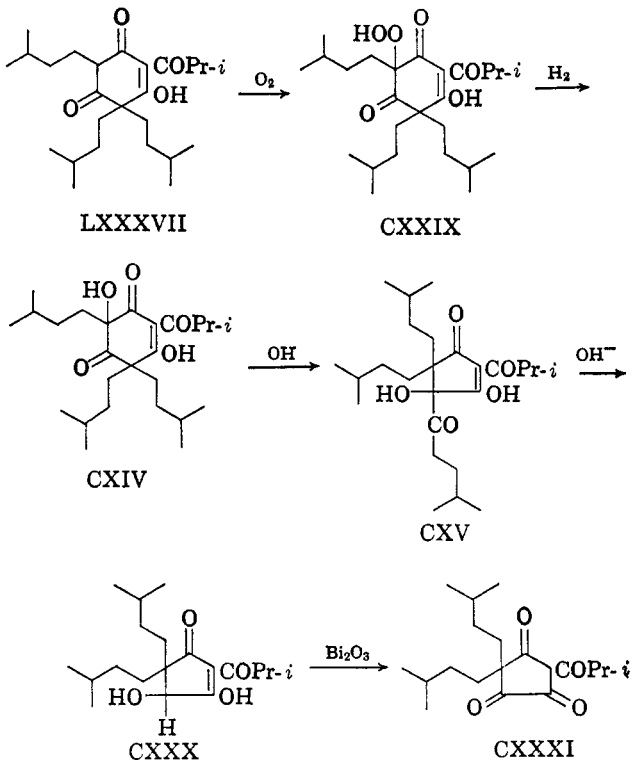
hydrolysis with sodium hydroxide gave as the major product an oil, $C_{18}H_{30}O$ (CXXV). Hydrogenation of this material gave a hexahydro derivative identical with the product obtained from hexahydrolupulenol with periodate. By oxidation of the hexahydro derivative, $C_{18}H_{36}O$, with nitric acid, 4-methylpentanoic acid was obtained. Lupulenol fails to reduce Fehling's solution, and on that ground an acyloin structure was at first excluded (185), but with bismuth oxide, a characteristic reagent for the acyloin group (313), it was smoothly oxidized to the triketone (CXXIV) in agreement with the structures (CXII) for lupulenol and (CXXI) for lupuloxinic acid (187). Neither lupuloxinic acid nor lupulenol have been found in hops or beers (239, 240).

b. Hulupones

Examination of the soft-resin fraction of hops by reversed-phase chromatography showed the presence of a group of analogs, other than humulones and lupulones, which were called hulupones (363). These were later isolated and examined by mass spectrometry and allocated structure XXVI (55). The hulupones are optically inactive bitter-tasting oils, relatively strong acids (pK = 2.7), which fail to form characteristic carbonyl derivatives (363). They were originally reported to be present in hops in concentrations of up to 3% (362), but later estimates (355, 368) were much lower (0.2–1.4%), and it has been claimed that in the method of extraction used, β acids were oxidized to hulupones (240, 368). Reduction of hulupone with sodium borohydride gave a dihydro derivative (CXXVII), while with platinum oxide a hexahydro derivative (CXXVIII) was obtained. One of the carbonyl groups was reduced with sodium borohydride as this product and the hexahydro derivative dehydrated much more readily than the parent compound in the vaporization chamber of the mass spectrometer (55, 362).



The relationship of lupulone to hulupone was established by the following series of degradations (367, 369). Autoxidation of hexahydrocolupulone (LXXXVII, R = *i*-Pr) afforded the peroxide (CXXIX) which was reduced to the corresponding alcohol (CXIV). Both of these products have ultraviolet light absorption in agreement with their formulation as tetrasubstituted acylphloroglucinols such as the essential oil component leptospermone (LXXXI, R = *i*-Bu). The alcohol (CXIV) readily rearranges with alkali to CXV, analogous to humulinone, and on hydrolysis gave the substituted humulinic acid (CXXX). Oxidation of this latter compound with bismuth oxide afforded tetrahydrocohulupone which had light absorption identical



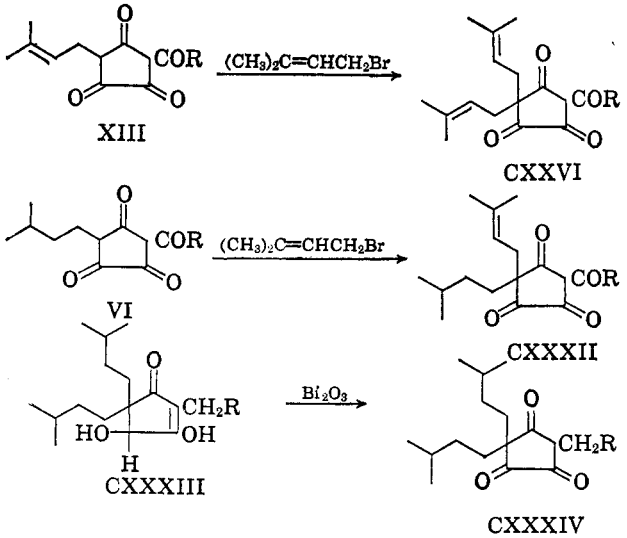
with the natural product. The product was characterized further by preparation of a sodium salt, mp 228°, and a quinoxaline, mp 150°, with 2,3-diaminonaphthalene, and by oxidation to di(3-methylbutyl)-malonic acid (369). Thermal decomposition of the peroxide (CXXIX) from hexahydrocolupulone gave tetrahydrocohulupone (CXXXI) directly, and this was thought to be a closer approach to the biosynthetic route (369).

The autoxidation of colupulone in hydrocarbon solvents is complex; more than 1 mole of oxygen is consumed and the double bonds in the dimethylallyl sides are attacked as indicated by a fall in the iodine number (420). It is not surprising, therefore, that attempts to prepare cohulupone itself by the route used to prepare tetrahydrocohulupone were unsuccessful. It was noted, however, that when an alcoholic solution of colupulone is shaken with oxygen in the presence of anhydrous sodium sulfite, the sodium salt of cohulupone is formed, from which the parent acid can be recovered in 30% yield (419, 420). Cohulupone (CXXVI, R = *i*-Pr), $C_{19}H_{26}O_4$, is a yellow oil, bp *ca.* 100° (10^{-4} mm), characterized as a sodium salt, mp 120°, and by forming quinoxalines with 1,2-diaminobenzene, mp 107°, and with 2,3-diaminonaphthalene. Hulupone (CXXVI, R = *i*-Bu), $C_{20}H_{28}O_4$, bp *ca.* 110° (10^{-4} mm), was similarly prepared from synthetic lupulone and gave a quinoxaline with 1,2-diaminobenzene, mp 110° (419).

Adhulupone (CXXVI, R = *sec*-Bu) was also prepared; quinoxaline, mp 95° (56), and 2,5,5-trimethylcyclohexane-1,3-dione and 3-methylpentane-2,4-dione were oxidized in this system (420), but the lupulone analog (XXX, R = Me) was stable to oxidation in the presence of sulfite (56). Other methods of oxidation were sought of which the use of sodium persulfate was the most successful. In ethanolic solution after 12 days at 20° or 15 min at 78° cohulupone was obtained in 30% yield while longer heating gave an uncharacterized product. Using sodium persulfate the hulupone analog (CXXVI, R = Me) was successfully prepared (56).

Hulupone was also synthesized in 54% yield by alkylation of dehydrohumulinic acid (XIII, R = *i*-Bu) which is the most convenient route to this analog (420). Cohulupone has also been prepared in this way (56) but is more readily available from colupulone. A series of hulupone analogs has been prepared by alkylation of dehydrohumulinic acid with methyl iodide (420) and allyl, propargyl, and benzyl bromides (56). Dihydrohulupone (CXXXII, R = *i*-Bu) could not be prepared in this way using isopentyl bromide, but was formed by alkylation of isohumulinic acid (VI, R = *i*-Bu) with 1-bromo-3-methylbut-2-ene (56).

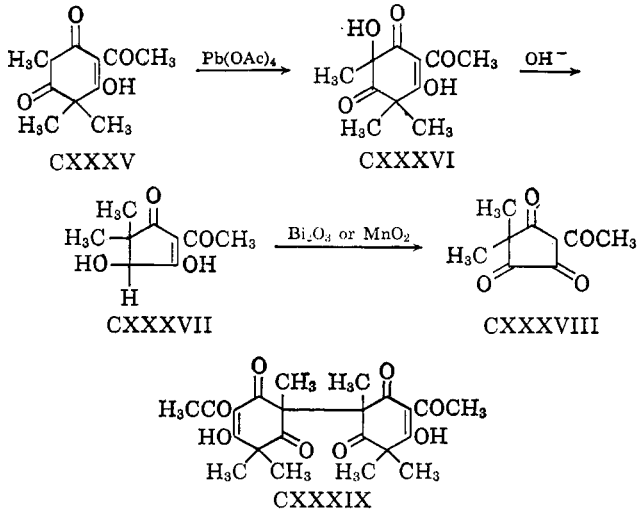
The hydrogenation of the hulupones was examined in more detail (69). In particular, cohulupone with palladium chloride as catalyst gave two products, one of which, hexahydrocohulupone, was volatile and identical with the product (CXXX) obtained from hexahydrocolupulone. Oxidation of hexahydrocohulupone with bismuth oxide afforded tetrahydrocohulupone. From the distillation residue the second hydrogenation product, $C_{19}H_{34}O_3$, mp 184°, was obtained in which the acyl side chain was reduced to alkyl. Similar "abnormal" reductions have been noted earlier. Using Adams catalyst this compound (CXXXIII, R = *i*-Pr) was the major product of the reduction, whereas with palladium–barium sulfate only hexahydrocohulupone was formed. The "abnormal" reduction product $C_{19}H_{34}O_3$ gave an acetate, mp 123°, and was oxidized with bismuth oxide or manganese dioxide to the triketone (CXXXIV, R = *i*-Pr). Hydrogenation of hulupone itself, using Adams catalyst, gave the "abnormal" product which was, as predicted, identical with hexahydrolupulenol (CXXIII = CXXXIV, R = *i*-Bu), thus confirming and interrelating the two series of oxidation products of lupulone (69).



Reduction of cohulupone with sodium borohydride afforded the 5-hydroxy compound (CXXVII, R = *i*-Pr) (69, 362) which was oxidized back to cohulupone with bismuth oxide.

As mentioned above, only low concentrations of hulupones are found in hops and they are probably not present in green hops (250, 368). They are formed, however, when lupulone is boiled with wort and so do occur as minor bittering principles in beer irrespective of whether or not they occur in hops, but the concentration seldom exceeds 1–2 ppm (161, 250, 409). This suggested that the hulupones were not the final stage in the oxidation process but were themselves oxidized further.

Attempts to convert the model lupulone (CXXXV) into a model hulupone by the methods used for the parent compounds were unsuccessful, but oxidation with lead tetraacetate gave *inter alia* the hydroxy compound (CXXXVI) which by hydrolysis to CXXXVII and oxidation gave the model CXXXVIII, mp 93°, the first crystalline hulupone (CXXXVIII). The major product of oxidation of CXXXV with lead tetraacetate was the biradical CXXXIX, and this suggests that similar products could be formed in hops during storage (27).

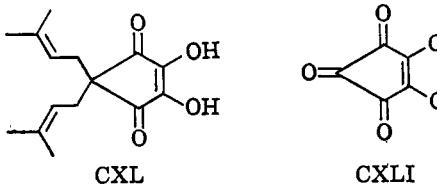


3. *Hulupone*

a. Hulupinic Acid

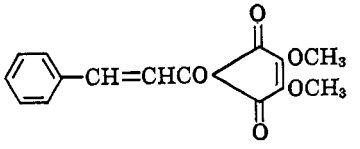
When oxygen is passed through an ethanolic solution of cohulupone at 78°, its disappearance and the

formation of a new product can be followed by paper chromatography. After 3 days this product, hulupinic acid, $C_{15}H_{20}O_4$, mp 168°, can be isolated (72). The same product is formed from hulupone itself (72) and from adhulupone (56) irrespective of the alcohol used as solvent, indicating the loss of the acyl side chain in the oxidation. Hulupinic acid was not bitter and formed an oily dimethyl ether and a crystalline tetrahydroderivative, mp 172°, which was oxidized to di(3-methylbutyl)malonic acid. On the basis of this data the ene–diol structure CXL was proposed for hulupinic acid, which was confirmed by proton magnetic resonance spectroscopy (72). Similarities between this structure and croconic acid (CXLI) (123) and linderone (CXLII) (229) were noted. Dihydrohulupinic acid, mp 124°, and an analog in which one of the dimethylallyl side chains was replaced by benzyl have also been prepared (56). Hulupinic acid is insoluble in light petroleum but soluble in ether and also forms an insoluble lead



CXL

CXLI



CXLII

salt. It was accordingly found in that portion of the hard-resin fraction of hops which gives an insoluble lead salt (the α hard resin). When this was examined further by ion-exchange chromatography, one of the fractions yielded crystalline hulupinic acid, albeit in yields of only 0.002–0.042% of the original hop, the higher values being found in old hops (71).

D. ACID DEGRADATION

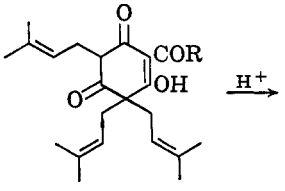
1. *Lupulone*

By treating the β acid from English hops with concentrated hydrochloric acid, Walker (400, 402) obtained two crystalline isomeric products, one a neutral substance, mp 91°, and the other a weak acid, mp 169–169.5°. They were formulated as $C_{21}H_{30}O_4$, but this was later corrected to $C_{20}H_{28}O_4$, when it was shown that Walker had been dealing with colupulone (187). The neutral product was synthesized from phlorisobutyrophenone and 2 moles of 1-bromo-3-methylbut-2-ene in dry chloroform and on the basis of its chemical and light absorption properties is regarded as 6-isobutyryl-3,4,9,10-tetrahydro-5-hydroxy-2,2,8,8-tetramethylbenzo[1,2-*b*:3,4-*b*']dipyran (CXLIII, R = *i*-Pr). By treatment with concentrated sulfuric acid this angular dipyran is rearranged to the weakly acidic linear isomer, namely 10-isobutyryl-3,4,6,7-tetrahydro-5-hydroxy-2,2,8,8-tetramethylbenzo[1,2-*b*:5,4-*b*']dipyran (CXLIV, R = *i*-Pr). The acyl side chain in this isomer is reduced to alkyl both by catalytic hydrogenation and by Clemmensen reduction (187) whereas the angular isomer (CXLIII) is reduced only by the latter method (26).

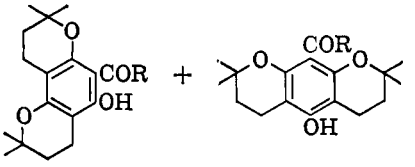
Acid degradation of synthetic lupulone gave the corresponding analogs, of which only the linear isomer (CXLIV, R = *i*-Bu), mp 104°, was crystalline (187) and which differed from either of Walker's products.

From the essential oil of a Tasmanian shrub, *Acradenia franklinii* (Kippist), a mixture of ketones was obtained, one of which, mp 128°, was called franklinone and assigned structure CXLV (34). Accordingly, the acetyl analogs of the acid degradation products of the β acids were prepared, the angular isomer (CXLIII, R = CH_3), mp 118°, being converted into the linear (CXLIV, R = CH_3), mp 159–159.5°, with concentrated sulfuric acid. Methylation of this last product then afforded the methyl ether identical with tetrahydrofranklinone (CXLVI, R = CH_3), mp 142°, from the natural product (57). Tetrahydrofranklinone has also been prepared by acylation and methylation of the benzodipyran (CXLVII) (116), but attempts to prepare franklinone itself have been unsuccessful (57).

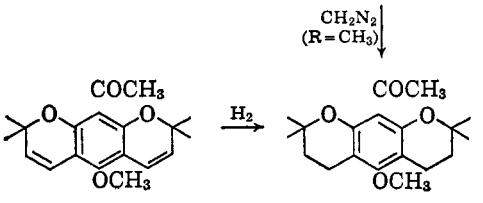
Two further related natural products are worthy of



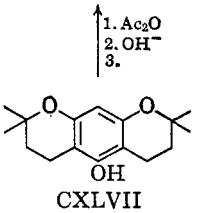
XXX



CXLIII CXLIV

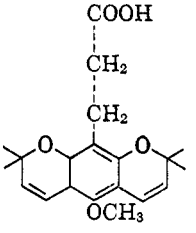


CXLV CXLVI

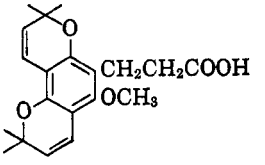


CXLVII

note. Eriostoic acid, mp 174°, from *Eriostemon* spp. (Rutaceae) has the linear benzodipyran structure CXLVIII (117), while eriostemoic acid, mp 101°, from other members of the same family has the angular structure CXLIX (116).



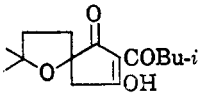
CXLVIII



CXLIX

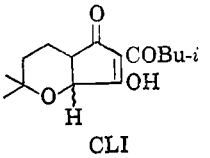
2. Humulinic Acid

Treatment of an alkaline hydrolysate of humulone with excess hydrogen chloride gave, in addition to humulinic acids A and B (see earlier), two further isomers, humulinic acids C and D, which could be separated by countercurrent distribution (20). Humulinic



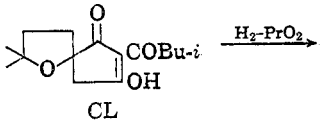
CL

acid C (CL) could be prepared more conveniently by treatment of the A isomer with acidic methanol under reflux (32% yield) (20) or with a mixture of perchloric and glacial acetic acids (54% yield) (68). Humulinic acid C is a pale yellow oil, bp 125–130° (10^{-4} mm), with similar light absorption to the other isomers and therefore contains the same enolized β-tricarbonyl system. It lacks, however, *sec*-hydroxy absorption in the infrared and fails to take up iodine or hydrogen in the presence of a palladium–barium sulfate catalyst. Using platinum oxide catalyst the acyl side was reduced to alkyl as with related compounds. Proton magnetic resonance spectroscopy excluded the tetrahydropyran structure (CLI) for humulinic acid C and suggested the

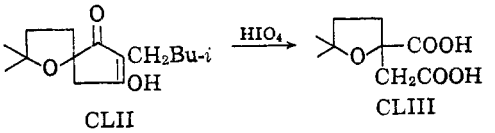


CLI

spiro tetrahydrofuran structure (CLII) for the reduced product. This was supported by oxidation of this

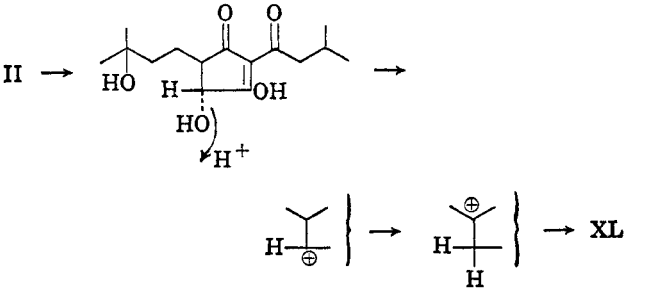


CL

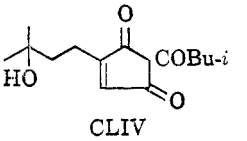


CLII CLIII

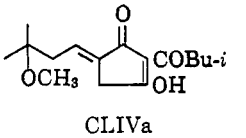
reduced product with periodate to the dibasic acid (CLIII). Humulinic acid C thus has structure CL (19, 67, 68), and the following mechanism was proposed for its formation (68).



Humulinic acid D is an unstable oil most conveniently isolated as its methyl ether, mp 57°. The parent acid is converted quantitatively in humulinic acid C by both acidic and basic reagents, and investigation of the proton magnetic resonance and the mass spectrum show it to have structure CLIV corresponding to one of the intermediates in the proposed scheme for the formation of humulinic acid C (19).



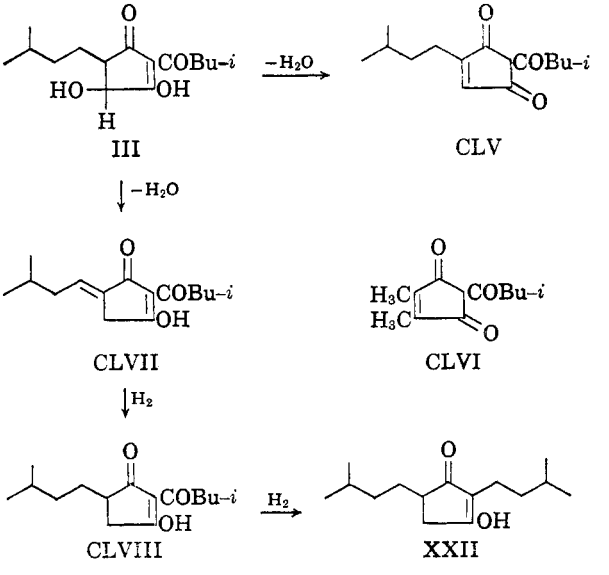
CLIV



CLIVa

A further product of acid isomerization of humulinic acid A or B has now been isolated by countercurrent distribution as its methyl ether. This product, methyl allohumulinic acid D, has been shown to have structure CLIVa (19a) which may be compared with CLVII, the corresponding product from dihydrohumulinic acid (see below).

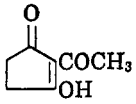
Birch and English (46) dehydrated dihydrohumulinic acid (A) with polyphosphoric acid and claimed to obtain CLV, mp 76°, which had the chromophore of



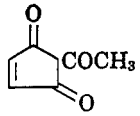
calythrone (CLVI), a component of the essential of *Calythrix tetragona* Lab. (44, 276). Other workers (68) were unable to repeat this work and obtained an isomeric product which failed to crystallize and was formulated as CLVII on the basis of its nmr spectrum and the formation of isovaleraldehyde by ozonolysis. Hydrogenation of this component under mild conditions saturated the exocyclic double bond to give 4-isopentyl-2-isovalerylcyclopentane-1,3-dione (CLVIII), mp 27°.

Methods for the synthesis of 2-acylcyclopentane-1,3-diones have recently been developed. Calythrone (CLVI) was obtained, albeit in only low yield, by condensation of dimethyl maleate with methyl isobutyl ketone (119).

2-Acetylcyclopentane-1,3-dione (CLIX) and 2-acetylcyclopent-4-ene-1,3-dione (CLX) have been pre-

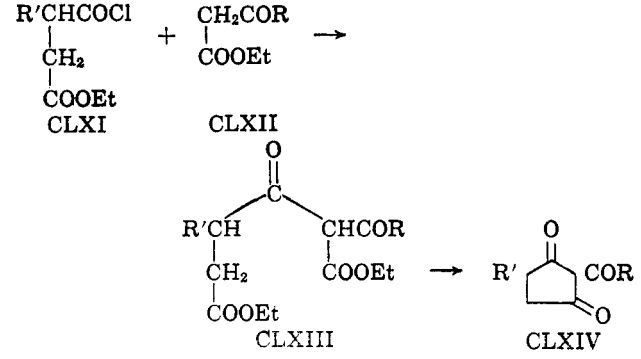


CLIX



CLX

pared in 45 and 10% yield, respectively, by condensation of succinic or maleic anhydride with isopropenyl acetate in the presence of aluminum chloride (263, 264, 273), but this scheme is not easily extended to homologs with larger acyl groups. An alternative scheme due to Vandewalle (381) involves condensation of hemisuccinoyl chloride (CLXI) with an acyl acetate (CLXII) followed by Dieckman cyclization of the diester.

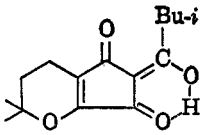


CLXI CLXII CLXIII CLXIV

By this route Vandewalle prepared the analogs CLXIV (R = H, R′ = Me; and R = $(CH_3)_2CHCH_2$, R′ = Me) (381), and it has been extended to product CLVIII as obtained by dehydration and hydrogenation of dihydrohumulinic acid (243).

3. *Dehydrohumulinic Acid*

Treatment of dehydrohumulinic acid with perchloric acid causes it to cyclize to the cyclopentenopyran (CLXV) which resisted attempts to reduce the central double bond (68).



CLXV

VI. Spectroscopic Properties of Hop Resins

A. Ultraviolet Spectroscopy

The light absorption of humulone and lupulone were first studied at the time when they were the only hop resins known, with a view to providing a convenient assay (8, 302). As with all other β-di- and β-triketones the light absorption of hop resins shows a pronounced bathochromic shift in basic media, and spectra are therefore best recorded both in acidic and basic media. Straightforward ethanolic solutions usually show the acidic spectrum, but in some cases, notably hulupone, such a solution is close to the isosbestic point and gives neither a characteristic nor reproducible spectrum without a buffer solution. Thus, in the spectrophotometric method for the estimation of α and β acids, measurements are made in alkaline ethanol at 275 (background absorption), 325 (humulones), and 355 mμ (lupulones) (8). The results obtained from the derived regression equations, when applied to natural mixtures of hop resins, do not always agree with the values obtained for the α-acid concentration, either by polarimetry or by conductometric determination of the end point during titration with lead acetate. This is no doubt due to the fact that the α and β acids are not the only species present in such hop extracts which absorb specifically in the ultraviolet. This can be seen from Table III in which the ultraviolet (and infrared) light absorption data of the compounds discussed in this review are collected. As far as possible molecular extinction coefficients (ε) have been calculated from the published data. Certain spectra are illustrated in the literature, and these are indicated by an asterisk. Despite the limitations of the spectrophotometric assay of the α and β acids, the measurement of ultraviolet absorption has played an important role in hop chemistry, not only by characterizing the chromophores present in hop resins but as the preferred method in following separations carried out by countercurrent distribution and column chromatography.

Campbell and Coppinger (81) carried out the first survey of the light absorption of phloroglucinol derivatives. In neutral ethanolic solution phloroglucinol itself (CLXVI) shows two weak absorption bands at λ_{max} 267.5 mμ (ε 450) and 271 mμ (ε 388), but these are much stronger in basic solution: λ_{max} 252.2 mμ (ε 20,000) and 349.5 mμ (ε 8730). Substitution with one (CLXVII), two (CLXVIII), or three methyl groups causes a slight bathochromic shift, but this is

TABLE III
ULTRAVIOLET AND INFRARED ABSORPTION OF HOP RESINS AND RELATED COMPOUNDS

Formula no.		Ultraviolet absorption λ_{max}, mμ (ε)			Infrared absorption ν_{max}, cm^{-1}			
		In EtOH or acidic EtOH	In basic EtOH	Ref[a]	DMS no.	OH	>C=O	Ref[a]
I	Humulone	235 (13,700), 281–5 (8000), 310–325 sh (5330)	228 (14,730), 324 (11, 520), 350–361 sh (8750)	150	11,637	3335	1667, 1626	11,* 150*
	Humulone–*o*-phenylenediamine complex	230 (8500), 242 (5700), 280 (5700), 330 (5700), 360 (4300)	...	150	...	~3400	1645	18*
II	Humulinic acid (A)	226 (11,200), 266 (9300)	257 (20,700), 266 sh (12,300)	20	11,632 (a)	...	...	20*
	Oxime	251 (16,700), 303 (18,000)	...	150				
	Humulinic acid (B)	226 (10,500), 266 (9300)	257 (20,000), 266 sh (16,900)	20	...	...	...	20*
III	Dihydrohumulinic acid (A)	259 (9500), 267 (10,500)	...	150	11,633 (a)	...	...	150*
	Oxime	251 (16,400), 303 (17,200)	...	150				
IV	Humuloquinol	293 (22,000), 350 (2400)	...	150				
	Humuloquinol tetrabenzoate	234 (72,000)	...	150				
V	Humuloquinone	292 (25,500), 381 (725), 530 (268)	218–220 sh (10,600), 299 (17,000), 344 (10,200)	150, 391*				
VI	Isohumulinic acid	251, 258 (25,000), 280 (22,000)	...	150				
X	2-Acetyl-4,6-dimethyl-4-hydroxycyclohexane-1,3,5-trione	230 (10,800), 240 (10,200), 283 (9650), 315 (6400)	257 (19,400), 310 (12,800)	55, 80*	11,617	3355, 3145	1661, 1639 sh	58
XI	3-Isopentylcyclopentane-1,2,4-trione	275.5 (12,230)	233.5 (10,500), 326 (10,800)	150				
XIII	Dehydrohumulinic acid	255 (21,700), 280 (18,000)	275 (25,800), 303 (25,900)	189	11,625	3225	1707, 1650 sh, 1616	189
XIV	4-Hydroxy-2,5-diisopentylcyclopentane-1,3-dione	250 (15,700)	273 (23,500)	66				245*
XVI	2-Isopentyl-7-methyl-3,5-dioxooctanal	268	218, 293	295				
XVII	2-Isobutyl-5-isopentyl-γ-pyrone	220, 265	220, 265	245				
XIX	2-Hydroxy-3-isopentyl-6-isobutyl-γ-pyrone	220	260	245				
XXII	"Deoxydihydrohumulinic acid"	252 (16,700)	273 (27,400)	68				
XXX	(Co)lupulone			8,* 11*	11,639	3130	1666, 1588	185*
XXXV	Deoxyhumulone	290	...	292*	...	...	...	
	Deoxycohumulone	290 (18,260)	...	210	...	...	...	
XXXVI	Xanthohumol	370	438	397*	...	3300	1600	397*
XXXVII	Isoxanthohumol	290	332	397*	...	3300	1650, 1600	397*
XXXIX	2-Isopentenyl-5-methoxyresorcinol	270	340	397	...	3400	1620, 1600	397*
XL	2-Isopentyl-5-methoxyresorcinol	270	340	397	...	3400	1620, 1000	397*
LX, R = R′ = Me	3-Methylphloracetophenone	222.5, 290	...	398				
LXI, R = R′ = Me	3,5-Dimethylphloracetophenone	227.5, 291.5	...	398				
LXII, R = R′ = Me	3-Acetylfilicinic acid	232.5, 275, 335	...	398				
LXIII, R = R′ = Me	2-Acetyl-4,4,6-trimethylcyclohexane-1,3,5-trione	275 (5820), 330 (8500)	355 (16,400)	27				
LXIV, R = R′ = Me	2-Acetyl-4,4,6,6-tetramethylcyclohexane-1,3,5-trione	237.5, 277.5	...	398				
LXVIII	Filicinic acid	245 (16,900), 295 (3923) 343 (1145)	235 (21,760), 253 (19,040), 353 (9070)	175*				
LXIX	Diacetylphloroglucinol	270 (36,700), 300 (3940)	290 (28,700), 370 (7140)	175				
LXX	3,5-Diacetylfilicinic acid	245 (13,800), 297 (15,460)	240 (16,400), 300 (21,220)	175*				
LXXII	Isopentylphlorisovalerophenone	294 (14,790) 335 sh (2690)	327 (19,050)	128				
LXXVII	Isopentenylphloroglucinol	270 (790)	...	265				
LXXVIII	2,2-Dimethylchroman-5,7-diol	270 (680)	...	265				
LXXX	Colupone	245 sh, 285 (9500)	240 sh, 280 (19,600)	242	...	3400, 3000	1700, 1660, 1550	242

TABLE III (*Continued*)

Formula no.		Ultraviolet absorption λ_{max}, mμ (ε)			Infrared absorption ν_{max}, cm^{-1}			
		In EtOH or acidic EtOH	In basic EtOH	Ref[a]	DMS no.	CH	>C=O	Ref[a]
LXXXI	Leptospermone (R = *i*-Bu)	238 (8300), 279 (10,800)	278 (17,350)	86	...	...	1715, 1660, 1550	86
	Flavesone (R = *i*-Pr)	234 (39,800), 280 (31,600)	...	43	...	...	...	...
LXXIV	2,4-Diacetyl-3,4-dihydroxy-5-methylcyclopent-2-enone	227 (10,900), 265 (9100)	253 (19,200), 267 infl (18,100)	58	...	...	...	
	Anil	245 (12,700), 315 (22,000)		58				
LXXXV	2-Acetyl-3,4-dihydroxy-5-methylcyclopentane-1,3-dione	...	...	58	11,613	3495	1724, 1639	58
LXXXVI	3-Acetyl-5-methylcyclopentane-1,2,4-trione	253 (21,900), 275 infl (16,300)	272 (26,000), 300 (22,100)	58	...	...	...	
LXXXVII	Hexahydrocolupulone	...	355.5 (20,400)	82, 297*	...	...	1655, 1587	369
XCIV	Isohumulone (mixed isomers)	225 (12,670), 268 (10,100), 280 (9054)	255 (18,824)	11,* 102 308	...	...	...	11*
XCVI	Tetrahydroisohumulone	230 (9150), 275 (9150)	253 (16,700)	59	...	3508	1709, 1574–1626	59
XCVIII	Neohydroisohumulone	253 (11,800)	274 (17,200)	59		3448, 3000 br	1709, 1538–1639	59
CII	Isohumulone (B)	232, 273	254, 271 sh	14				
CIII	(A)	226, 281	254, 271 sh	14				
CVIII	Oxyhumulinic acid (R = *i*-Bu)	253 (13,100), 266 (12,400)	253 (18,200), 268 infl (16,100)	103				11*
	(R = *i*-Pr)	253 (11,300), 266 (10,400)	255 (18,000), 270 infl (15,550)	103				
CXI	Humulinone, (R = *i*-Bu)							11*
	Cohumulinone (R = *i*-Pr)	228 (10,500), 269 (12,150)	262 (17,800), 273 (16,000)	103				
	Adhumulinone (R = *sec*-Bu)	230 (11,400), 278 (9400)	255 (18,900), 273 (18,850)	104				
CXIV	2-Hydroxy-6-isobutyryl-2,4,4-triisopentylcyclohexane-1,3,5-trione	245 (7700), 280 (10,600)	255 (13,900), 270 sh (12,300)	369		3448	1725, 1670	369
CXV	4-Hydroxy-2-isobutyryl-4,γ-methylvaleryl-5,5-diisopentylcyclopentane-1,3-dione	250 (7450), 280 (10,100)	255 (15,850), 270 sh (12,100)	369	11,640	3500	1697, 1625	369
CXVI	Tetrahydrohumulinone (R = *i*-Bu)	230 (9500), 277.5 (10,600)	255 (18,300), 270 sh (16,800)	369	11,638	3500	1710, 1650	369
	Tetrahydrocohumulinone (R = *i*-Pr)	230 (9160), 270 (10,200)	255 (22,500), 270 (20,400)	191				
CXVIII	α-Dihydrohumulinone	230, 282	265	261	...	3400	1710, 1650, 1600	261
CXIX	Isohumulinone A	228 (10,000), 267 (8850)	250 (18,300), 270 (16,900)	30				
CXX	Isohumulinone B	250 (12,000), 273 (10,450)	253 (17,000), 269 (15,000)	30				
CXXI	Lupuloxinic Acid	252 (10,000)	...	185				
CXII	Lupulenol	253 (12,340)	275 (22,500)	185				
CXXIII	Hexahydrolupulenol	250 (14,000), 256 infl (12,000)	275 (24,900)	69, 185	11,603	3500	1560	69
CXXIV	3,3,5-Triisopentylcyclopentane-1,2,4-trione	280 (9800)	230 (12,500), 330 (10,500)	187	11,604	...	1740, 1685, 1645	69
CXXVI	Hulupone (R = *i*-Bu)	280 (8900)	255 (14,800), 325 (10,000)	420	11,606	...	1706, 1645	362,* 420
	Cohulupone (R = *i*-Pr)	280 (8790)	255 (12,800), 325 (8760)	420	11,628	...	1770, 1724, 1661	420
CXXVII	5-Hydroxy-2-isobutyryl-4,4-di(3-methylbut-2-enyl)-cyclopentane-1,3-dione	260 (8250)	270 (12,550), 260 sh (11,750)	69	11,605	3030	1715, 1626	69
CXXVIII	5-Hydroxy-2-isobutyryl-4,4-diisopentylcyclopentane-1,3-dione	245 (19,500), 275 (14,800)	250 (20,500)	69	11,607	2975, 2900 sh	1705, 1620	369
CXXIX	...	245 (7950), 280 (9400)	278 (12,400)	369				
CXXXI	Tetrahydrocohulupone	285 (9200)	255 (15,700), 325 (10,800)	369	11,626	...	1755, 1710, 1650	369
	Na salt		255 (12,000), 325 (9050)	369	11,627		1740, 1680, 1620	369
	Diisopentylmalonic acid				11,622		1701	369

TABLE III (*Continued*)

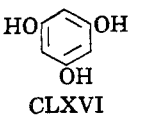
Formula no.		Ultraviolet absorption, λ_{max}, mμ (ε)			Infrared absorption, ν_{max}, cm^{-1}			
		In EtOH or acidic EtCH	In basic EtOH	Ref[a]	DMS no.	OH	>C=O	Ref[a]
	Diethyl diisopentylmalonate				11,623		1730	369
CXXXIII, R = Pr	5-Hydroxy-2-isobutyl-4,4-diisopentylcyclopentane-1,3-dione	250 (14,150)	275 (24,000)	69	11,600	3460	1563	69
	Acetate	250 (13,700)	275 (22,300)	69	11,601	...	1750, 1575	69
CXXXIV	5-Isobutyl-3,3-diisopentylcyclopentane-1,2,4-trione	280 (3480)	230 (4310), 330 (3570)	69	11,602	...	1740, 1680, 1645	69
CXXXV	2-Acetyl-4,4,6-trimethylcyclohexane-1,3,5-trione	275 (5820), 330 (8500)	355 (16,400)	27	...	...	...	...
CXXXVI	2-Acetyl-6-hydroxy-4,4,6-trimethylcyclohexane-1,3,5-trione	237.5 (7563), 275 (10,370)	270 (17,030)	27	...	...	...	...
CXXXVII	2-Acetyl-4-hydroxy-5,5-dimethylcyclopentane-1,3-dione	225 (10,100), 267.5 (9700)	250 (19,360), 270 (16,140)	27	...	...	...	...
CXXXVIII	3-Acetyl-5,5-dimethylcyclopentane-1,2,4-trione	282.5 (10,220)	250 (13,720), 322.5 (10,300)	27	...	...	...	...
CXXXIX	6,6′-Bis(2-acetyl-4,4,6-trimethylcyclohexane-1,3,5-trione)	240 (21,500), 275 (20,520)	275 (31,510)	27	...	...	...	...
CXL	Hulupinic acid	301 (10,230)	261 (14,450), 393 (12,300)	72	...	3300, 3000	1750, 1685, 1660	72
CXLI	Croconic acid	231 (13,490), 298 (28,180)	...	123	...	...		123*
CXLII	Linderone	244 (19,500), 262 sh (14,100), 357 (29,500)	252 (22,900), 285 sh (15,850) 352 (20,430)	229	...	...	1715, 1630	229
CXLIII, R = *i*-Pr	6-Isobutyryl-3,4,9,10-tetrahydro-5-hydroxy-2,2,8,8-tetramethylbenzo[1,2-*b*:3,4-*b*′]dipyran	298 (16,500)	300 (19,900)	187	11,634	...	1610	187
CXLIII, R = Me	6-Acetyl-3,4,9,10-tetrahydro-5-hydroxy-2,2,8,8-tetramethylbenzo[1,2-*b*:3,4-*b*′]-dipyran	...	...	57	11,631	...	1610, 1592	57
CXLIV, R = *i*-Pr	3,4,6,7-Tetrahydro-5-hydroxy-10-isobutyryl-2,2,8,8-tetramethylbenzo[1,2-*b*:5,4-*b*′]dipyran	282 (4000)	282 (4280)	87	11,636	3490	1690, 1605	187
CXLIV, R = Me	10-Acetyl-3,4,6,7-tetrahydro-5-hydroxy-2,2,8,8-tetramethylbenzo[1,2-*b*:5,4-*b*′]-dipyran	...	...	...	11,629	3425	1667, 1645, 1582	57
CXLV	Franklinone	220 (10,000), 252 (50,120), 262 (50,120), 339 (3160)	...	34	...	...	1740, 1643, 1632	34
CXLVI, R = *i*-Pr	3,4,6,7-Tetrahydro-10-isobutyryl-5-methoxy-2,2,8,8-tetramethylbenzo[1,2-*b*:5,4-*b*′]dipyran	275 (3980)	...	57	11,635	...	1698, 1600	57
CXLVI, R = Me	10-Acetyl-3,4,6,7-tetrahydro-5-methoxy-2,2,8,8-tetramethylbenzo[1,2-*b*:5,4-*b*′]-dipyran (tetrahydrofranklinone)	275 (3980)		57	11,630	...	1701, 1597	57
CXLVIII	Eriostoic acid	...	...	117*	...	...	1740, 1700	117
CXLIX	Eriostemoic acid	206 (17,500), 254 sh (24,000), 258 (24,000), 322 sh (2260)	...	116	...	...	1708, 1640	116
CL	Humulinic acid C	225 (10,400), 264 (7650)	251 (18,900), 268 (15,600)	20	...	...	1715, 1630, 1600	20*
	Sodium salt	...	...	...	...	...	1685, 1615, 1580	20*
CLII	...	252 (18,700)	274 (26,300)	68	...	...	...	
CLIV	Humulinic acid D	235 (16,900), 266 (17,400)	235 (16,900), 275 (21,000) 405 (765)	20	...	3400	1715, 1655, 1630	20*
CLV	2-Isobutyryl-4-isopentylcyclopent-4-ene-1,3-dione	240 (16,600), 266 (18,200)	...	46	...	3560	1710, 1670	46

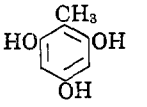
TABLE III (*Continued*)

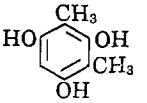
Formula no.		Ultraviolet absorption λ_{max}, mμ (ε) In EtOH or acidic EtOH	In basic EtOH	Ref[a]	Infrared absorption ν_{max}, cm^{-1} DMS no.	OH	>C=O	Ref[a]
CLVI	Calythrone	240 (21,500), 265 (19,500)	...	119	...	...	1703, 1680, 1642, 1612	119
CLVII	2-Isovaleryl-4-(3-methylbutylidene)-cyclopentane-1,3-dione	238 (13,620), 305 (10,280)	240 (13,020), 270 (16,880)	68	...	...	...	...
CLVIII	2-Isovaleryl-4-(3-methylbutyl)cyclopentane-1,3-dione	225 (11,100), 265 (8500)	245 (23,900), 265 (17,100)	68	...	...	...	...
CLIX	2-Acetylcyclopentane-1,3-dione	220 (11,800), 265 (7040)	...	263, 264	...	...	1710, 1635, 1595	263, 264
CLX	2-Acetylcyclopent-4-ene-1,3-dione	223 (14,000), 259 (13,800), 317 (820)	...	273	...	...	1700, 1630, 1595	273
CLXV	...	260 (25,700), 280 sh (21,500)	265 (31,700), 280 (33,900)	68	...	...	...	...
CLXVI	Phloroglucinol	267.5 (450), 271 (388)	252.5 (20,000), 349.5 (8730)	81	...	...	...	...
CLXVII	Methylphloroglucinol	271.5 (835), 278 (769)	256 (16,400), 356.5 (6650)	81	11,612	3595, 3510	1637, 1613	58
CLXVIII	Dimethylphloroglucinol	275 (1200)	261 (16,000), 363 (6100)	81	...	...	...	
CLXIX	Phloracetophenone	227 (12,400), 285 (14,700)	318 (26,400)	81	...	...	...	...
CLXX	Methylphloracetophenone	222.5, 290	...	298	...	...	...	...
CLXXI	Dimethylphloracetophenone	227.5, 291.5	...	298	11,616	3510	1613, 1575	58
CLXXIII	Cyclopentane-1,3-dione	242 (20,700)	257 (29,400)	50	...	...	...	...
CLXXIV	Cyclopentane-1,2,4-trione	267 (10,850)	350 (13,450)	50	...	...	...	...
CLXXV	Cyclopent-4-ene-1,3-dione	222 (14,450), 322 (20), 367 (20)	Decompn	371	...	...	1745, 1715	371

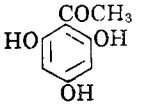
[a] Those references marked with an asterisk denote that the complete spectrum is reproduced.

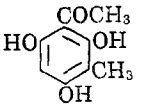
much more pronounced with an acyl group: phloracetophenone (CLXIX) has λ_{max} 226 mμ (ε 12,400) and 285 mμ (ε 14,700) in neutral ethanolic solution and λ_{max} 318 mμ (ε 26,400) in basic solution. Dimethylphloracetophenone (CLXXI) and desoxyhumulone (XXXV),

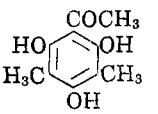

CLXVI


CLXVII


CLXVIII


CLXIX


CLXX


CLXXI

which retain the β-triketone system within an aromatic framework, exhibit λ_{max} 290 mμ (ε 18,000) in ethanolic solution (80, 210, 292). Campbell and Coppinger also showed (80) that oxygenation of dimethylphloracetophenone in the presence of lead acetate gave a 2-acetyl-4-hydroxy-4,6-dimethylcyclohexane-1,3,5-trione (X) with the chromophore of humulone (I, R = *i*-Bu). Although these structures cannot be aromatic they still show λ_{max} 237 and 282 mμ together with bands at higher wavelengths (325 mμ). Replacement of the hydroxyl groups in humulone with a further β,β-dimethylallyl group, as in lupulone (XXX), causes a further bathochromic shift (to 340 mμ).

Leptospermone (LXXXI), in which the isolated β-tricarbonyl system cannot be conjugated further, does not show this band at higher wavelengths, but has λ_{max} 238 mμ (ε 7520) and 279 mμ (ε 11,530) (86) as in the simpler 2-acetylcyclohexane-1,3-dione (235 mμ (ε 14,700) and 275 mμ (ε 11,100)) (345). The light absorption properties of leptospermone have been examined in some detail (86). In particular, the effects of solvent, dilution, and pH have been studied. At pH 1.22 maxima can be seen at 238 and 279 mμ while at pH 13 only the latter is present. The spectra show a single sharp isosbestic point at 256 mμ (ε 6918). It was suggested that the enol shows maxima at 238 and 279 mμ while the ion has a maximum only at 279 mμ (86). Similar detailed studies of the hop resins would no doubt prove of value.

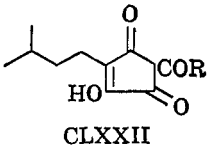
The simplest β-triketone with a five-membered ring, 2-acetylcyclopentane-1,3-dione (CLIX), has only recently been prepared (262, 264) and has λ_{max} 220 mμ (ε 11,800) and 265 mμ (ε 7040), and thus shows a hipsochromic shift compared with the six-membered ring analog. On the other hand, the acyclic analog, 3-acetylpentane-2,4-dione, with λ_{max} 277.5 mμ (ε 8128) (226) shows a slight bathochromic shift.

Hop resin derivatives which contain the 2-acylcyclopentane-1,3-dione system, *e.g.*, isohumulone, humulinic acid, humulinone, and oxyhumulinic acid, all show λ_{max} *ca.* 280 mμ in acid solution and λ_{max} 255 mμ with a

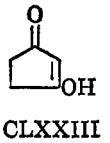
shoulder at 280 mμ in a basic solution (11). This absorption provides the basis for spectrophotometric methods for the estimation of isohumulone in beer. Thus, while the method of Rigby and Bethune (308) is based on measurements made in basic methanol solution at 255 mμ, the methods of Brenner, *et al.* (51), and Klopper (231) use the absorption at 280 mμ in acid solution (for a more detailed discussion see ref 48 and 212).

3-Acylcyclopentane-1,2,4-triones which can exist in dienolic forms, such as isohumulinic (VI) and dehydrohumulinic (XIII) acids, show λ_{max} 255 and 280 mμ in acid solution and λ_{max} 235, 275, and 300 mμ in basic solution together with weak absorption at 430 mμ (120). On the other hand, 5,5-disubstituted 3-acylcyclopentane-1,2,4-triones, which can only form monoenols such as hulupone (CXXVI), show simpler spectra with λ_{max} 280 mμ in acid solution and λ_{max} 255 and 325 mμ in basic solution (420). In between these two forms 2-acetylcyclopent-4-ene-1,3-dione (CLX), which contains the chromophore of an enolic tautomer of 3-acylcyclopentane-1,2,4-trione, *e.g.*, CLXXII, has λ_{max} 223 and 259 mμ in ethanolic solution with a weak absorption at 317 mμ (273). The enolization of these compounds will be discussed further in the light of their proton magnetic resonance spectra (see section VI-D).

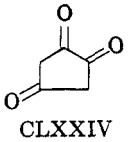
Catalytic hydrogenation or Clemmensen reduction of 2-acylcyclopentane-1,3-diones affords 2-alkylcyclopentane-1,3-diones which show a single absorption maxima at *ca.* 250 mμ in acidic media displaced to *ca.* 270 mμ in basic solution (59, 66, 69, 245, 261). This represents a bathochromic shift compared to the parent compound, cyclopentane-1,3-dione (CLXXIII), which shows λ_{max} 242 mμ in acid and λ_{max} 257 mμ in basic solution (50), while cyclopentane-1,2,4-trione (CLXXIV) has λ_{max} 267 mμ in acid and λ_{max} 310 mμ in basic solution (50). On the other hand, cyclopent-4-ene-1,3-dione (CLXXV), which is not enolic, only shows λ_{max} 222 mμ with weak absorption at 322 and 367 mμ in acid solution and decomposes in alkaline media (281).



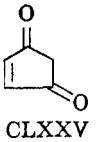
CLXXII



CLXXIII



CLXXIV



CLXXV

The spectral behavior of flavonoid compounds, which are also derivatives of phloroglucinol, have been studied under a wide range of conditions (225). Thus, valuable structural information has been obtained not only from spectra measured in acidic and basic ethanolic solution but also for spectra measured in the presence of, for example, sodium acetate, aluminum chloride, and boric acid. An extension of these techniques to hop compounds could be fruitful.

B. INFRARED SPECTROSCOPY

The infrared spectra of hop extracts were discussed in 1961 (334), and the following year the spectra of humulone (150), lupulone (185), and dihydrohumulinic acid (150) were recorded. Later the spectra of humulone, isohumulone, humulinone, humulinic acid, and oxyhumulinic acid were recorded and briefly discussed (11). In Table III are collected references to the available infrared data on hop constituents. Some of these spectra have been included in the Documentation of Molecular Spectroscopy Collection in which case the relevant number is included. Also included in Table III are the bands present in the hydroxyl and carbonyl frequency area of the spectra. There has been no detailed analysis of the infrared spectra of the hop resins, but studies have been made on other β-triketones. Thus, 2-acetylcyclohexane-1,3-dione as a liquid film shows a diffuse hydrogen-bonded hydroxyl absorption at 2630 cm^{-1} and in chloroform solution carbonyl absorption at 1680 (conjugated carbonyl) and 1565 cm^{-1} (conjugated chelate carbonyl) (86, 345).

The corresponding carbonyl absorptions in the acyclic analog, triacetylmethane, were at 1678 and 1580 cm^{-1} (130) compared with 1710, 1635, and 1595 cm^{-1} in 2-acetylcyclopentane-1,3-dione (129). In the six-membered ring series leptospermone (LXXXI) shows carbonyl absorption at 1715 (unconjugated C=O), 1660 (conjugated C=O), and 1550 cm^{-1} (conjugated chelate C=O) (86), the first band being absent in the spectra of 2-acetyl-5,5-dimethylcyclohexane-1,3-dione and 2-acetyl-4,4,6-trimethylcyclohexane-1,3-dione (86). Similar assignments were made with ceroptene and other β-triketones (130) and with the hop substances. Lupulone exhibits carbonyl bands at 1666 and 1588 cm^{-1} (185), while the corresponding absorption in humulone are at 1663 and 1626 cm^{-1}. Humulone (150) and (co)lupulone (11) show weak absorption at 3300 and 3130 cm^{-1}, respectively, associated with hydrogen-bonded hydroxyl.

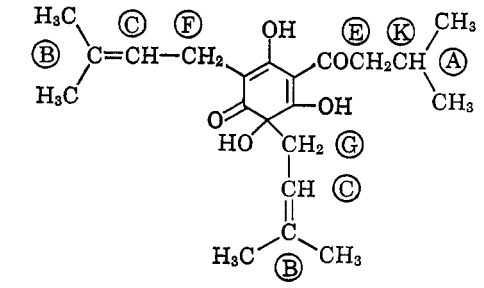
In the five-membered ring series dihydrohumulinic acid (III) was one of the first spectra recorded. This showed sharp hydroxyl absorption at 3420 cm^{-1} and carbonyl frequencies at 1690 and 1629 cm^{-1} (150). Similar bands were seen in the spectra of humulinic and oxyhumulinic acids, the latter showing an additional hydroxyl absorption at 3250 cm^{-1} (11). Comparison of the infrared spectra of humulinic acids A, B, C, and D (20) demonstrated the absence of hydroxyl absorption in the C isomer and in the methyl ether prepared from humulinic acid D.

C. NUCLEAR MAGNETIC RESONANCE SPECTROSCOPY

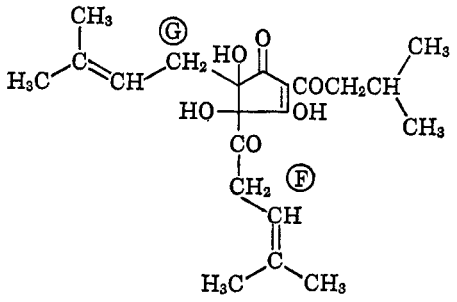
Proton magnetic resonance spectroscopy was first applied to hop chemistry in 1959 in order to distinguish between the five- (CXI) and six-membered (CX) ring structures for humulinone (340). The spectra of humulone (I), dihydrohumulone (CLXXVI), and humulinone were recorded in deuteriochloroform at 60 Mc/sec, and by comparison of the spectra of humulone and dihydrohumulone it was possible to allocate all the signals to the relevant protons, in particular, the signals associated with the methylene groups of the dimethylallyl side chains F and G, which in humulone were doublets at τ 6.79 and 7.41, respectively. If the six-membered ring structure (CX) for humulinone were correct, the methylene groups F and G would be in similar environments and should give a signal as for G in humulone of double intensity. In fact, in the spectrum of humulinone the doublet associated with the methylene group (F) is displaced to a lower field (τ 6.58) than in humulone. This is to be expected if the five-membered ring structure (CXI) is correct, because a methylene group between a double bond and a carbonyl group, as in (CXI), is less shielded than one situated between a double bond and a carbinol function as in CX. Thus the proton magnetic resonance spectrum supports the five-membered ring structure (CXI) for humulinone now substantiated by further chemical evidence (see section VC1a).

It was noted that the signals associated with hydroxylic protons were broad and concentration dependent and therefore not suitable for accurate structural assignments. Further, in the spectrum of humulone the enol proton was observed at very low field (τ −9.2) (340).

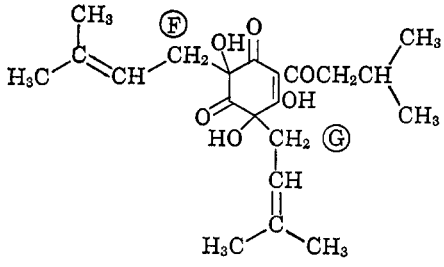
Forsén and Nilsson (130) examined the spectra of a group of enolized β-triketones and found enol signals at similar positions. Thus, in triacetylmethane the enol signal was at τ −7.41, compared with τ −6.81 in the β-diketone, acetylacetone, while in 2-acetyl-5,5-dimethylcyclohexane-1,3-dione (CLXXVII, R = Me) and the analog (CLXXVII, R = $CH_2CH_2C_6H_5$) it was at τ −8.11. Even lower values were found in the unsaturated analog (CLXXVII, R = $CH{=}CHC_6H_5$) (τ −8.16), cereoptene (CLXXVIII) (τ −8.91) (130), and usnic acid (CLXXIX) (τ −9.20) (132).



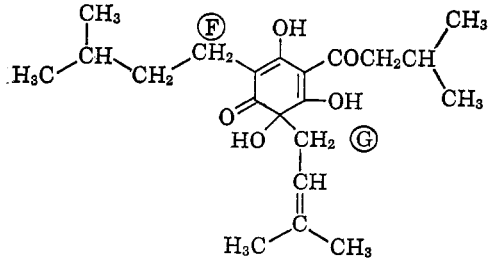
I



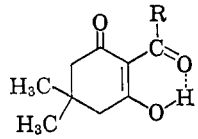
CXI



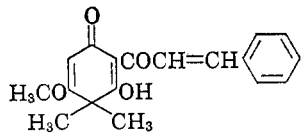
CX



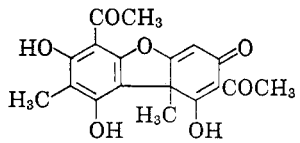
CLXXVI



CLXXVII



CLXXVIII



CLXXIX

The phenolic aromatic chelated proton in usnic acid was found at τ −3.28, similar values being found in 2-hydroxyacetophenone (τ −2.32), 2,4-dihydroxyacetophenone (τ −2.70), and 2,4-dimethoxy-6-hydroxyacetophenone (τ −4.03) (315). In phloracetophenone (τ −0.65) and phloroglucinaldehyde (τ −0.40) with two alternative phenolic hydroxyls available for chelation, the signal is not at such a low field (315).

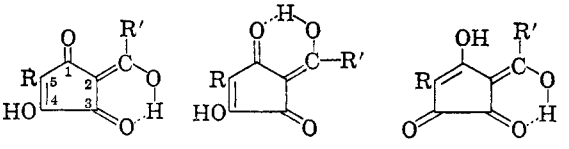
Forsén and Nilsson (129, 130) also drew attention to the linear relationship which exists between the enolic proton shift and the frequency of the chelate carbonyl frequency in the infrared spectrum, a relationship also discussed by Hay and Williams (164).

It was subsequently found that in substances which contain a β-triketone system in a five-membered ring the enol proton was at higher field than in comparative compounds with six-membered rings. Thus, with cohulupone (CXXVI) the enolic proton lies between τ −2 and −3 (28), while in 2-acetylcyclopentane-1,3-dione and 2-acetylcyclopent-4-ene-1,3-dione, it is at τ −4.75 and −2.10, respectively (129).

To return to humulone, it is reported that the enol signals of the different analogs (co-, ad-, and humulone) are at different τ values so that by integrating the areas under the relevant peaks the composition of the α acid can be rapidly evaluated (13). Lupulone similarly shows two signals at τ −1 and −9 due to two enolic forms so the β acids must be removed before the α-acid fraction can be analyzed in this manner (13).

After being applied to the structure of humulinone, proton magnetic resonance spectroscopy was next applied in 1963 to confirm the structure of hulupinic acid (CXL) (72), and since that time it has been widely used. In hulupinic acid the ene–diol protons give a broad (1.2 ppm) signal at τ 2.6 of intensity corresponding to two protons and the remaining signals in the spectra can readily be assigned to the methyl groups, methylene group, and vinyl protons of the dimethylallyl side chains. Of particular interest was the application of proton magnetic resonance spectroscopy to a study of the fine structure of isohumulinic acid and of the product obtained by oxidizing humulinic acid (A or B) with bismuth oxide (189), now called dehydrohumulinic acid (131). These compounds, formerly 3-acylcyclopentane-1,2,4-triones, are found to be dienolic with signals between τ −2 to −3 and 2.3 to 2.5, each of the intensities corresponding to one proton. The former signal is from the normal chelated enol proton found in five-membered ring β-triketones while the latter may be compared with the weaker enolic signal found in the monoenolic 3-methylcyclopentane-1,2-dione (τ 3.28) (41). Further evidence for the dienolic structure of isohumulinic acid came from the chemical shift (τ 7.63) of the lower field of the two methylene groups in the isopentyl side chain which indicates proximity to a double bond, necessarily in the ring.

This conclusion implies that there is no proton at C_5, and in agreement the methylene signal is split merely as a triplet by the adjacent methylene. The absence of further splitting proves there can be no proton at C_5. The corresponding signal in dehydrohumulinic acid is at τ 6.95 because the methylene is between two double bonds and is split as a doublet by the adjacent olefinic proton, so, again, there cannot be a proton at C_5. Of the possible dienolic structures (CLXXX) the exocyclic forms are preferred (131), as

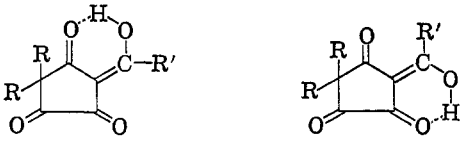


a b c

CLXXX

alternative forms are derivatives of cyclopentadienone. Such compounds are unstable and rapidly dimerize, a tendency not observed with the hop derivatives. Further, cyclopentene-3,5-dione does not enolize to form 3-hydroxycyclopentadienone (144, 281).

Cohulupone can only exist as a monoenol and for similar reasons the exocyclic enolic forms (CLXXXI) are preferred (131). In one sample of cohulupone the enol signal was split into two signals at (τ −3.08 and −2.90) which could be due to the simultaneous presence of two enolic forms (131).

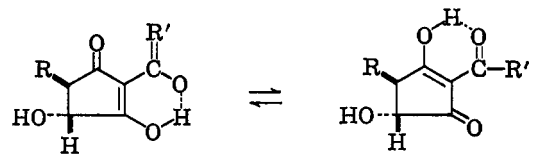


CLXXXI

Use was made of proton magnetic resonance spectroscopy to determine the fine structures of the humulinic acids (10, 66). In the spectra given by humulinic acids A and B the major difference is seen in the signals due to the hydrogens at C_4 and C_5. In humulinic acid A the signal from the 4-proton is split as a doublet by the 5-proton. The splitting is 4.5 cps, pointing to a dihedral angle of 120° between the 4- and 5-hydrogens so that humulinic acid A is 4,5-*trans*. In humulinic acid B, the corresponding doublet signal has $J = 7.5$ cps so the dihedral angle is 0° and this acid has the *cis*-4,5 configuration (66).

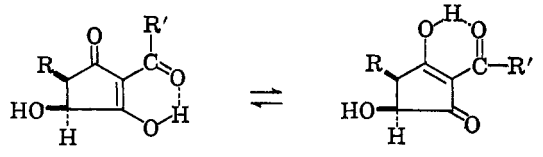
It is also noteworthy that the signal for the 2-methylene group of the isovaleryl chain in humulinic acids A and B is 0.18 ppm to lower field than the corresponding signal in dehydrohumulinic acid. This indicates that the isovaleryl carbonyl group is not enolized, leading to structures CLXXXII and CLXXXIII for humulinic acids A and B and to CLXXXIV and CLXXXV for the dihydro acids (66).

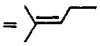
With this background it was possible to allocate all of the signals in the spectrum of isohumulone A (73). In particular, the signal associated with the hydrogen atom at C_5 showed that it was a mixture of two stereochemical forms. Further support for this view came from the signals of the methylene groups attached to C_5 which can be resolved as two overlapping triplets. It could thus be estimated that isohumulone A consisted of a mixture of two isomers of which 60% was the *trans*



CLXXXII, R = 

CLXXXIV, R = 



CLXXXIII, R = 

CLXXXV, R = 

(with humulinic acid B stereochemistry) (CII) and 40% the *cis* isomer (with humulinic acid A stereochemistry) (CIII) (73). Later when these isomers were separated and the individual spectra recorded, most of the assignments made for the mixture (14) were substantiated. The signal associated with the proton on C_5 was found as a triplet in isohumulone B; in isohumulone A it appeared as a doublet of doublets (14).

Chemical evidence, loss of double bond, and loss of secondary hydroxyl suggested structure CLI for humulinic acid C, but this was immediately rejected on the basis of the pmr spectrum (68). No signals attributable to the C_4 proton could be found. Further study of the pmr spectrum of the reduction product of humulinic acid C in which the acyl side chain reduced to alkyl suggested the spiro structure CLII for the reduction product. In particular, signals at τ 8.68 and 8.65 were assigned to the almost equivalent geminal methyl groups adjacent to ethereal oxygen, the symmetrical A_2B_2 pattern centered at τ 7.98 to the 3- and 4-methylene protons of the tetrahydrofuran ring, and the broadened singlet at τ 7.33 to the isolated methylene of the monoenolized cyclopentadione ring.

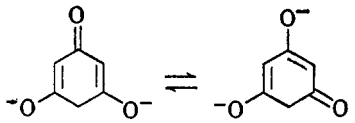
Confirmation of these assignments came from a study of the product (CLIII) obtained by oxidation of CLII with periodate. This showed lines at τ 8.73 and 8.67 from the nonequivalent geminal methyl groups adjacent to oxygen and a near-symmetrical pattern centered at τ 7.97 for the 3- and 4-methylenes in the ring. From the structures, that of CL for humulinic acid C follows. Graphical substitution of the signal attributable to the methylene group of the isovaleryl side chain from the spectrum of humulinic acid C left the lines from the methylene group at C_5 (68).

Further, the proton magnetic resonance spectra of humulinic acid D and its methyl ether led to the acid being formulated as CLIV, an intermediate in the formation of humulinic acid C (19).

Further observations on the enolization of cyclopentane triones have been made. Hydrolysis of isohumulinic acid leads to the monoenolic 3-isopentylcyclopentane-1,2,4-trione (68), and in this compound and the 3-methyl analog enolization occurs only in the oxygen functions which flank the alkyl group (120).

The proton magnetic resonance of some model compounds have been measured. In 3-acetyl-5,5-dimethylcyclopentane-1,2,4-trione, the enol signal (τ −2.03) is at the same field as in cohulupone (τ −2 to −3) (27). 2-Acetyl-4-hydroxy-5-methylcyclopentane-1,3-dione represents a simple model of humulinic acid. Oxidation of the form first encountered, mp 112°, with bismuth oxide gave the dienolic 3-acetyl-5-methylcyclopentane-1,2,4-trione which was reduced with sodium borohydride to a second form of the model humulinic acid, mp 81°. These were assumed to be *trans* and *cis* isomers, respectively, as with the parent acids until proton magnetic resonance spectroscopy revealed that both forms were mixtures. The form, mp 112°, consisted of two parts of the *trans* isomer to one part of the *cis*, while the form with mp 81° consisted of two parts of *cis* to one of *trans* (27).

Finally, it may be mentioned that nmr studies have established that phloroglucinol (CLXVI) and its monosodium salt exist in an aromatic structure, while the disodium salt exists as the cyclic tautomer (CLXXXVI) (169).



CLXXXVI

D. MASS SPECTROMETRY

Mass spectrometry was first applied to hop resins by the Swedish workers (55) who recorded the spectra of humulone, lupulone, hulupone, and dihydrohulupone and were thus able to assign structures to the last two compounds. The parent peak was shown strongly by hulupone and lupulone but only very feebly by humulone. The strongest peak in all the spectra was at *m/e* 69 due to the dimethylallyl ion, and peaks at M − 69 were also observed. In the lupulone spectrum peaks at M − 2 × 69 + 1 and M − 2 × 69 − 1 were prominent and the former was also present in hulupone, indicating the presence of two dimethylallyl side chains. The second largest peak in all the spectra was at *m/e* 41 due to the isopropenyl ion. The acyl side was also cleaved in the inlet chamber of the spectrometer giving with humulone and lupulone peaks at *m/e* 85 and M − 85 due to loss of an isovaleryl group, while cohulupone gave peaks at *m/e* 71 and M − 71 due to loss of an isobutyryl group. Reduction of hulupone with sodium borohydride gave a dihydro derivative which was partially dehydrated in the spectrometer as was the hexahydro derivative obtained using Adams catalyst.

The mass spectra of the humulinic acid A, B, C, and D isomers and of the methyl ether of humulinic acid D have been recorded (19). All show the parent peak (*m/e* 266) and peaks at *m/e* 69 and M − 69. Peaks at *m/e* 18 and M − 18, less intense in humulinic acid C, indicate ready dehydration. The mass spectra of hashish constituents have also been studied (60).

VII. Essential Oil Constituents

The composition and influence of the essential oil of hops in brewing have been the subjects of a number of earlier reviews (61, 182, 193, 325).

A. INTRODUCTION

Hops contain 0.5–1.5% of essential oil which can be separated by distillation in steam and recovered from the distillate either by solvent extraction or by the use of an oil trap which allows the aqueous phase to return to the boiler (54, 190, 321, 365, 422). In the first method the oil will contain water-soluble aliphatic acids which are washed out in an oil trap. The bulk of the essential oil is distilled out within 2–4 hr, but small quantities of additional material will separate if the distillation is continued for a further 24 hr; under these conditions significant amounts of the β acids are steam volatile (230, 258). The α acids are also slightly steam volatile but are isomerized during the course of the distillation to isohumulones which are not appreciably steam volatile (230, 258). In the oil which separates during 2–4 hr, steam-distillation compounds usually classified as hop resins are only present in trace amounts, the bulk of the oil being made up of terpene hydrocarbons and oxygenated components.

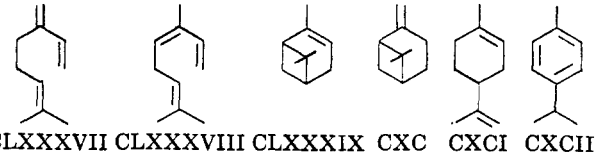
The first systematic examination of the essential oil of hops was due to Chapman (87–89, 91–93) who in the period 1895–1929 described the presence of myrcene, humulene, caryophyllene, and the oxygenated components linalool, geraniol, "luparone," "luparenol," "luparol," and linalyl isononoate. The next important study took place many years later when Šorm and his colleagues (346–348) investigated the oil from Zatec hops and confirmed the presence of myrcene, humulene, and caryophyllene. They also isolated a further hydrocarbon, farnesene, and undecan-2-one (methyl nonyl ketone) which they believed to be identical with Chapman's luparone.

Examination of the essential oil by thin layer chromatography ("chromatoplates") demonstrated that it was more complex than the classical studies indicated. In fact, it was too complex to be resolved on one chromatoplate and preliminary fractionation by countercurrent distribution was necessary before the presence of the compounds listed above could be confirmed, together with twenty unknown constituents (306). This technique has not until recently been applied further to the examination of the essential oil, as it was largely superseded by gas–liquid chromatography (309, 310) first applied to the study of hop oil by Howard (179) in 1956.

Again, the chromatograms obtained were very complex, and, in order to improve the resolution, the essential oil is usually first separated into two fractions by chromatography on silica gel. The first fraction, eluted with light petroleum, consists of the hydrocarbons, while the second, eluted with ether, contains oxygenated components (180, 306). In later studies the oxygenated constituents have been fractionated further on the basis of their functional groups before examination by gas–liquid chromatography (221, 222, 323). The components resolved by gas chromatography have been identified by retention data and, after recovery in a suitable trap, by physical techniques such as infrared and proton magnetic resonance spectroscopy and/or mass spectrometry. The advances in gas chromatographic technique over the past decade have led to the recognition of an increasing number of trace constituents in hop oil (146, 147, 221, 222, 321, 323). The most refined technique used to date consists of gas chromatography using a high-resolution capillary column coupled directly to a time-of-flight mass spectrometer (74, 77–79). It will be convenient to consider the individual fractions separately.

B. HYDROCARBONS

On the chromatograms of the hydrocarbon fraction from all hop varieties examined, the most important monoterpene is myrcene, which may account for 30% of the whole oil (190). It is also found in the essential oil of bay and its structure (CLXXXVII) was elucidated in 1924 (333). Much smaller amounts of other monoterpenes are present and, for example, the essential oil from "Sunshine" hop (V-94) contains at least ten hydrocarbons with retention time comparable to myrcene (188). One of these components was identified on the basis of retention time as ocimene (137, 188), the structure of which (CLXXXVIII) was provided by Sutherland (374). Other monoterpenes identified by retention time and mass spectra include α- (CLXXXIX) and β-pinene (CXC), limonene (CXCI),



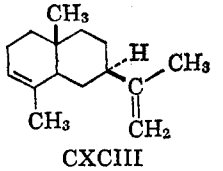
CLXXXVII CLXXXVIII CLXXXIX CXC CXCI CXCII

and *p*-cymene (CXCII) (77–79). By this technique the occurrence of the hydrocarbons pentane, 2-pentene, isoprene, and octane in hop oil has also been demonstrated (77–79).

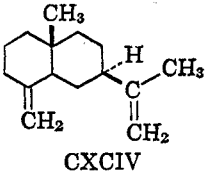
Turning to the sesquiterpenes, the major peaks on the chromatograms are due to farnesene (0–19% of the hop oil), caryophyllene (4–22%), and humulene (8–33%),

which are eluted from Apiezon M in this order at temperatures above 150°. At 100°, however, farnesene is eluted after caryophyllene, and the two components have the same retention time at 125° (320). Farnesene was also eluted after caryophyllene by using temperature programming on a capillary column of Tween −20 (78). Possibly because of this behavior farnesene was not characterized in the earliest gas chromatographic investigations (180) and was confused with isocaryophyllene (190) before final confirmation of its identification (188, 193, 325).

In the oil of some varieties of hops no sesquiterpenes are eluted after humulene (180), but in others one or more "post-humulene" components are observed (188, 319). Howard and Slater noted five compounds eluted after humulene in "Alsace" hops (188), while, by the more refined technique of capillary column gas chromatography and mass spectrometry, twelve unidentified sesquiterpenes have been detected (77–79). Of these, the one eluted immediately after humulene had an infrared spectrum resembling that of α-selinene (CXCIII), but the nmr spectrum was not in complete agreement with this assignment. The oil from the abnormal variety OW-153 contained little humulene and caryophyllene and was rich in a posthumulene component characterized as β-selinene (CXCIV) (365). From Japanese "Shinshu-wase" hops Shigematsu and Kitazawa (339) isolated a sesquiterpene which gave cadalene on dehydrogenation, but the published infrared spectrum of this hydrocarbon is very similar to that of β-selinene (339).

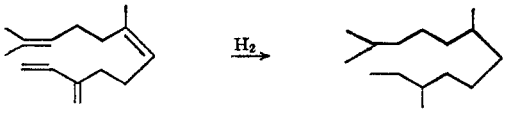


CXCIII



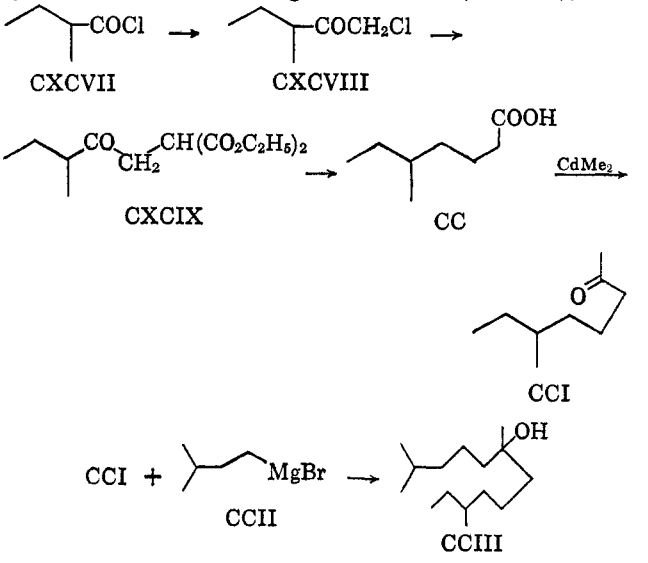
CXCIV

The structure of farnesene (CXCV) followed from ozonolysis which gave formaldehyde (2 moles), acetone (1 mole), and succinic acid (2 moles) (348), and was confirmed by synthesis of 2,6,10-trimethyldodecane (also called octahydrofarnesene) (CXCVI) (346).



CXCV CXCVI

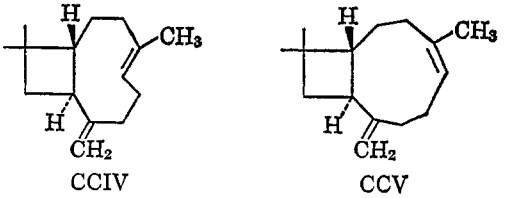
The synthesis started with 2-methylbutyryl chloride (CXCVII) which by treatment with diazomethane and hydrochloric acid gave the chloro ketone (CXCVIII). This in turn was condensed with ethyl malonate and the product (CXCIX) electrolytically reduced. Hydrolysis and decarboxylation then gave 5-methylheptanoic acid (CC) converted to the methyl ketone (CCI) using dimethylcadmium. The methyl ketone (CCI) was then condensed with isohexylmagnesium bromide (CCII) and the resultant tertiary alcohol (CCIII) dehydrated and reduced to give farnesane (CXCVI), identical with that obtained from the natural product. 2,6,10-Trimethyldodecane made by a variation of the above route had a different infrared spectrum, easily differentiated from the 2,6,10 isomer.



CXCVII CXCVIII CXCIX CC CCI CCII CCIII

CCIII $\xrightarrow[\text{ii. } +H_2]{\text{i. } -H_2O}$ CXCVI

Three caryophyllenes, α, β, and γ, were originally isolated from clove bud oil, of which α-caryophyllene is identical with humulene (91, 93) and γ-caryophyllene with isocaryophyllene so that the prefixes have fallen into disuse and the name caryophyllene refers to the β isomer. The long series of investigations leading to structures CCIV for caryophyllene and CCV for isocaryophyllene were carried out on material from clove oil and have been reviewed elsewhere (36, 145, 343) and will not be considered further here. On the other hand, the structure of humulene, which was largely deduced from work carried out on material from hops, will be discussed here.

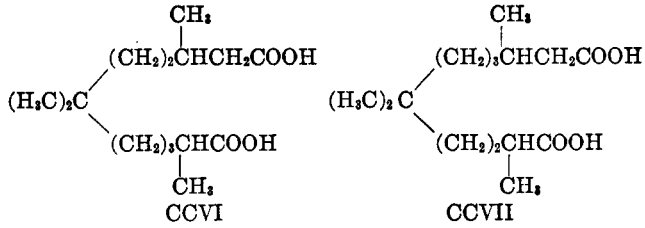


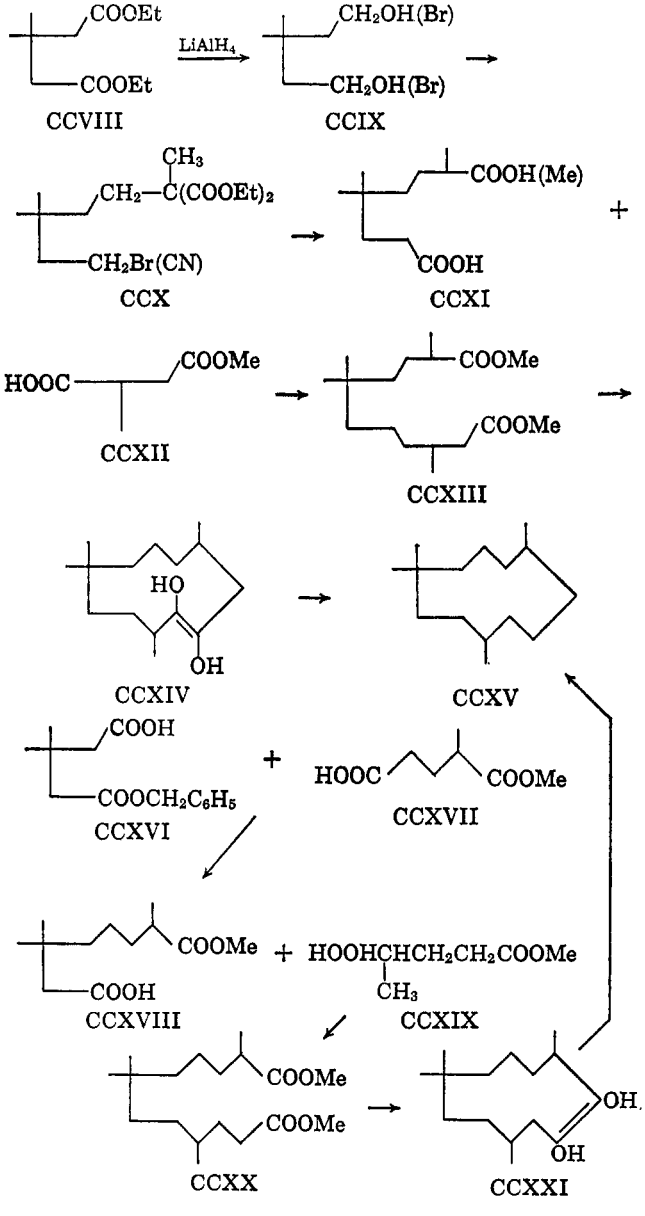
CCIV CCV

Chapman (87–89) first isolated humulene, showed it to have the formula $C_{15}H_{24}$, and prepared a series of derivatives: nitroso chloride, mp 176°; nitrosate, mp 163°; nitrolpiperidide, mp 153; nitrolbenzylamine, mp 136°; nitrosonitrosite, mp 116°; and a dinitrosite, mp 166° dec. Humulene has three double bonds and is therefore monocyclic (99). Ozonolysis gave levulinic aldehyde (or acid), 2,2-dimethylsuccinic acid, and in certain cases formaldehyde (see below).

In a series of investigations, Harris (99, 100, 125–127, 155) reduced the crystalline nitroso chloride to

hexahydroaminohumulene, which by methylation and Hoffman degradation afforded a tetrahydrohumulene. Ozonolysis of this compound gave a C_{15} dibasic acid which was assigned the alternative structures CCVI or CCVII, which found support in synthetic studies (124, 125). This led to the 1,1,4,8-tetramethylcycloundecane structure for humulene (CCXV), independently proposed by Šorm and his colleagues (351). The Czech group confirmed the skeleton assigned to humulene by two syntheses of humulane (349, 350). In the first, reduction of diethyl 3,3-dimethylglutarate (CCVIII)

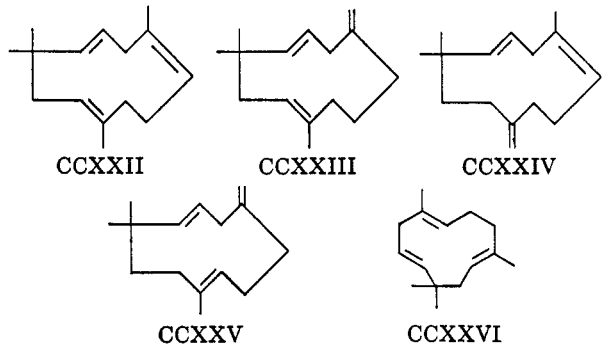




with lithium aluminum hydride gave 3,3-dimethylpentane-1,4-diol (CCIX), the dibromide of which was treated with 0.5 mole of diethyl methylmalonate and the unreacted bromo group condensed with cyanide (CCX). Hydrolysis and decarboxylation then afforded 2,5,5-trimethyloctanedioic acid (CCXI) which was converted to the 1-methyl ester and condensed under Kolbe electrolytic conditions with methyl hydrogen methylsuccinate (CCXII) to give CCXIII. Acyloin condensation of this product gave the enediol (CCXIV) which was reduced to humulane (CCXV).

In the alternative synthesis, 1-benzyl 5-hydrogen 3,3-dimethylglutarate (CCXVI) was condensed with 1-methyl 5-hydrogen 2-methylglutarate (CCXVII) under Kolbe conditions to give, after removal of the benzyl group by hydrogenolysis, the acid (CCXVIII). This by means of a further Kolbe synthesis with 5-methyl 1-hydrogen 2-methylglutarate (CCXIX) gave the diester (CCXX). Acyloin condensation of this ester followed by reduction of CCXXI then afforded humulane (CCXV).

Šorm, *et al.* (349, 350), on the basis of this nucleus and the fragments obtained by oxidation, proposed the structure CCXXIII for humulene itself, but this was regarded as untenable by Fawcett and Harris (127) as it did not explain the formation of their C_{15} dicarboxylic acid. They therefore suggested either CCXXIV "with some co-existent endocyclic form" (CCXXII) or the unique structure CCXXV. Clarke (97) proposed the structure CCXXII but later he and



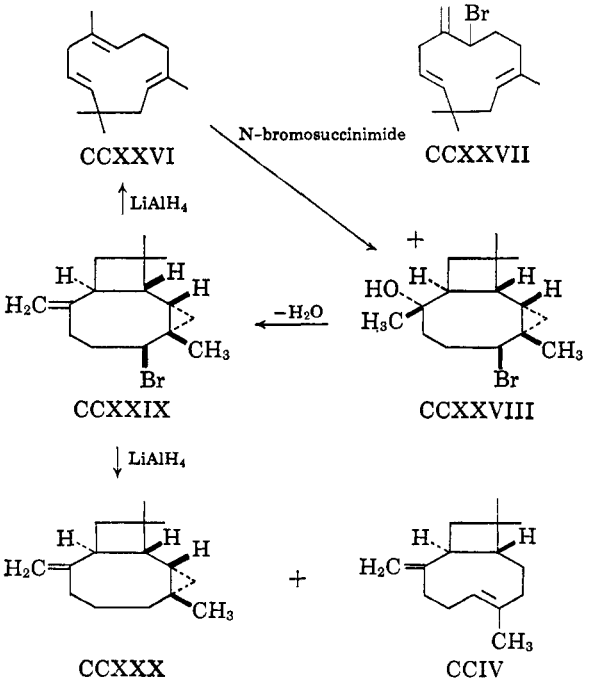
Ramage (98) stated that while the chemical evidence supported CCXXII the infrared spectrum required an exocyclic double bond.

Five years later, Hildebrand, Sutherland, and Waters (172) isolated humulene from *Agonis abnormis* and prepared a crystalline adduct with silver nitrate, mp 175° dec (171). In ozonolysis experiments on humulene purified in this way the ozonide was reduced with lithium aluminum hydride to 1,3-butanediol, 1,4-pentanediol, and 2,2-dimethylbutane-1,4-diol (375). These fragments and quantitative infrared spectroscopy which excluded the presence of an excyclic methylene group led to the structure CCXXII for humulene (172, 375) in full agreement with nmr data (113).

With regard to the stereochemistry of humulene, the compound is undoubtedly derived from the farnesyl cation which has a *trans* central double bond. Hendrickson (168) thus concluded that humulene had the *trans,trans,cis* structure (CCXXII), whereas Sutherland and Waters (375) preferred the all-*trans* structure (CCXXVI). Final evidence in favor of the all-*trans* structure (CCXXVI) was recently provided by mass spectrometry (253) and X-ray crystallography of the silver nitrate adduct (158, 253).

From the essential oil of *Lindera strychnifolia* (E) Šorm, *et al.*, isolated the exocyclic isomer (CCXXIII), β-humulene, which could also be formed from normal α-humulene by contact with alumina (40). Whether this isomer is of widespread occurrence is not known. The mass spectrum of α-humulene from black currants given by Andersson and von Sydow (15) certainly differs from that of α- and β-humulene from grapefruit oil given by Hunter and Brodgen (217), but how far this is due to instrumental differences cannot be assessed.

Very recently the conversion of humulene into caryophyllene has been reported (140). With N-bromosuccinimide in aqueous acetone, humulene (CCXXVI) gives CCXXVII and CCXXVIII, both in about 20% yield. Dehydration of CCXXVIII using phosphorus oxychloride in pyridine gave the methylene tricyclic compound (CCXXIX) which was reduced with lithium hydride to give a mixture of hydrocarbons in 77% yield. Resolution of the mixture by preparative gas chromatography gave the tricyclic hydrocarbon (CCXXX) (50% of the mixture), caryophyllene (CCIV) (30%), and humulene (CCXXVI, possibly contaminated with CCXXIII) (10%).



The hydrocarbons, myrcene, caryophyllene, and humulene (344) have also been found in the essential oil of *Cannabis sativa*, and peaks with the retention times of farnesene and selinene are also present in published chromatograms (257) of this essential oil (325).

C. OXYGENATED COMPONENTS

The first chromatograms of this fraction obtained by Howard (180) showed it to be more complex than the hydrocarbon fraction. Further, whereas, under the conditions that he employed, the hydrocarbon fraction was completely volatile, only about 50% of the oxygenated fraction being eluted; *i.e.*, *ca.* 50% of the material boiled at a higher temperature than humulene. By saponification and methylation of the acid fraction obtained with diazomethane, Howard showed that at least 12 acids were present of which five had retention times identical with those of normal acids with six to ten carbon atoms while the remainder were accounted for as branched-chain components. The nonsaponifiable fraction was not examined in detail but eight components were seen on the chromatogram, one of which was characterized as undecan-2-one (180).

Further study of the low-boiling nonsaponifiable fraction demonstrated the occurrence of methanol and (−)-2-methylbutanol while (+)-2-methylbutyl isobutyrate was isolated from the oxygenated fraction by preparative gas chromatography (197). By a more refined gas chromatographic technique, Roberts (321) showed the presence of 26 oxygenated components, about half of which had retention times greater than humulene. Tentatively identified were 2-methylbutyl isobutyrate, methyl nonoate, methyl decanoate, and undecan-2-one, the last two of which had the same retention time. Attention was drawn to the presence of two peaks due to unsaturated C_{10} methyl esters which had the same retention times as *cis* and *trans* isomers of methyl geranate. In a later paper (323) the carbonyl fraction was isolated by means of Girard's reagent T and at least 30 components were present. Undecan-2-one was, as expected, the major component, but this was accompanied by substantial amounts of nonan-2-one and tridecan-2-one and in one variety there was more of the latter compound than undecan-2-one. From the noncarbonyl fraction, linalool and geraniol and two high-boiling constituents were isolated. However, the essential oil contained little free geraniol which hence must occur in an esterified form. Shigematsu and Kitazawa (339) isolated generanyl isobutyrate from "Shinshu-wase" hops, and a synthetic sample of this ester had an infrared spectrum identical with a component isolated by Roberts (327).

The next advance was provided by Jahnsen (221, 222), who used temperature-programmed gas chromatography after subjecting the oil to a more systematic chemical fractionation. The oxygenated components

were treated with Girard's reagent T to remove the carbonyl constituents, and then the free alcohols separated from the esters in the noncarbonyl fraction by chromatography on alumina. The ester fraction was then subjected to methanolysis and by further chromatography separated from the liberated alcohols. The methyl esters were divided further into two fractions by complex formation with urea, and the saturated methyl esters were obtained from the mixtures by bromination.

Examination of the carbonyl fraction so obtained by temperature-programmed gas chromatography showed the occurrence of a whole homologous series of methyl ketones from acetone to hexadecanone and a similar series of normal aldehydes from propyl to undecyl. Evidence was also obtained for eight branched ketones and several unsaturated and branched aldehydes including citral. From the alcohol fraction the major constituents were 2-methylbutanol and linalool with lesser amounts of geraniol, nerolidol, nerol, and terpineol. Evidence was also obtained for the occurrence of a series of normal alcohols with 5–11 carbon atoms. The ester fraction was very complex; tentatively identified were linalyl acetate and the formate, acetate, and isobutyrate of geraniol. After methanolysis about 60 peaks were recognized on the chromatogram of the methyl esters. Normal and iso acids from butyric to hexadecanoic were identified together with numerous branched unsaturated acids, including the two regarded as the isomeric forms of methyl geranate.

Jahnsen agreed with this identification, but later he and the Western Regional Laboratory group separated these two components by preparative gas chromatography and showed by analysis, ozonolysis, and nuclear magnetic resonance spectroscopy that they were methyl dec-4-enoate (CCXXXI) and methyl deca-4,8-dienoate (CCXXXII) (76).

$$CH_3[CH_2]_4CH{=}CHCH_2CH_2COOCH_3$$
CCXXXI

$$CH_3CH{=}CH[CH_2]_2CH{=}CHCH_2CH_2COOCH_3$$
CCXXXII

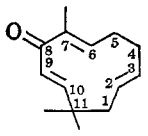
Roberts and Stevens (327) also isolated these two components and showed that the infrared spectrum did not agree with that of authentic methyl geranate. Hydrogenation of the mixture gave 80% of methyl decanoate and 20% of material with the same retention time as methyl 3,7-dimethyloctanoate obtained by hydrogenation of methyl geranate. They therefore concluded that traces of methyl geranate were present in the oxygenated fraction. This would also be expected from the co-occurrence of geraniol and citral.

To return to the classic examination of hop oil by combined gas capillary column gas chromatography and mass spectrometry (74, 77–79), the esters originally identified in this way included 2-methylpropyl isobutyrate, 2-methylbutyl isobutyrate, methyl heptanoate, methyl hept-4-enoate, methyl 6-methylheptanoate, methyl octanoate, methyl nonanoate, methyl octenoate, methyl deca-4-enoate, methyl deca-4,8-dienoate, geranyl acetate, geranyl propionate, and geranyl isobutyrate.

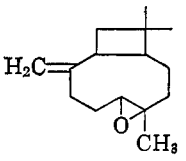
In two subsequent notes (74, 75) identifications of additional components of the oxygenated fraction have been reported. The chromatogram showed 86 peaks of which 44 have been definitely characterized and the structures of a further 30 components have been predicted from the mass spectra (74). In addition to the compounds already mentioned the following esters were characterized by comparison of the infrared and mass spectra with those of authentic samples: methyl hexanoate, butyl isobutyrate, 2-methylpropyl propionate, methyl 5-methylhexanoate, 2-methylpropyl 2-methylbutyrate, 3-methylbutyl isobutyrate, methyl 4-methylhex-2-enoate, pentyl isobutyrate, methyl thiohexanoate, ethyl heptanoate, hexyl propionate, 2-methylbutyl isovalerate, 2-methylbutyl 2-methylbutyrate, hexyl isobutyrate, methyl thioheptanoate, octyl acetate, heptyl isobutyrate, 2-methylbutyl hexanoate, octyl isobutyrate, neryl acetate, methyl undecanoate, neryl propionate, neryl isobutyrate, and methyl dodecanoate (74). Compounds predicted on the basis of the mass spectra include methyl thio-2-methylbutyrate, 2-methylpropyl (branched) pentenoate, methyl (branched) thiohexanoate, pentenyl isobutyrate, methyl 2,5-dimethylhexanoate, methyl 6-methylheptanoate, 2-methyl-5-pentenylfuran, methyl thioisoheptanoate, methyl (branched) nonanoate, methyl nonenoate, methyl 2- and 8-methylnonanoate, methyl decenoate, methylbutyl heptanoate, methyl undecenoate (four isomers), methyl 9-methyldecanoate, methyl undecadienoate, methyl dodecanoate (branched), methyl dodeca-8-enoate, methyl dodecadienoate, linalyl propionate, methyl dodecenoate, and two isomers of methyl tridecenoate (74). The carbonyl components revealed by this technique include, acetone, 2-nonanone, 2-decanone, 2-undecanone, 2-tridecanone, 2-tetradecanone, 2-pentadecanone (77–79), tetradec-9-en-2-one, and pentadeca-6,9-dien-2-one (74).

While there seems little doubt that this technique will eventually lead to a complete proximate analysis of hop oil, it has not yet provided much information about the occurrence of oxygenated sesquiterpenoids no doubt on account of their high boiling points and retention times. Roberts found that the majority of components boiling higher than humulene were due to unsaponifiable constituents (321). It had been observed earlier by Howard and Slater that as hops aged the oil became richer in oxygenated components (190), and Roberts was able to show that autoxidation products of caryophyllene and humulene were present in the oxygenated fraction (323). No evidence was found for

the occurrence of zerumbone (CCXXXIII), a constituent of the oil of wild ginger (*Zingiber zerumbet*) shown to have the structure and stereochemistry of 8-oxohumulene (2,6,9,9-tetramethyl-2,6,10-cycloundecan-

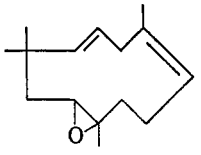


CCXXXIII

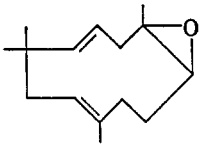


CCXXXIV

trien-1-one) (106, 112), but peak 18 was identified as being due to caryophyllene epoxide (CCXXXIV). This compound was previously shown by Treibs (377) to be a constituent of clove oil and the primary product of autoxidation of caryophyllene. It was also formed by treatment of caryophyllene with peracids, and similar treatment of humulene gave a monoepoxide with the same retention time as peak 20. The isolation of these two components by preparative gas chromatography was unsuccessful because of decomposition of the compounds in the injection flash heater (323). The isolation of humulene mono- and diepoxide from the essential oil of *Zingiber zerumbet* was reported (283), but it was later found that the first of these products was heterogeneous (105). By a combination of fractionation and column chromatography over neutral alumina and over silica gel–silver nitrate, it was separated into caryo-

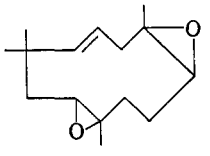


CCXXXV

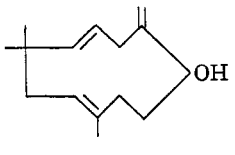


CCXXXVI

phyllene epoxide and two humulene epoxides, CCXXXV and CCXXXVI, in approximately equal amounts. Both humulene epoxides on treatment with perbenzoic acid gave humulene trioxide, and the respective structures were assigned on the basis of infrared and proton magnetic spectroscopy; that of CCXXXVI was confirmed by ozonolysis to *as*-dimethylsuccinic acid (105). Humulene diepoxide, mp 105°, contained a disubstituted double bond and must therefore have structure CCXXXVII (283). Damodaran and Dev (105) also isolated from *Zingiber zerumbet* a sesquiterpenoid alcohol which they called humulenol, shown to have the allylic structure CCXXXVIII; it was also obtained from the humulene epoxide (CCXXXVI) by treatment with alumina (Brockman scale I). It seems probable that these



CCXXXVII



CCXXXVIII

oxidation products of humulene will also be found in hop oil.

Flavor and Detection of Hop Oil Constituents in Beer.— By definition the essential oil of hops is volatile in steam and under normal brewing conditions will largely be lost from the beer. To obviate this some brewers add a portion of their choicest hops, as judged by aroma, at the end of the boil, either in the kettle or hop back. British brewers practice "dry hopping" in which hops are added to beer in the cask or conditioning tank. This may be advantageous as hop oil constituents may be more soluble in a dilute alcoholic solution (beer) than water, but, whatever brewing conditions are used, the amount of hop oil components in beer is very small.

By tasting trials Howard and Stevens (196) found that 1.0 ppm of the essential oil can be detected by taste in water, but 3.0 ppm were necessary for detection in unhopped beer bittered with isohumulone. The oxygenated fraction was found to be a more potent flavoring agent than the hydrocarbon fraction. In water, 1 ppm of the hydrocarbon fraction was just detected while all the tasters could pick out 1 ppm and a significant number 0.1 ppm of the oxygenated fraction; 1 ppm of myrcene, however, was unacceptable, a result also found by Rigby (300). Schilfarth (337), on the other hand, reported that 0.1–1.0 ppm of sesquiterpene hydrocarbons imparted a pleasant aroma to beer. In view of the low concentrations necessary to influence flavor it is not surprising that early attempts to isolate hop oil components from beer were not successful.

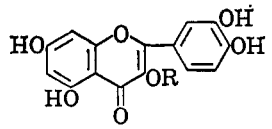
To a group of Australian workers goes the distinction of being the first to demonstrate the occurrence of hop oil in beer (146, 147). Starting with 400 l. of beer they obtained 57 g of neutral material soluble in ether which largely consisted of the higher alcohols and esters produced during fermentation. After removal of these components by careful fractionation, only 1.2 ml of steam-volatile material remained which, when examined by gas chromatography, showed clearly the presence of myrcene (0.5 ppm) and sesquiterpene hydrocarbons (0.75–1.0 ppm) at the concentrations indicated in the original beer.

In a later study Likens and Nickerson (248) designed a special extraction apparatus so that the steam distillate could be extracted with an immiscible solvent. By means of this apparatus they were able to demonstrate good recoveries from 0.5 ppm of hop oil. They were thus able to demonstrate the presence of myrcene, humulene, methyl dec-4-enoate, methyl deca-4,8-dienoate, and undecan-2-one in hopped wort but, with one exception, not in experimental or retail beers. In the exception, a beer with a strong hop aroma, all the above components were found together with caryophyllene and unidentified compounds. In this beer, both hydrocarbon and oxygenated components were present at concentrations above the threshold values found by

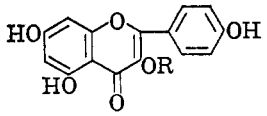
Howard and Stevens (196), and Likens and Nickerson (248) concluded that in the remaining beers there was little likelihood that hop oil components (in their original form) exert an influence on the flavor of finished beer. This may be too facile a view as the additive effect of subthreshold concentrations of certain organic compounds has been demonstrated (143), and thus the flavor of beer may be due to synergism between subthreshold levels of hop oil components and volatile products of fermentation.

VIII. Polyphenolic Constituents (Tannins)

The importance of this group of hop compounds in the brewing process is due not only to their contribution to flavor (*cf.* ref 196) but also to their part in the production, by protein–polyphenol interactions, of nonbiological haze, which limits the shelf life of bottled beers. It has been estimated that hops contain 2–4% of tannin. After removal of the hop resins by ether extraction. Harris (149) found the optimum yield of tannin was obtained by extraction with 70% ethanol. Examination of this and other fractions by two-dimensional paper chromatography demonstrated the presence of at least 77 components (149). Long before this, however, in 1820, gallic acid was shown to be present in hop tannin (219) and not much later, in 1859, quercetin (CCXXXIX, R = H) was characterized (399).



CCXXXIX



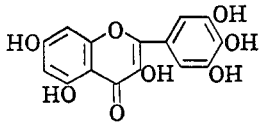
CCXL

The presence of gallic acid was later confirmed (149) and other phenolic acids identified include protocatechuic acid (149), *p*-hydroxycoumaric acid (38), and caffeic acid (38, 149). Chlorogenic acid, neochlorogenic acid, and a third component, which gave caffeic and quinic acid on hydrolysis, were also observed (149). In contrast, only *p*-coumaric and ferulic acids were detected in the leaves of *Cannabis sativa* (38). Harris observed 18 compounds on his chromatographs of hop tannin which gave color reactions indicative of flavonols. After hydrolysis many of these disappeared, but the spots due to quercetin, kaemferol, and myricetin intensified. He was thus able to identify quercitrin (CCXXXIX, R = rhamnosyl), isoquercitrin (CCXXXIX, R = glucosyl), and rutin (CCXXXIX, R = rutinosyl), three compounds found earlier in Japanese hops (272, 378). He also made tentative identification of kaempferitrin (CCXL, R = rhamnosidorhamnosyl) and myricitrin (CCXLI, R = rhamnosyl).

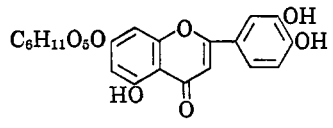
Further studies of the flavonol glucosides in hops were reported by Lebreton (244), Hubáček and Trojna (208, 209), and Bhandari (42). Lebreton hydrolyzed the mixture of flavonoids and identified quercetin (CCXXXIX, R = H), kaempferol (CCXL, R = H), glucose, and rhamnose in the hydrolysate (244). In a similar way, Bate Smith (38) examined the leaves of *H. lupulus* and *C. sativa* and found that while hops contained quercetin, kaempferol, and myricetin (CCXXLI, R = H), only traces of kaempferol were present in *Cannabis*. The Czech workers (208, 209) by paper chromatography confirmed the presence of isoquercitrin and rutin, and showed the presence of a 3-rhamnodiglucoside, a 3-rhamnoglucoside, and a 3-glucoside (astragalin) of kaempferol, and a 3-rhamnodiglucoside of quercetin. By thin layer chromatography Bhandari (42) isolated isoquercitrin, rutin, and astragalin.

Advance notice of a communication of Vancraenenbroeck, Vanclef, and Lontie (379) reports the presence of two 3-monoglucosides of quercetin, one with the sugar in the furanose form and the other in the pyranose form, and a similar pair of 3-monoglucosides of kaempferol. The 3-rhamnoglucosides of quercetin and kaempferol were also found together with a glucoside of phlorisobutyrophenone which is of especial interest with regard to the biogenesis of the hop resins (see later).

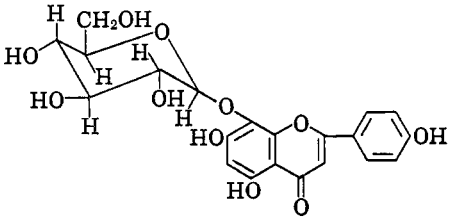
As mentioned above, Japanese hops contain quercitrin, isoquercitrin, and rutin (378). In contrast, the leaves of *Humulus japonicus* contain glucoluteolin (luteolin 7-glucoside) (CCXLII) (163), cosmosiin, which is a glucoside of apigenin (25), and vitexin (25), the structure of which (CCXLIII) has been settled recently (176).



CCXLI

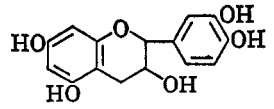


CCXLII



CCXLIII

Closely related to flavonols, the presence of catachin (CCXLIV) and epicatachin in hops has been established (153), but more important from the point of view of nonbiological haze are the leucoanthocyanins (anthocyanogens (153)). Many groups of workers have demonstrated the presence of substances which, on acid treatment, give rise to the anthocyanidin pigments cyanidin and delphinidin (37–39, 149, 232, 251, 282). Although it generally has been accepted that leucoanthocyanins derived from malt are more important than those from hops in haze formation, Gaeng and



CCXLIV

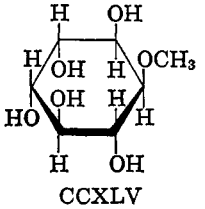
Delizée claim that in certain cases they are of equal importance (133). The structures of the hop and malt leucoanthocyanins which have similar chromatographic properties have not been elucidated. They are extremely labile substances converted even by atmospheric acidity into polymeric substances, while stronger acid gives the anthocyanidin pigments.

Harris and Ricketts (153) pointed out that most of these leucoanthocyanins were not true leuco compounds. They therefore suggested that the term "leucoanthocyanin" should be reversed for derivatives of Δ^3-flaven-3-ol, and the term "anthocyanogen" should be used to describe other compounds capable of forming anthocyanidins on treatment with acid. This has been largely adopted in the literature of the brewing industry, but elsewhere the synonymous term "proanthocyanidin" suggested about the same time by Freudenberg and Weinges has had wider acceptance.

Small amounts of anthocyanins themselves are found in the seeds of hops (149, 251).

IX. Carbohydrates

The characterization of glucose (or fructose) in hops, as the ozazone, was first accomplished in 1913 by Power, Tutin, and Rogerson (280). Later, MacWilliam (254) carried out the first chromatographic examination of the hop sugars and isolated the following sugars: fructose (0.51%), glucose (0.41%), sucrose (0.22%), raffinose (0.09%), stachyose (0.06%), and an unknown sugar (0.06%) (figures in parentheses refer to per cent in dry hops *var.* Early Choice). He also found that hops contained 1–2% of pectin (255). Hydrolysis of hop pectin afforded galacturonic acid, galactose, and arabinose, indicating that the fractions were contaminated with araban and galactan. On the basis of viscosity measurements the molecular weight of purified hop pectin samples was only one-tenth of that of apple pectin (255). The inositol derivative, quebrachitol (CCXLV), was found in the leaves of *Humulus lupulus* (0.035%),



CCXLV

by Plouvier (278), and it is noteworthy that it had been found earlier in *Cannabis sativa* (7), thus providing a further biochemical connection between the two species. Vitamin C has also been found in hop leaves (227).

X. Nitrogenous Constituents

Hops contain 2.0–3.5% of nitrogen of which about 0.5% is in a soluble form. As long ago as 1874 the presence of an alkaloid resembling coniine was suspected (142), and although later workers confirmed this observation (90, 280) it has not been characterized. The first systematic examination of the nitrogenous constituents of the hop cone was made by Chapman in 1914 (90) who isolated betaine, adenine, hypoxanthine, choline, and the amino acids histidine arginine, and asparagine. Of these, choline (141) and asparagine (62, 280) had been isolated earlier. With the advent of paper chromatography Harris (148) reexamined the soluble nitrogen fraction of commercial hops and found the following amino acids: alanine, γ-aminobutyric acid, arginine, asparagine, aspartic acid, cystine, glutamic acid, glutamine, glycine, histidine, isoleucine, leucine, lysine, phenylalanine, proline, serine, threonine, tyrosine, tryptophane, and valine. The absence of methionine was noteworthy as was the presence of an unknown amino acid, which was characterized as L-(−)-pipecolic acid (151, 152). This amino acid was also found in germinating barley and almost simultaneously found in Rhodesian teak, beans, apples, potatoes, and an edible mushroom (*Psalliota campestris*), and has subsequently been found in many plant species.

Harris and Tachell (154) later investigated the amino acids present in green, unkilned hops and found, in addition to those amino acids listed above, β-alanine, α-aminoadipic acid, γ-methyleneglutamic acid, and γ-methyleneglutamine. The last two compounds, having previously been found only in groundnut seedlings (114) and tulip leaves, could not be found in kilned hops. Mori (266) has investigated the changes in amino acid composition in the ripening hop cone and reports that leucine disappeared at the time resin was first formed.

Other nitrogenous compounds isolated from hops included riboflavin (401) and histamine, the amount of the latter being 30–40 μg/g (398). The seeds of hops contain 32% of protein (326) while those of *Humulus scandens* (Lour.) Merr. have 24% (118).

XI. Lipids

The lipids present in hops consist of hydrocarbons, triglycerides, and hop wax. Lermer (246) obtained a wax-like substance which he regarded as myristyl palmitate, but this identification could not be confirmed by Power, Tutin, and Rogerson (280). Instead, they characterized hentriacontane, ceryl alcohol, cerotic acid, β-sitosterol, and β-sitosteryl glucoside. A crystalline hydrocarbon similar to hentriacontane was deposited when certain samples of the essential oil of hops were stored at 0° (190), but analytical data were in better agreement with triacontane than the C_{31} hydro-

carbon. A similar hydrocarbon together with ceryl alcohol and sitosterol were isolated by ion-exchange chromatography (194). A more extensive study of hop wax has been made in Australia (95) and by Czech workers (224, 417).

Hop wax is deposited as a dark green solid from a cold methanolic extract of hops (1.35%) (95). The quantitative recovery of the wax is difficult. Thus, if the wax is removed from a cold methanolic solution and α acids are precipitated from the filtrate as their lead salts, these salts, and the α acids on regeneration, will still be contaminated with wax. Two further precipitations and regeneration of the α acids fail to remove the last traces of wax, and the α acids are only obtained pure through the complex with 1,2-diaminobenzene (95).

Examination of hop wax by gas–liquid chromatography, on a column of 25% silicone oil at 260°, showed the presence of 17 peaks due to hydrocarbons. The hydrocarbon fraction of the wax, when separated by chromatography on dehydrated silica gel, gave the same gas chromatographic pattern. None of the hydrocarbons were identified (95), but by comparison with the data of Kranz, *et al.* (234), it was suggested that these hydrocarbons could correspond *inter alia* with triacontane and hentriacontane (366).

The results of the Czech investigation have recently been published (417) after a preliminary communication in which they announced the occurrence of dimethyl paraffins in hop and other waxes (224). From the wax of Saaz hops they separated the hydrocarbon fraction (7.9%) by chromatography on silica gel and examined it further by gas–liquid chromatography on Apiezon L (417); 47 peaks were characterized of which a homologous series of *n*-alkanes from C_{12} to C_{33} accounted for 98.7% of the hydrocarbon fraction. Of these, nonacosane (52.4% of the hydrocarbon fraction), hentriacontane (22.2%), heptacosane (8.7%), triacontane (3.5%), and pentacosane (3.1%) were present in the greatest amount. Smaller amounts (1.28%) of homologous isoparaffins from C_{13} to C_{33} were present together with traces (0.02%) of the C_{18}, C_{19}, and C_{23} diisoparaffins. No unsaturated hydrocarbons were found (417).

The oxygenated components of hop wax were not volatile in the gas chromatographic system mentioned above (25% silicone oil at 260°), and only the hydrocarbons were volatile from the neutral fraction obtained after saponification (95). It is noteworthy that myristyl alcohol, if present, should be volatile under these conditions. The neutral fraction after saponification was examined on a 3% silicone rubber SE-30 column at 220° when seven compounds with retention times comparable to cholesterol were observed (95). It has been suggested that squalene and β-sitosterol could account for two of the unidentified peaks (325). Hop wax would thus appear to consist largely of sterol esters.

The acid fraction obtained by saponification of hop wax contained the following acids: formic, acetic, propionic, butyric, isobutyric, valeric, hexanoic, heptanoic, octanoic, nonanoic, decanoic, dodecanoic, tetradecanoic, hexadecanoic, octadecanoic, oleic, and linoleic acids together with 16 which were not identified (95).

Fertilized hops contain 18–30% w/w of seeds which yield about 32% of a liquid seed fat when the ground seeds are extracted with light petroleum. Only traces of this seed fat are liberated when intact seeds are boiled either with wort or with light petroleum. The seed fat is relatively unstable, and after only 2 weeks at 0° it forms a skin and develops a racid odor (326). The fatty acid composition of the seed fat of English Goldings hops is given in Table IV, together with data given for *Humulus scandens* (Lour.) Merr. (118) and the range of values found for the seed fat of *Cannabis sativa* (hemp) (173). The over-all similarity of the composition of these seed fats is in agreement with the botanical relationship of the three species. It is noteworthy that of the fatty acids present in hop seed fat, hexadecanoic, octadecanoic, oleic, and linoleic acids have already been encountered among the saponification products of hop wax and together with linolenic acid have been found in beer (32, 94). It is not suggested, however, that hydrolysis of hop wax (or seed fat) is the major source of these acids in beer (95).

TABLE IV
ANALYSES OF HOP AND HEMP SEED FAT (%)

Fatty acid	*Humulus lupulus* (326)	*Humulus scandens* (118)	*Cannabis sativa* (173)
16:0 (hexadecanoic)	7	14	5.8–9.9
18:0 (octadecanoic)	3		1.7–5.6
18:1 (9) (oleic)	10	15	6–17
18:2 (9, 12) (linoleic)	60	54	46–70
18:3 (6, 9, 12)	5	...	...
18:3 (9, 16, 15) (linolenic)	15	13	14–28
18:4	Trace	...	...
20:0 (eicosanic)	...	...	1.0–1.9

XII. DEVELOPMENT AND BIOGENESIS OF HOP CONSTITUENTS

The development of the hop resins and essential oil components during maturation of the hop cone has been the subject of a number of investigations (31, 33, 122, 206, 324, 393, 404–406) and has been reviewed (371).

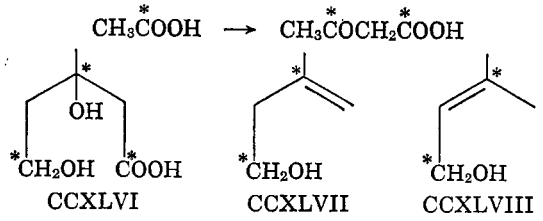
With regard to the resins it is now well established that the lupulones appear a few days before the humulones (33, 324, 404–406), but both are present at a very early stage and are subsequently produced at about the

same rate (206). ^{14}C-Labeled carbon dioxide is incorporated into both humulones and lupulones throughout the ripening process although in an over-ripe hop only labeled lupulone was produced (122). It is noteworthy that the different analogs develop at different rates. In very immature hops the percentage of the co-component in both α and β acids was much lower than that in normal ripe hops, although the relationship (198)

$$\%\ \text{colupulone in}\ \beta\ \text{acid} = 20.2 + 0.943(\%\ \text{cohumulone in}\ \alpha\ \text{acid})$$

appears to hold throughout the growing period (206). After about August 20, under English conditions, the proportion of the co-components remained constant (206). Similarly, it was found in the United States that the incorporation of $^{14}CO_2$ into cohumulone (25.5–32.9%), humulone (48.1–54.6%), and adhumulone (19.0–22.5%) remained constant over the period August 18–29 although the synthesis of co- and adhumulone had ceased by September 3 (122). These results do not lend support to the view that the α acids are formed by oxidation (or photolysis) of the β acids (33, 371, 404, 406) but rather that the α and β acids are formed from a common precursor. Support for the latter view came with the isolation of desoxyhumulone (XXXV) from hops (292, 293). Oxidation of this compound affords humulone while condensation with a further isoprene residue gives lupulone.

Birch (45) has proposed that phloroglucinol derivatives are formed by cyclization of a poly-β-keto acid formed by head-to-tail linkage of acetyl fragments. Further modification of the basic skeleton can then be achieved by methylation or, as in the case of the hop resins, by condensation with an isoprene residue. According to modern views the isoprene residue will also be produced from acetic acid *via* acetoacetate and mevalonic acid (CCXLVI). Dehydration and decarboxylation of mevalonic acid 5-pyrophosphate then gives isopentenyl pyrophosphate (CCXLVII), and it is this or the isomeric γ,γ-dimethylallyl pyrophosphate (CCXLVIII) which is the "active isoprene" unit.

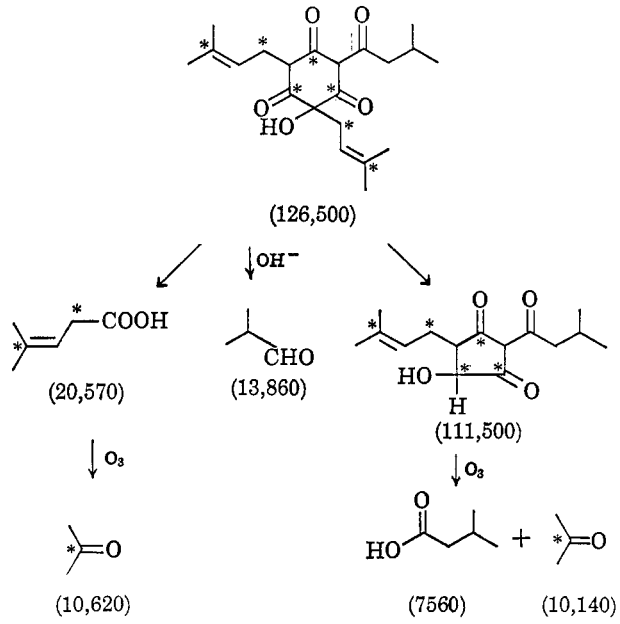


CCXLVI CCXLVII CCXLVIII

Support for these biogenetic pathways was obtained by degradation of the humulone formed after injection of $CH_3{}^{14}COONa$ into a hop bine. Wright and Howard (421) carried out the degradations (Scheme II) and found that the ^{14}C was strongly incorporated into the phloroglucinol nucleus and to a lesser extent into the isoprenyl side chains; little activity was found in the acyl side chain. These results support the view that the phloroglucinol nucleus is readily formed from acetyl fragments and that acetate is also incorporated into the isoprene residues by a longer biochemical pathway. It is suggested that the acyl side chain arises from intermediates involved in amino acid synthesis; thus leucine intermediates give rise to humulone and lupulone, isoleucine to adhumulone, and valine to cohumulone, etc. The incorporation of ^{14}C-leucine into hop resins has been demonstrated (267). It is not known whether the acyl group is attached before cyclization of the polyacetate or after,

SCHEME II

DEGRADATION OF ^{14}C-LABELED HUMULONE (421)



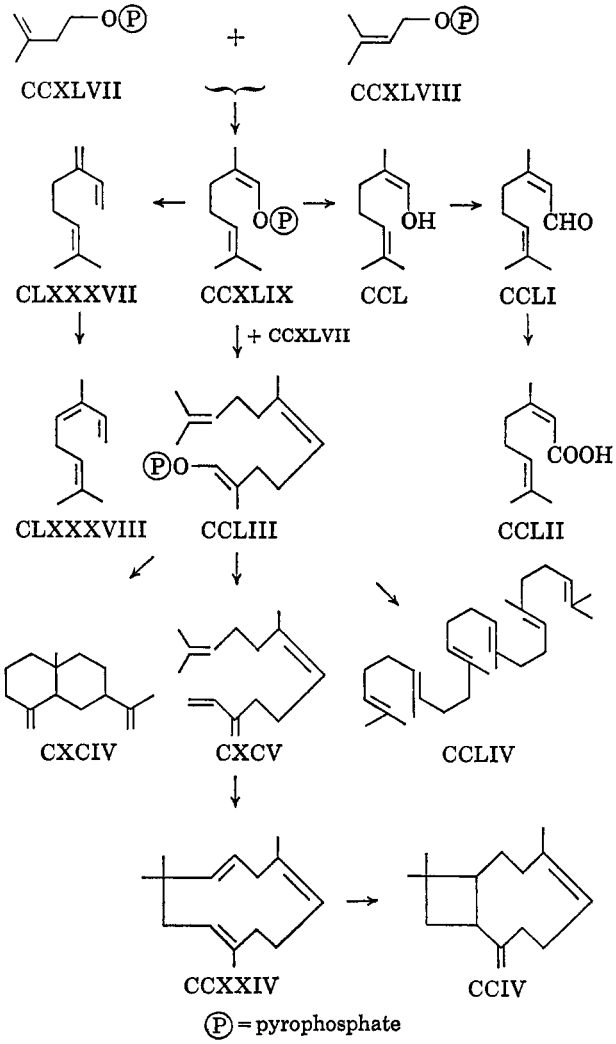
and, if after, whether before or after isoprenylation. The isolation of phlorisobutyrophenone glucoside from hops (379) suggests that the acyl group is present before isoprenylation, and the failure to detect free phloroglucinol (or the glucoside) (65) implies that the phloracylphenone is formed by cyclization of an acylpolyacetate.

The development of the essential oil constituents and the influence of kilning was investigated by Howard and Slater (192). At the earliest stage of development only traces of oxygenated material are present, and these develop almost to their final level before hydrocarbon synthesis begins. The hydrocarbon first produced is rich in humulene and contains relatively little myrcene. As ripening proceeds the proportion of humulene falls and that of myrcene increases so that in the final stages of ripening the synthesis of myrcene is quantitatively the most important process taking place. Similar observations were made by Bullis and Likens (61). During the kilning process some essential

oil is lost but the over-all composition does not appear to change, suggesting that oil is only lost from ruptured lupulin glands.

No radiochemical studies have been carried out on the development of essential oil components. Roberts and Stevens (325) reviewed current biogenetic theory and showed how it could explain the biogenesis of the essential oil. The condensation of isopentenyl pyrophosphate (CCXLVII) with γ,γ-dimethylallyl phosphate (CCXLVIII) leads to geranyl pyrophosphate (CCXLIX). Elimination of pyrophosphoric acid from this reactive intermediate will give rise to myrcene (CLXXXVII) and ocimene (CLXXXVIII), while transesterification can produce the formyl, acetyl, propionyl, and isobutyryl esters of geraniol (CCL) which are found. Oxidation can give citral (CCLI) and geranic acid (CCLII).

Further condensation of geranyl pyrophosphate (CCXLIX) with isopentenyl pyrophosphate (CCXLVII) gives farnesyl pyrophosphate (CCLIII), the parent compound of farnesene (CXCV), humulene (CCXXIV), caryophyllene (CCIV), and selinene (CXCIV). Head-to-head linkage of two molecules of farnesyl pyrophosphate leads to squalene (CCLIV) and the sterol nucleus.

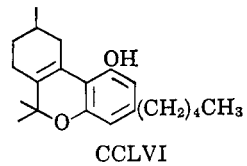


It is noteworthy that resin synthesis is almost complete before the synthesis of the essential oil starts (61, 325); *i.e.*, when the plant has isoprene residues surplus to the requirements of resin synthesis, they combine together to form terpene hydrocarbons. At first sesquiterpenes are elaborated, but as ripening proceeds only monoterpenes are found. A comparison of the development of resins of essential oils in seeded and seedless hops, grown as far as possible under identical conditions, showed that seedless hops produced 20% more α acid than seeded ones and twice as much essential oil. There was no corresponding increase in the β-acid fraction and resin synthesis was complete at the same time in seeded and seedless hops. On the other hand, the production of essential oil in the seedless hops continued for a further week after it was complete in the seeded samples (157).

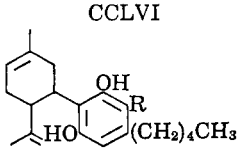
XIII. Hashish Constituents

Mention has been made earlier of the botanical relationship between hops and *Cannabis sativa* L., and similarities in the seed fat and essential oil of the two species have been discussed. The resins of the two plants, however, are completely distinct, but brief mention will be made here of the resins of *C. sativa* which contain the active constituents of the drug known variously as hashish, charus, ganja, bhang, kif, or marihuana. Earlier work has been well reviewed (2, 115, 376) and the report of a recent symposium has appeared (418).

The first homogeneous compound to be described was cannabinol whose structure (CCLV) was confirmed by two independent synthesis (3, 136). Neither this compound nor cannabidiol subsequently isolated (4, 220) showed any psychotomimetic activity, but an intermediate in the synthesis of cannabinol, tetrahydrocannabinol (CCLVI), had such activity, although it was not so active as the natural product. It was therefore thought that the active principles of hashish consisted of a mixture of isomeric tetrahydrocannabinols (376). Confirmation of this view came from the production of active principles by acid treatment of cannabidiol (6). This compound was originally written as CCLVII (R = H) (5), but recently



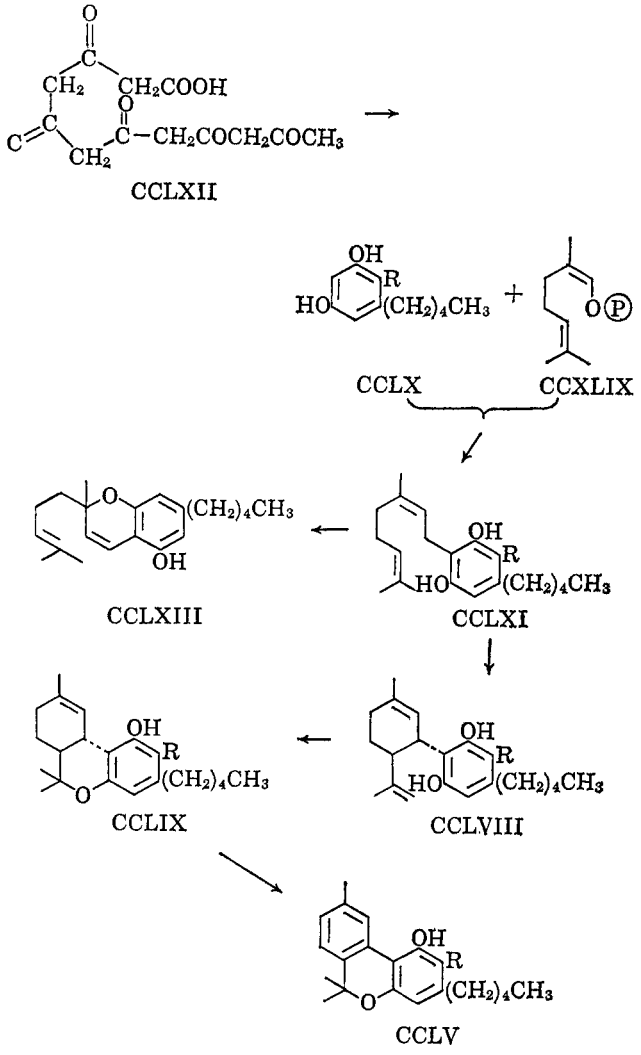
CCLVI



CCLVII

Mechoulam and his colleagues have shown the correct structure is CCLVIII (R = H) (260). They have also isolated from hashish a crystalline derivative of tetrahydrocannabinol which has very high activity (CCLIX, R = H) and can be formed from cannabidiol (CCLVIII, R = H) under very mild acidic conditions (135).

These structures receive support from biosynthetic theory. It was postulated (220, 407) that the hashish resins were formed from olivil (CCLX, R = H) and geranyl pyrophosphate (CCXLIX) and this view was largely confirmed with the isolation of cannabigerol (CCLXI), the primary condensation product (134).



The first compound to be characterized from the acidic fraction of hashish resin was cannabidiolic acid (235, 236, 338) originally thought to be CCLVII (R = COOH), but this structure was later revised to CCLVIII (R = COOH) (259). Subsequent work has led to the isolation of cannabigerolic acid (CCLXI, R = COOH) and cannabinolic acid (CCLV, R = COOH) (259), although tetrahydrocannabinolic acid (CCLIX, R = COOH) has not yet been characterized.

Returning to the biosynthesis of hashish resins, the formation of olivil can be readily explained by the cyclization, reduction, and decarboxylation of the polyketo acid (CCLXII), and condensation of the cyclized and reduced acid (CCLX, R = COOH) with geranyl pyrophosphate will lead to cannabigerolic acid (CCLXI, R = COOH), cannabidiolic acid (CCLVIII, R = COOH), and cannabinolic acid (CCLV, R = COOH).

Thus, it would appear that both hop and hashish resins arise by a polyacetate pathway followed by condensation with isoprenoid fragments. Except in this broad outline, however, there is little similarity. Hashish resins are formed solely from the linear polyacetate (CCLXII), while hop resins utilize a shorter polyacetate chain but require the acyl side chain from another source. The basic nucleus of the hop resins is then substituted with one or more molecules of γ,γ-dimethylallyl phosphate while hemp resins use only 1 mole of the diisoprenoid, geranyl pyrophosphate, to give a product which then undergoes further cyclization.

Very recently a further active component of hashish, cannabichromene, has been isolated, the structure (CCLXII) of which represents an alternative mode of cyclization of cannabigerol (CCLXI, R = H) *via* the hypothetical intermediate, 8-hydroxycannabigerol (135a).

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