action mixture was diluted with water. The aqueous solution was decanted from the resulting oil, and the oily residue was washed with water twice by decantation. The residue was boiled with ethanol in an attempt to isolate the desired XV, but cooling of the solution yielded only a resinous solid. This solid was redissolved by boiling, and the hot solution was poured into 1800 cc. of mixed Fehling solution at 50°. The resulting mixture was boiled under reflux for 2 hours and allowed to cool. The cooled mixture was extracted with ether, and the ether was dried and distilled to yield an orange-colored heavy oil. The oil was covered with methanol, warmed to effect solution, boiled with carbon and filtered. Upon cooling, the solution deposited light yellow crystals of XVI melting at 108–109°. Recrystallization from methanol raised the melting point to 110–111°. The ultraviolet absorption spectrum indicated one maximum: $\lambda_{\max.}$ 292 mμ, ϵ 18800.

Anal. Calcd. for $C_{36}H_{34}O_6$: C, 76.85; H, 6.09. Found: C, 76.96; H, 6.13.

Debenzylation of 5,5'-Dipropenylvanillil Bis-benzyl Ether.—The crystals of XVI were covered with acetic anhydride and treated with a few drops of perchloric acid as described earlier.[6] After standing for 30 minutes at room temperature, the mixture was poured into excess water. The precipitate was filtered and recrystallized twice from ethanol to give crystals of XI melting at 179–180° and having an ultraviolet absorption spectrum with the maxima: $\lambda_{\max.}$ 275 mμ, ϵ 15900; $\lambda_{\max.}$ 322 mμ, ϵ 11700.

Anal. Calcd. for $C_{22}H_{26}O_8$: C, 66.94; H, 5.62. Found: C, 66.86; H, 5.69.

Acknowledgment.—The author wishes to thank Mr. Harold Willemsen for the analyses and spectra reported in this paper.

[6] I. A. Pearl, This Journal, **76**, 3635 (1954).

Appleton, Wisconsin

[Contribution from the Research Division, Canadian Breweries Limited]

Components of the Lead-precipitable Fraction of *Humulus lupulus*. Adhumulone

By F. L. Rigby and J. L. Bethune

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Adhumulone, a new component detected earlier in the lead-precipitable fraction of a hop extract, has now been isolated by countercurrent distribution followed by crystallization as the *o*-phenylenediamine complex. The empirical formula, $C_{21}H_{30}O_5$, shows adhumulone to be an isomer of humulone. Close structural similarity to humulone is indicated by other properties, including infrared spectra, which are reported. When boiled in mildly alkaline solution, adhumulone is transformed readily to isoadhumulone, comparable to but not identical with isohumulone. Hydrolysis of adhumulone in 1.5 *N* NaOH yields a new compound, *adhumulinic acid*.

The authors have shown previously[1,2] that the lead-precipitable fraction of a hop extract is a mixture containing significant amounts of two new components, cohumulone and adhumulone, in addition to humulone which formerly was believed to comprise most of this fraction. The first of these new substances to be detected, cohumulone, has been isolated earlier and some of its properties have been studied.[2,3] The investigation of the composition of the lead-precipitable fraction has been continued and the present report describes the isolation and properties of adhumulone.

The countercurrent distribution pattern, Fig. 1, for the lead-precipitable fraction (604 mg.) of California Seedless hops clearly shows the presence of the three principal components, cohumulone, humulone and adhumulone, with partition coefficients of 0.15, 0.29 and 0.48, respectively. This pattern was obtained after 350 transfers in a 100-tube apparatus using the single withdrawal procedure. The solvent system consisted of 2,2,4-trimethylpentane over a buffer composed of 74 volumes of *M* K_2HPO_4 adjusted to *p*H 7.25 with concentrated H_3PO_4, plus 26 volumes of reagent grade methanol.

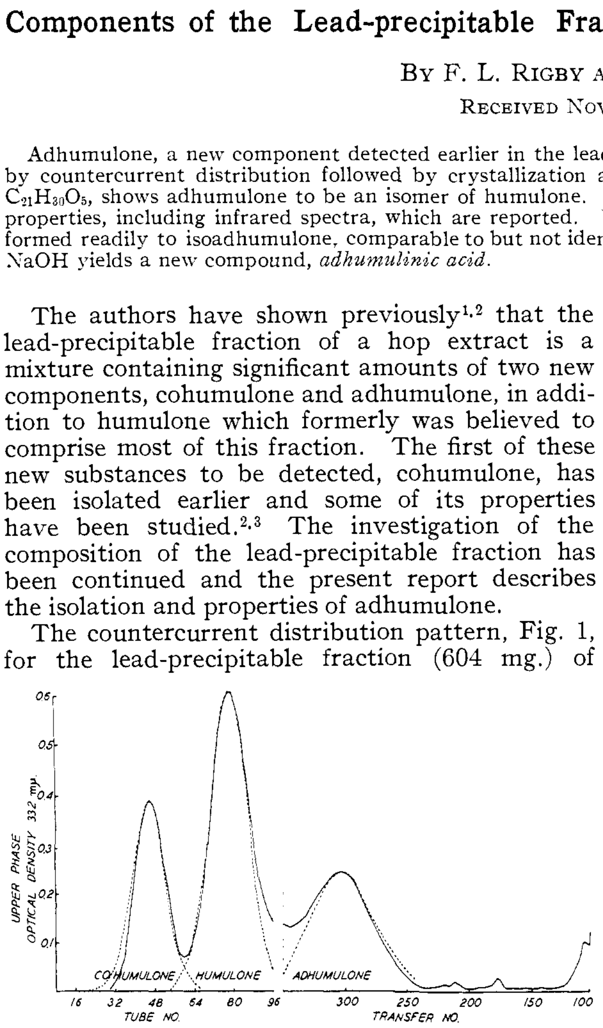

Fig. 1.—Distribution pattern for the lead-precipitable fraction of California Seedless hops, 1952. Solid line shows experimental pattern and dotted lines show theoretical curves.

Traces of minor components emerged from the train from transfer 125 to transfer 225. A mixture of substances having very high partition ratios, and amounting to approximately 5% of the total material distributed, was withdrawn in transfers 100 to 120. A mixture of substances having low partition ratios, also amounting to about 5% of the total material, did not move beyond the first ten tubes of the apparatus.

Distribution of the lead-precipitable fraction of extracts of other hop varieties yields similar patterns, the combined humulone, cohumulone and adhumulone usually comprising 85 to 90% of this fraction. The adhumulone content, expressed as percentage of the total of the three components, ranged from 10 to 15% among the samples examined.

The partition isotherms for these substances become non-linear at high concentration levels due to the low solubility of the substances in the aqueous phase. In consequence, when large loads of material (20–30 g.) are distributed, as in the isolation of substantial amounts of the components, such distributions depart considerably from the theoretical behavior shown in Fig. 1, and a much

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[1] F. L. Rigby and J. L. Bethune, This Journal, **74**, 6118 (1952).

[2] F. L. Rigby and J. L. Bethune, *Am. Soc. Brewing Chemists Proc.*, 119 (1953).

[3] G. A. Howard and A. R. Tatchell, *Chemistry & Industry*, 436 (1953).

greater number of transfers must be applied (700–800), with recycling, in order to separate the adhumulone band (see Experimental).

Adhumulone, after separation from the other components of the lead-salt fraction by countercurrent distribution was obtained as a yellow viscous oil which could not be induced to crystallize. Like humulone and cohumulone it forms a complex, m.p. 97.5–98° dec., with *o*-phenylenediamine (*cf.* humulone complex m.p. 118–118.5° dec. and cohumulone complex m.p. 92.4–93° dec.). Since the adhumulone complex can form mixed crystals with either of the other two complexes, adhumulone must be separated completely from the other two components by countercurrent distribution before attempting crystallization of the complex.

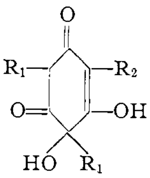
The empirical formula for adhumulone, $C_{21}H_{30}O_5$, is identical with that of humulone, both of these substances containing one more methylene group than cohumulone, $C_{20}H_{28}O_5$. Adhumulone is optically active, $[\alpha]^{26}D$ -187 in methanol, this value being slightly lower than those of humulone and cohumulone ($[\alpha]^{26}D$ -211 and -208.5, respectively). The iodine value for adhumulone, 133 (theor. 140), as with humulone and cohumulone, is equivalent to two double bonds. The ultraviolet absorption spectra of adhumulone in alkaline methanol, maxima at 325 and 360 mμ, and in acid methanol, maxima at 236, 288 and 323 mμ, are indistinguishable from those of humulone.

Adhumulone, when boiled in aqueous solutions under conditions comparable to those required for the conversion of humulone to isohumulone (see Experimental), also is transformed to a colorless, odorless, viscous oil, $C_{21}H_{30}O_5$, which is isomeric with the parent compound and is therefore called isoadhumulone. This isomer has a higher solubility in water than its precursor, adhumulone, and, like isohumulone and isocohumulone, contributes the characteristic hop bitter flavor to beer. The ultraviolet absorption spectrum of isoadhumulone in alkaline methanol shows the same maximum, 255 mμ, an isohumulone and isocohumulone but the specific extinction is slightly lower than that of the other two iso compounds, $E^{1\%}_{1\text{ cm. } 255\text{m}\mu}$ = 500, 520 and 540 for isoadhumulone, isohumulone and isocohumulone, respectively.

Hydrolysis of adhumulone by refluxing in 1.5 *N* NaOH yields adhumulinic acid $C_{15}H_{22}O_4$, m.p. 83–83.2° (*cf.* humulinic acid $C_{15}H_{22}O_4$, m.p. 92° and cohumulinic acid $C_{14}H_{20}O_4$, m.p. 78–79°).

Infrared spectra were recorded for adhumulone, cohumulone and humulone, for their isomers produced on boiling in aqueous solution and for their crystallizable products after alkaline hydrolysis. The spectra for each of these groups of substances confirm the high degree of structural similarity indicated by their other properties.

The structures I, II and III, R_2 = $COCH_2CH(CH_3)_2$, are the formulas now accepted for humulone,[4] isohumulone[5] and humulinic acid,[6] respectively.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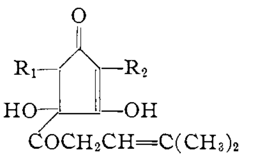
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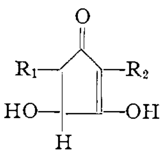
III

$R_1 = CH_2CH = C(CH_3)_2$

Howard and Tatchell[3] have shown recently that cohumulone differs from humulone only with respect to the side chain R_2 of I, which is $COCH(CH_3)_2$ for cohumulone. This difference is retained during hydrolysis to the humulinic and cohumulinic acids, respectively, and also may be assumed to be the only difference between isohumulone and isocohumulone. This is in accord with the infrared spectra which show no significant differences between humulone and cohumulone in the region of OH, C–H and C=O stretching vibrations, 2.7 to 6.5 μ or, similarly, between the iso-compounds or the degradation acids in this region. The differences which appear between the spectra of these pairs of compounds at wave lengths longer than 6.5 μ therefore must be due to the additional methylene group in the side chain of humulone. The most noticeable difference between the humulone and cohumulone spectra occurs at 12.25 μ where an additional absorption band for cohumulone is apparent.

The spectra of adhumulone and its reaction products closely parallel those of humulone and cohumulone and their respective reaction products indicating that the structure of adhumulone does not differ from that of humulone with respect to any major functional group, but differs most probably in the structure of one of the side chains.[7]

For each parent compound, the transformation to the iso compound is accompanied by a shift to shorter wave lengths for the C=O stretching vibration (6.0 μ for the parent compounds and 5.8–5.9 μ for the iso compounds) which is consistent with the change from a six- to a five-membered ring. The transformation to the iso compounds is accompanied also by a substantial decrease in the absorption intensity due to OH stretching vibration. This spectral change can best be explained by predominance of the enol form in a six-carbon ring and the keto form in a five-carbon ring.[8]

The spectra for the acids produced on hydrolysis of the parent compounds also show a decrease in the intensity of the OH stretching absorption band and a shift to lower frequency for the C=O stretching absorption band. These C=O bands in the hydrolytic products also differ from those of the parent compounds and iso compounds in that they appear as a single absorption band for the former and a multiple band for the latter, which can be attributed to the loss of the isohexenoyl group on hydrolysis. Another consistent difference between the spectra of each iso compound and the corre-

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[4] A. H. Cook and G. Harris, *J. Chem. Soc.*, 1873 (1950).

[5] M. Verzele and F. Govaert, *Congr. intern. ind. ferment. Confs. et commun. Ghent, Belg.*, 295 (1947).

[6] G. Harris, G. A. Howard and J. R. A. Pollock, *J. Chem. Soc.*, 1906 (1952).

[7] It has recently been reported by G. A. Howard and A. R. Tatchell, *Chem. & Ind.*, **32**, 992 (1954), that, during the course of synthesis of some analogs of cohumulone, a compound has been prepared in which R_2 (above) is $COCH(CH_3)(CH_3CH_2)$ and which they have shown to be identical with adhumulone isolated from natural sources by us.

[8] D. N. Shigarin, *Zhur. Fiz. Khim.*, **24**, 924 (1950); *C. A.*, **45**, 1422 (1951).

sponding hydrolytic product is the substantial decrease in the intensity of the absorption band at 12μ in the latter.

Experimental[9]

Isolation of Adhumulone.—The lead-precipitable fraction of 1952 California Seedless hops was used as source material. Three distributions were carried out, each with 33 g. of material, in a 100-tube, 10 ml. per phase, countercurrent apparatus, using the solvent system 2,2,4-trimethylpentane over a buffer composed of 74 volumes of *M* K_2HPO_4 adjusted to *p*H 7.25 with concd. H_3PO_4 plus 26 volumes of methanol. The more rapidly moving material, which is principally adhumulone, was recycled twice, the contents of the tubes containing the more slowly moving material being replaced, as necessary, to accommodate the advancing adhumulone band. After 800–900 transfers the adhumulone appeared as a band which occupied all the tubes of the apparatus. The contents of the tubes were removed and the phases were separated. The adhumulone was removed from the trimethylpentane phase (500 ml.) by extraction with two successive 100-ml. portions of 90% methanol. This methanol was then combined with the buffer phase and the combined solutions, after being made strongly acid, were extracted twice with petroleum ether. Evaporation of the petroleum ether, under vacuum at room temperature, yielded a yellow, oily adhumulone concentrate, 16% of the original material. The adhumulone concentrate was redistributed, *n* = 650, using the solvent system 2,2,4-trimethylpentane over *M* phosphate buffer *p*H 7.8 (8 vol.) plus methanol (2 vol.). The center portion of the adhumulone band was withdrawn and the adhumulone (6 g.) recovered as before. The adhumulone was then combined in dry benzene with one equivalent of *o*-phenylenediamine, assuming an equivalent weight of 360 for adhumulone. Crystallization occurred readily and after three recrystallizations the melting point was constant at 97.5–98° dec. *Anal.* Calcd. for $C_{27}H_{38}O_5N_2$: C, 68.91; H, 8.14; N, 5.95; mol. wt., 470. Found: C, 69.2, 69.3; H, 8.1, 8.3; N, 5.9, 6.0; mol. wt. (from % N), 470. Calcd. for $C_{21}H_{30}O_5$ (free acid): C, 69.59; H, 8.34; mol. wt., 362. Found: C, 69.9, 70.0, 69.9; H, 8.3, 8.3, 8.2; mol. wt., 362 (from % N in complex).

Isoadhumulone.—Adhumulone (1.3 g.) was dissolved in 50 ml. of 95% ethanol and made alkaline with 50 ml. of 0.1 *N* NaOH, giving a final excess alkali concentration of 0.014 *N*, after neutralization of the acid. The solution was refluxed on a boiling water-bath. Samples of 0.2 ml. were taken at 5-min. intervals, diluted to 100 ml. with methanol, and read in the spectrophotometer at 325 and 255 mμ. Refluxing was continued until minimal 325 and maximal 255 mμ values were obtained (approx. 30 min.). The reaction mixture was acidified, extracted with petroleum ether and the solvent removed by vacuum evaporation at room temperature. The isoadhumulone was purified by two successive countercurrent distributions, in a nitrogen atmosphere, using a solvent system consisting of 2,2,4-trimethylpentane over a buffer composed of 8 volumes of 0.5 *M* K_2HPO_4 adjusted to *p*H 4.6 with 0.25 *M* citric acid plus 2 volumes of methanol. The isoadhumulone was recovered using the procedure described earlier for adhumulone with the exception that all vessels were flushed with nitrogen and exposure of the product to atmospheric oxygen was reduced to a minimum. The isoadhumulone obtained in this way was a colorless, odorless, viscous oil. *Anal.* Found: C, 69.9, 70.0, 69.9; H, 8.1, 8.1, 8.3, corresponding to $C_{21}H_{30}O_5$ (*cf.* adhumulone).

Adhumulinic Acid.—Adhumulone (1.2 g.) was refluxed for 2.5 hours in 1.5 *N* NaOH containing 40% methanol. Acidification of the reaction mixture yielded an oily layer from which no crystals could be formed on chilling. The oily material was separated and dissolved in cyclohexane but only slight crystal formation could be induced. Purification of the product then was accomplished by concentrating the crystallization liquor to a small volume and distributing the material in the countercurrent apparatus, *n* = 40, using the solvent system cyclohexane over a buffer consisting of *M* NaH_2PO_4 adjusted to *p*H 4.6 with 6 *N* KOH. The position of the adhumulinic acid band (*K* = 0.3 approx.) was established by reading the optical density, at 252 mμ, of aliquots of the upper phase diluted with alkaline methanol. The band was withdrawn and the adhumulinic acid, after recovery in the usual manner (see isoadhumulone) was crystallized from petroleum ether, separating as fine needles, m.p. 83–83.2°. *Anal.* Calcd. for $C_{15}H_{22}O_4$: C, 67.64; H, 8.33; O, 24.03. Found: C, 67.6, 67.4; H, 8.2, 8.0; O, 24.4, 24.5.

Further quantities of adhumulinic acid were prepared by refluxing the entire lead-precipitate fraction in alkali and separating the mixture of acids by distributing the product using the solvent system diethyl ether over 0.5 *M* K_2HPO_4 adjusted to *p*H 5.5 with 0.25 *M* citric acid.

Infrared and Ultraviolet Absorption Spectra.—Samples of humulone, cohumulone and adhumulone for spectral examination were prepared from crystalline *o*-phenylenediamine complex immediately prior to recording the spectra (melting points for the complexes, 118–118.5, 92.4–93 and 97.5–98°, respectively). Humulinic, cohumulinic and adhumulinic acid solutions were prepared from crystalline products, melting points 91.4–91.8, 78.5–79 and 83–83.2°, respectively. The three iso compounds were prepared and purified immediately prior to use by the procedure described under "Isoadhumulone."

Solutions of the parent compounds and iso compounds were prepared in 5% concentration (g. of solute per 100 ml. of solution) in each of two solvents, perchloroethylene for examination in the range 2.5 to 7.75 μ, and carbon disulfide for the range 7.0 to 15 μ. Humulinic, cohumulinic and adhumulinic acids were prepared in 2.5% concentration as they were not soluble to the extent of 5%. The spectra were recorded using a Perkin-Elmer model 12C spectrophotometer with a 0.213 mm. fixed cell and a sodium chloride prism.

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Toronto, Ontario, Canada

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(9) The infrared spectra referred to in this article have been deposited as Document number 4464 with the ADI Auxiliary Publications Project, Photoduplication Service, Library of Congress, Washington 25, D. C. A copy may be secured by citing the Document number and by remitting $1.25 for photoprints or $1.25 for 35 mm. microfilm in advance, by check or money order payable to Chief, Photoduplication Service, Library of Congress.