351. *The Chemistry of Hop Constituents. Part III.* The Structures of Humulinic and* iso*Humulinic Acids.*

By G. Harris, G. A. Howard, and J. R. A. Pollock.

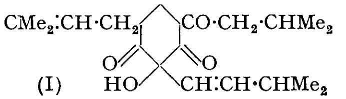
Humulone has been degraded through *iso*humulinic acid to 3-4′-methylbutyl*cyclo*pentane-1 : 2 : 4-trione which has been synthesized. New oxidative degradations of humulinic and dihydrohumulinic acids have been carried out and the structures of these compounds and of humulone are discussed.

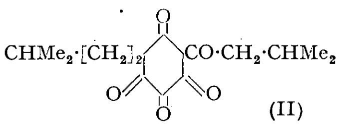
Although the hop constituent humulone, $C_{21}H_{30}O_5$, has recently been synthesized by Riedl (*Brauwiss.*, 1951, 85), the method used was equivocal and, moreover, the reactions of the compound are difficult to reconcile with the proposed formulation (I). It is essential for an understanding of these reactions that the structures of the reaction products should be firmly established and the present work was undertaken in an attempt to confirm the structures of humulinic and *iso*humulinic acids, two important degradation products of humulone.

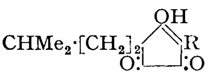
* Part II, preceding paper.

Wöllmer observed (*Ber.*, 1916, **49**, 780) that humulone was very readily reduced in the presence of palladium chloride to a volatile hydrocarbon and humuloquinol, $C_{16}H_{24}O_5$, the latter being readily oxidised by air to the corresponding quinone (II). This reduction, typical of an allyloxy-compound, remains without analogies if humulone has the structure (I) which was assigned to it when Wieland (*Ber.*, 1925, **58**, 102) extended Wöllmer's work on humulone and humuloquinone and degraded the latter to *iso*humulinic acid, $C_{15}H_{22}O_4$, for which the structure (III; R = $CO{\cdot}CH_2{\cdot}CHMe_2$) was postulated. In agreement with this formulation *iso*humulinic acid forms an azine with *o*-phenylenediamine, a dioxime, and a mono-oxime methyl ether. As explained below, however, the conversion of (III) into dihydrohumulinic acid by reduction or of dihydrohumulinic acid into (III) by oxidation could not be effected; these transformations should be possible if *iso*humulinic acid and dihydrohumulinic acid have the structures (III; R = $CO{\cdot}CH_2{\cdot}CHMe_2$) and (IV; R′ = $CH_2{\cdot}CH_2{\cdot}CHMe_2$) respectively ascribed to them by Wieland. A verification of the Wieland structure for *iso*humulinic acid was, therefore, considered desirable.

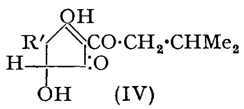
Treatment of the compound with cold alkali gave *iso*valeric acid and a crystalline substance, $C_{10}H_{14}O_3$, which gave a strong colour with methanolic ferric chloride and had pK 6·9 in 50% aqueous acetone. This compound formed an azine with *o*-phenylenediamine and, unlike its precursor, readily gave a benzylidene derivative with benzaldehyde; it was thus thought likely that it was 3-3′-methylbutyl*cyclo*pentane-1 : 2 : 4-trione (III; R = H) and its synthesis was effected by the following method (cf. Diels, Sielisch, and Muller, *Ber.*, 1906, **39**, 1328). Two equivalents of ethyl oxalate were condensed with one of 6-methylheptan-2-one in boiling ethanolic sodium ethoxide to give 3-ethoxalyl-5-3′-methylbutyl*cyclo*pentane-1 : 2 : 4-trione which was hydrolysed by aqueous methanolic hydrogen chloride to the required 3-3′-methylbutyl*cyclo*pentane-1 : 2 : 4-trione, identical with the material isolated by hydrolysis of *iso*humulinic acid. There can be no doubt, therefore, that the latter is correctly represented by (III; R = $CO{\cdot}CH_2{\cdot}CHMe_2$). Preliminary attempts to acylate (III; R = H) to (III; R = $CO{\cdot}CH_2{\cdot}CHMe_2$) have been unsuccessful.







(III)



Attention was next turned to the structure of humulinic acid and its dihydro-derivative. The former is readily obtained, together with acetone, *iso*butaldehyde, and an unsaturated acid, by alkaline hydrolysis of humulone (Lintner and Schnell, *Z. ges. Brauwesen*, 1904, **27**, 668; Wieland, *loc. cit.*). The unsaturated acid was incorrectly described by Wieland as 4-methylpent-2-enoic acid * and its correct identification as the 3-enoic acid by Cook and Harris (*J.*, 1950, 1873) requires the Wieland structure (I) for humulone to be modified to (V). The reactions of humulinic acid were investigated by Wieland (*Ber.*, 1925, **58**, 2012) and by Wieland and Martz (*Ber.*, 1926, **59**, 2352) who postulated the structure (IV; R′ = $CH_2{\cdot}CH{:}CMe_2$) for it although the formulation (VI; R′ = $CH_2{\cdot}CH{:}CMe_2$) is equally possible on Wieland's evidence. Since diphenyl*cyclo*pentanetrione (Ruggli and Schmidlin, *Helv. Chim. Acta*, 1946, **29**, 383) and methyl*cyclo*pentanetrione (Orchin and Butz, *J. Amer. Chem. Soc.*, 1943, **65**, 2296) can be hydrogenated to hydroxy*cyclo*pentanediones it was expected that partial hydrogenation of *iso*humulinic acid would lead smoothly to a dihydro-derivative likely to be identical with (IV; R′ = $CH_2{\cdot}CH_2{\cdot}CHMe_2$). Although the course of the hydrogenation of *iso*humulinic acid is not simple the dihydro-derivative isolated was different from dihydrohumulinic acid. This dihydro*iso*humulinic acid should be either (IV; R′ = $CH_2{\cdot}CH_2{\cdot}CHMe_2$) or (VI; R′ = $CH_2{\cdot}CH_2{\cdot}CHMe_2$) since the method of prepar-

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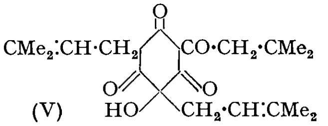
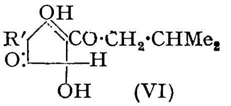
* Geneva convention, CO_2H = 1.

ation could equally well lead to either compound and the properties of dihydrohumulinic acid are as well explained by one structure as by the other.

Since *iso*humulinic acid could not be reduced to dihydrohumulinic acid, oxidation of the latter to the former was investigated. The oxidation of a secondary α-ketol group is normally easy and, if dihydrohumulinic acid is (IV) or (VI), should readily afford *iso*humulinic acid (III; $R = CO{\cdot}CH_2{\cdot}CHMe_2$). Fehling's solution unexpectedly failed to affect either humulinic or dihydrohumulinic acid. In view of the stability of *iso*humulinic acid to hypobromite (Wieland, *loc. cit.*) the action of this reagent on humulinic acid was investigated. Both this acid and its dihydro-derivative readily reduce three equivalents of hypobromite but the cyclic structure is destroyed, bromoform and *iso*valeric acid being formed in both cases. With dihydrohumulinic acid, a crystalline acid, $C_9H_{16}O_5$, was also obtained. This compound behaved as an α-hydroxy-acid and may well be the 1-hydroxy-5-methylhexane-1 : 2-dicarboxylic acid (VII) expected if dihydrohumulinic acid has the structure (IV; $R' = CH_2{\cdot}CH_2{\cdot}CHMe_2$). The oxidation of cyclic β-diketones with hypohalites is well known (cf. Ingold and Thorpe, *J.*, 1922, 157) but the ready oxidations of humulinic and dihydrohumulinic acids are in strong contrast to the stability of *iso*humulinic acid to hypobromite.

Humulinic acid readily reduces one equivalent of periodate with evolution of carbon dioxide and the formation of an enolic compound which is hydrolysed by alkali to *iso*valeric acid and 5-methylhex-4-enoic acid, together with *iso*butyl methyl ketone and 6-methylhept-5-en-2-one. A compound with structure (IV; $R' = CH_2{\cdot}CH{:}CMe_2$) would reduce one equivalent of periodate and give the di-β-keto-carboxylic acid (VIII). Decarboxylation of this would lead to 2-(3-keto-5-methylhexanoyl)-5-methylhex-4-enal (VIII, with H in place of CO_2H) which would then be further hydrolysed by alkali to the products actually isolated. Although the results of the periodate and hypobromite oxidation of humulinic acid are explicable on the basis of (IV; $R' = CH_2{\cdot}CH{:}CMe_2$), this structure still leaves unexplained its stability towards Fehling's solution and also to alkali and acid. Equally inexplicable is the ready reduction of both humulinic and dihydrohumulinic acids to hydrocarbons by the Clemmensen procedure (Wieland and Martz, *loc. cit.*). In the case of dihydrohumulinic acid an intermediate deoxy-compound was obtained and ascribed by Wieland and Martz the structure (IX). The stability of the deoxy-compound to both acid and alkaline hydrolysis, coupled with the absence of ketonic reactions and of coloration with methanolic ferric chloride, are, however, inconsistent with this formulation. Present results, in fact, support a formula, $C_{14}H_{24}O_2$, rather than $C_{15}H_{26}O_2$ as required by (IX). Other properties of humulinic and dihydrohumulinic acid are also difficult to reconcile with formulations such as (IV); *e.g.*, their ultra-violet absorption spectra are quite unlike those of hydroxy*cyclo*pentanediones (Bastron, Davis, and Butz, *J. Org. Chem.*, 1943, **8**, 215). Further they resist hydrolysis by acid or alkali, are even stronger acids (p*K* 3·2 and 3·8 in 50% aqueous acetone) than the *cyclo*pentanetriones (p*K* 4—5 and showing one active H), and yet contain only one of the two active hydrogen atoms to be expected from the formulations (IV). They also fail to give derivatives characteristic of secondary alcohols. These properties, incompatible with (IV), find a ready explanation on the basis of the structure (X; $R' = CH_2{\cdot}CH{:}CMe_2$ and $CH_2{\cdot}CH_2{\cdot}CHMe_2$) for humulinic and dihydrohumulinic acids respectively. Periodate oxidation of such compounds would lead to hydroxy*cyclo*propanones which would rearrange readily to (VIII). Furthermore, the general properties and hypobromite oxidation of (XI) described by Ingold, Seeley, and Thorpe (*J.*, 1923, 860) are closely analogous to the behaviour observed with humulinic acid. The synthesis of model compounds is being investigated.


(V)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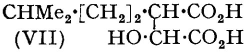
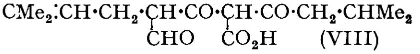
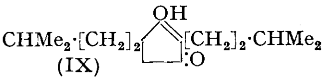

(VII)

$$\begin{array}{l} CHMe_2\cdot[CH_2]_2\cdot CH\cdot CO_2H \\ \qquad\qquad\quad\ \ HO\cdot CH\cdot CO_2H \end{array}$$

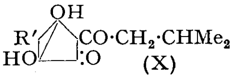

(VIII)

$$\begin{array}{l} CMe_2{:}CH\cdot CH_2\cdot CH\cdot CO\cdot CH\cdot CO\cdot CH_2\cdot CHMe_2 \\ \qquad\qquad\qquad\ \ CHO \quad\ \ CO_2H \end{array}$$

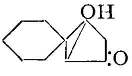
Finally, it is pertinent to place on record further experiments designed to test the validity of structure (V) for humulone. A compound with structure (V) would give 4-methylpent-3-enoic acid when oxidised by periodate. While humulone is oxidised by this reagent the formation of 4-methylpent-3-enoic acid does not occur. Again, the fact that humulone undergoes hydrogenolysis in the presence of palladium chloride is incompatible with its having the structure (V). It is now suggested that the above difficulties find accommodation in formula (XII). In agreement with this structure it has been ascertained that humulone forms only a monophenylurethane and is a monobasic compound with one active hydrogen atom. The formation of humulinic and 4-methylpent-3-enoic acids from humulone is then readily explained by an allylic rearrangement of (XII), followed by hydrolytic fission of the product.



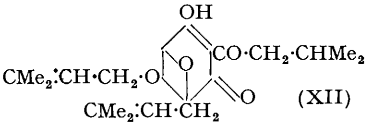
(IX)



(X)



(XI)



(XII)

EXPERIMENTAL

Microanalyses are by Drs. Weiler and Strauss. M. p.s are uncorrected.

Humulone.—To humulone (Wöllmer, *Ber.*, 1916, **49**, 780) (23·1 g.) in acetic acid (440 c.c.), ice-cold water (500 c.c.) was added slowly at 0° with scratching and stirring, to give pale yellow rhombs, m. p. 63° (Found: C, 69·7; H, 8·4; active H, 0·78%; equiv., 379; I.V., 95·5. Calc. for $C_{21}H_{30}O_5$: C, 69·6; H, 8·3; 3 active H, 0·83%; equiv., 362; IV., 70). Ultra-violet absorption: Max., in basic ethanol, 324, 228, and 350—361 shoulder mμ (ε = 11,520, 14,730 and 8750 respectively); in acidic ethanol, 235, 281·5, and 310—325 (shoulder) mμ (ε = 10,100, 8000, and 5330 respectively). The *o*-phenylenediamine complex had m. p. 117—118·5° (Found: C, 69·0; H, 8·3; N, 5·9. Calc. for $C_{27}H_{38}O_5N_2$: C, 68·9; H, 8·1; N, 6·0%). Ultra-violet absorption in ethanol: Max., 230, 242, 280, 330, and 360 mμ (ε = 8500, 5700 5700, and 4300 respectively).

Humulone had p*K* 4·5 in 50% acetone and reduced 1·97 equivs. of periodate producing 3·3 equivs. of acid but none of the expected 4-methylpent-3-enoic acid; it also reduced 4·95 equivs. of perbenzoic acid and absorbed 2·1 equivs. of bromine from a chloroform solution, hydrogen bromide being liberated.

A solution of humulone (500 mg.) and phenyl *iso*cyanate (550 mg.) in light petroleum (b. p. 80—100°) (12 c.c.) was boiled under reflux for 90 minutes under nitrogen, concentrated, and kept overnight at 0°. The product (90 mg.), m. p. 161° (decomp.), was twice recrystallized from light petroleum, giving *humulone phenylurethane*, m. p. 161° (decomp.) (Found: C, 70·0; H, 7·4; N, 3·3. $C_{28}H_{35}O_6N$ requires C, 69·9; H, 7·3; N, 2·9%), which gave a strong red to purple colour with alcoholic ferric chloride.

Humuloquinol.—When prepared by Wöllmer's method (*loc. cit.*), the quinol, was obtained as yellow granules, m. p. 126—128°, giving a red colour with alcoholic ferric chloride solution (Found: C, 64·3; H, 8·0; active H, 0·68. Calc. for $C_{16}H_{24}O_5$: C, 64·8; H, 8·2; 2 active H, 0·68%). Ultra-violet absorption: Max.: 293 and 350 mμ (ε = 22,000 and 2400 respectively). *Humuloquinol dimethyl ether*, prepared by an excess of diazomethane in ether, had b. p. 100—115°/10^{-5} mm. (Found: C, 67·1; H, 8·7; OMe, 19·9; active H, 0·25. $C_{18}H_{28}O_5$ requires C, 66·7; H, 8·7; OMe, 19·2; 1 active H, 0·31%), gave a green colour with alcoholic ferric chloride, and failed to form a 2 : 4-dinitrophenylhydrazone.

The *phenylurethane*, prepared in the usual way and recrystallized from benzene–light petroleum (b. p. 60—80°), had m. p. 131° (decomp.) (Found: C, 67·4; H, 7·0; N, 3·4; active H, 0·50. $C_{23}H_{29}O_6N$ requires C, 66·5; H, 7·0; N, 3·4; 2 active H, 0·49%). Benzoylation of humuloquinol in cold pyridine under nitrogen and recrystallizing the product from ethanol gave humuloquinol tetrabenzoate, m. p. 167° (Found: C, 73·6; H, 5·9; active H, 0·0. Calc. for $C_{44}H_{40}O_9$: C, 73·7; H, 5·6%). Ultra-violet absorption: Max., 234 mμ (ε = 72,000).

Humuloquinone.—Humuloquinone, prepared by Wöllmer's method (*loc. cit.*), had m. p. 84° after recrystallization from methanol (Found: C, 65·5; H, 7·5; active H, 0·73. Calc. for $C_{16}H_{22}O_5$: C, 65·3; H, 7·5; 2 active H, 0·66%). Ultra-violet absorption: Max., 292 and 510 mμ (ε = 29,000 and 1000 respectively). Treatment with Brady's reagent gave *humuloquinone* 2 : 4-*dinitrophenylhydrazone*, m. p. 215°, as violet laths (from acetic acid) (Found: C, 55·2; H, 5·7; N, 11·4. $C_{22}H_{26}O_8N_4$ requires C, 55·7; H, 5·5; N, 11·8%). Humuloquinone azine, recrystallized from methanol, had m. p. 110° (Found: C, 72·2; H, 7·2; N, 7·5; active H 0·44. $C_{22}H_{26}O_3N_2$ requires C, 72·1; H, 7·2; N, 7·6; 2 active H, 0·54%); Wöllmer gives m. p. 109°. Ultra-violet absorption: Max., in basic ethanol, 299, 344, and 218—220 (shoulder) mμ (ε = 17,000, 10,200, and 10,600 respectively); in acidic ethanol, 292 and 381 mμ (ε = 25,500 and 725 respectively); the band in the visible region is at 530 mμ with ε = 268.

iso*Humulinic Acid.*—Humuloquinone (1·4 g.) was dissolved in cold freshly boiled 2N-sodium hydroxide (300 c.c.), and the solution kept under nitrogen for 2 hours and acidified at 0° with concentrated hydrochloric acid. The acids were extracted with ether, and the extract was washed with water, dried, and evaporated under nitrogen. The residue was crystallized from methanol, giving *iso*humulinic acid (465 mg., 37%), m. p. 141°. Recrystallization gave pure material, m. p. 144° (Found: C, 67·7; H, 8·4; active H, 0·30. Calc. for $C_{15}H_{22}O_4$: C, 67·6; H, 8·3; 1 active H, 0·38%). The compound did not depress the m. p. (143°) of the *iso*humulinic acid prepared by Wieland's method (*loc. cit.*). Ultra-violet absorption: Max., 251, 258 (double peak), and 280 mμ (ε = 25,000 and 22,000 respectively).

To a solution of diazomethane (0·002 mole) in dry ether (40 c.c.) was added *iso*humulinic acid (0·266 g., 0·001 mole). After 10 minutes at room temperature the ether was distilled off leaving a residue which could not be crystallized. Conversion into the oxime in the usual way gave iso*humulinic acid methyl ether oxime*; recrystallized from ether–light petroleum (b. p. 40—60°), it had m. p. 100° (Found: C, 65·0; H, 8·4; N, 4·6. $C_{16}H_{25}O_4N$ requires C, 65·1; H, 8·6; N, 4·8%).

*iso*Humulinic acid (0·133 g.) was refluxed in aqueous methanol (10 c.c.) with hydroxylamine hydrochloride (0·1 g.) and anhydrous sodium acetate (0·15 g.) for 10 minutes. Dilution with water (20 c.c.) and cooling gave the crude *dioxime*, m. p. 202° (decomp.). Recrystallized from aqueous dioxan, this had m. p. 205° (decomp.) (Found: C, 61·4; H, 7·8; N, 9·5. $C_{15}H_{24}O_4N_2$ requires C, 60·8; H, 8·1; N, 9·5%).

Hydrogenation of iso*Humulinic Acid.*—*iso*Humulinic acid (670 mg.) in methanol (30 c.c.) in the presence of Adams's platinum oxide slowly absorbed 160 c.c. of hydrogen overnight. The filtered solution was evaporated and extracted with boiling light petroleum (10 c.c.). Insoluble material (99 mg.), m. p. 160°, was filtered off, and the filtrate concentrated to small bulk and allowed to crystallize at 0°. A pale yellow *dihydro-acid* (140 mg.), m. p. 81°, separated and was filtered off. Recrystallized from light petroleum it had m. p. 88° (Found: C, 67·2; H, 8·8. $C_{15}H_{24}O_4$ requires C, 67·2; H, 9·0%). Ultra-violet absorption: Max., 250 mμ (ε = 15,000).

Alkaline Hydrolysis of iso*Humulinic Acid.*—A solution of *iso*humulinic acid (1 g.) in freshly boiled 2N-sodium hydroxide (60 c.c.) was steam-distilled for 45 minutes. The residue was acidified at 0° and then set aside in a refrigerator. Crystalline material, m. p. 98°, separated and was filtered off. The filtrate was extracted with ether and the bicarbonate-soluble portion obtained from this extract was cooled to −80° in light petroleum. Material, m. p. 95°, separated and was combined with the compound, m. p. 98°, whose m. p. it did not depress. The filtrate from the low-temperature crystallization was evaporated, to give 173 mg. of an acid. This acid gave only one band on a buffered silica column in 1% butanol–chloroform (cf. Moyle, Baldwin, and Scarisbrick, *Biochem. J.*, 1948, **43**, 308) and was converted through the acid chloride into *iso*valeranilide, m. p. and mixed m. p. 110° (Found: C, 74·2; H, 8·4. Calc. for $C_{11}H_{15}ON$: C, 74·6; H, 8·5%). The combined crops of crystalline material of m. p. 95 and 98° were recrystallized from light petroleum (b. p. 40—60°), giving 3-3'-*methylbutyl*cyclo*pentane*-1 : 2 : 4-*trione* (21 mg.), m. p. 102° (Found: C, 66·2; H, 7·9. $C_{10}H_{14}O_3$ requires C, 66·0; H, 7·7%).

With *o*-phenylenediamine in hot methanol the trione gave a yellow azine (77%), m. p. 210° (decomp.).

3-*Ethoxalyl*-5-3'-*methylbutyl*cyclo*pentane*-1 : 2 : 4-*trione.*—A mixture of ethyl oxalate (11 g.) and 6-methylheptan-2-one (10 g.) was added to a solution of sodium (1·8 g.) in dry ethanol (40 c.c.), cooled in ice. A solution of ethyl oxalate (11 g.) in a sodium ethoxide solution, prepared from sodium (1·8 g.) and dry ethanol (40 c.c.), was then added and the mixture heated under reflux for 15 minutes. The hot mixture was saturated with dry hydrogen chloride, cooled, and filtered. The deposited sodium chloride was washed with dry ethanol and the combined filtrates and washings were evaporated *in vacuo*. The residue was dissolved in ethyl acetate and ether, and the solution extracted with saturated sodium hydrogen carbonate solution. The extracts were acidified and the acids extracted with ether. The ethereal extract was washed, dried, and evaporated to give the ethoxalyl-trione (9 g., 40%), m. p. 74°. This could not be purified for analysis but treatments with Brady's reagent and methanolic *o*-phenylenediamine gave respectively a 2 : 4-*dinitrophenylhydrazone* as yellow plates, m. p. 156° (Found: C, 51·9; H, 4·6; N, 12·4. $C_{20}H_{22}O_9N_4$ requires C, 52·0; H, 4·8; N, 12·1%), and an *azine* as a deep red powder, m. p. 284° (decomp.) (Found: C, 66·8; H, 5·4; N, 8·6. $C_{18}H_{18}O_4N_2$ requires C, 66·3; H, 5·6; N. 8·4%).

3-3′-*Methylbutyl*cyclo*pentane*-1 : 2 : 4-*trione*.—A solution of the foregoing ethoxalyl-trione (9 g.) in methanol (200 c.c.) and 12N-hydrochloric acid (200 c.c.) was refluxed for 3 hours until evolution of gas ceased. The solution was concentrated to half its bulk in a vacuum and then set aside in a refrigerator. The product (6 g.) was filtered off and a further crop (1 g.) was obtained by concentration of the mother liquors. The combined crops were recrystallized from benzene and light petroleum, giving 3-3′-methylbutyl*cyclo*pentane-1 : 2 : 4-trione (4 g., 69%), m. p. 102° undepressed by admixture with a specimen prepared from *iso*humulinic acid (Found: C, 65·7; H, 7·8%). The 2 : 4-*dinitrophenylosazone* crystallized in scarlet needles, m. p. 228° (decomp.), from ethyl acetate (Found: C, 48·6; H, 3·6; N, 20·5. $C_{22}H_{20}O_9N_8$ requires C, 48·9; H, 3·7; N, 20·7%). The *benzylidene* derivative, m. p. 216°, crystallized from aqueous methanol (Found: C, 75·0; H, 6·6. $C_{17}H_{18}O_3$ requires C, 75·6; H, 6·7%). The *azine* had m. p. 230° (decomp.) (Found: C, 75·8; H, 7·4; N, 10·7. $C_{16}H_{18}ON_2$ requires C, 75·6; H, 7·1; N, 11·2%). Ultra-violet absorption: Max., in acidic ethanol, 275·5 mμ ($\varepsilon = 12{,}230$); in basic ethanol, 326 and 233·5 mμ ($\varepsilon = 10{,}800$ and 10,500 respectively).

Humulinic Acid.—The compound, prepared by Wöllmer's method (*loc. cit.*) and recrystallized from *cyclo*hexane (68% yield), had m. p. 93° (Found: C, 67·4; H, 8·4; active H, 0·3%; equiv., 278. Calc. for $C_{15}H_{22}O_4$: C, 67·6; H, 8·3; 1 active H, 0·38%; equiv., 266). Ultra-violet absorption: Max., in acidic ethanol, 266 and 225·5 mμ ($\varepsilon = 9620$ and 9630 respectively); in basic ethanol, 250 and 262·5—269 mμ (shoulder) ($\varepsilon = 20{,}600$ and 16,420 respectively). Wöllmer (*loc. cit.*) gives m. p. 92°.

Humulinic acid decolourised bromine in chloroform, gave a red colour with alcoholic ferric chloride solution, and was soluble in sodium hydrogen carbonate solution. It did not reduce hot Fehling's solution, failed to form an azine with *o*-phenylenediamine, and was recovered unchanged in excellent yield when boiled under reflux for 3 hours with methanolic hydrochloric acid (5 vols. of methanol with 2 vols. of concentrated hydrochloric acid). Humulinic acid was stable indefinitely to boiling 2N-sodium hydroxide. It reduced a total of 0·8 equiv. of periodate with the formation of 1·8 equivs. of acid. With excess of lithium aluminium hydride in boiling ether it gave a neutral oil which gave no colour with alcoholic ferric chloride and gave a resinous urethane and an oily dark red 2 : 4-dinitrophenylhydrazone.

The oxime was prepared in aqueous solution in the usual way, the crude product being extracted with boiling ether and recrystallized from methanol; it had m. p. 150° (Found: C, 63·5; H, 7·9; N, 5·1. Calc. for $C_{15}H_{23}O_4N$: C, 64·0; H, 8·2; N, 5·0%). Wieland (*loc. cit.*) gives m. p. 152—153°. The ultra-violet absorption spectrum in ethanol was very similar to that of dihydrohumulinic acid oxime: Max., 251 and 303; Min., 272 mμ ($\varepsilon = 16{,}700$, 18,000, and 6800 respectively).

Hypobromite Oxidation of Humulinic Acid.—To a cold solution of humulinic acid (5 g.) in N-sodium hydroxide (200 c.c.) was slowly added, dropwise with shaking, a saturated aqueous solution of bromine (300 c.c.) until a slight excess of hypobromite was present. The solution was extracted with ether, and the ethereal extract washed with water, dried, and evaporated, to give bromoform (b. p. 149°; 2·6 g., 55%). The alkaline oxidised solution was acidified and extracted with ether. The ethereal extract was washed with water and then extracted with saturated aqueous sodium hydrogen carbonate solution. The alkaline extract was acidified, the acids were extracted with ether, and the ethereal extract washed with water, dried, and evaporated, giving an oil which was extracted with boiling light petroleum. The petroleum solution, on evaporation, gave *iso*valeric acid (0·94 g., 50%) (*p*-bromophenacyl ester, m. p. 67°, undepressed by admixture with an authentic specimen).

Periodate Oxidation of Humulinic Acid.—(*a*) To a solution of humulinic acid (5·02 g.) in ethanol (80 c.c.) was added one of sodium metaperiodate (4·4 g.) in water (80 c.c.). After 5 minutes sodium iodate began to separate and carbon dioxide was evolved (baryta test). After 6 hours the sodium iodate (3·7 g., 90%) was filtered off and washed with a little ethanol. The combined filtrate and washings were diluted with water and extracted with ether. The aqueous solution was made slightly acid, saturated with sodium chloride, and extracted with ether. The combined ethereal extracts were washed with water, dried, and evaporated. The oily residue was dissolved in 2N-sodium hydroxide (100 c.c.) and the solution refluxed for 2 hours. The cooled solution was extracted with ether and the extract washed with water, dried, and evaporated, to give the ketonic fraction. The alkaline solution was acidified and extracted with ether; the bicarbonate-soluble fraction from this extract was converted into *p*-bromophenacyl 5-methylhex-4-enoate, m. p. 83° (from ethanol) undepressed by admixture with a specimen prepared from the authentic acid (Staudinger, *Helv. Chim. Acta*, 1922, **5**, 751) [Found: C, 54·6; H, 5·2%; *M* (Rast), 314. $C_{15}H_{17}O_3Br$ requires C, 55·2; H, 5·5%; *M*, 326]. The ketonic fraction was distilled, to give a fraction, b. p. 80—83°, and a residue. The distillate gave a 2 : 4-dinitrophenylhydrazone, m. p. 83° (Found: C, 51·6; H, 5·7; N, 19·7. Calc. for $C_{12}H_{16}O_4N_4$: C, 51·4; H, 5·7; N, 20·0%), undepressed by admixture with the 2 : 4-dinitrophenylhydrazone of *iso*butyl methyl ketone. The residual ketone was converted into a 2 : 4-dinitrophenylhydrazone, m. p. 87°, undepressed by admixture with that of authentic 6-methylhept-5-en-2-one prepared from citral (Verley, *Bull. Soc. chim.*, 1897, **17**, 176).

(*b*) In another experiment, with humulinic acid (17 g.), the oily oxidation product was divided into neutral (2·4 g.), bicarbonate-soluble (1·1 g.), and caustic-soluble (9·5 g.) fractions in the usual way. No recognisable products were obtained from the neutral and acidic fractions. The enolic fraction was hydrolysed in boiling 2N-sodium hydroxide (50 c.c.) under reflux for 3 hours and then divided into neutral (4·7 g.), acidic (1·85 g.), and enolic (0·38 g.) fractions in the usual way. The acidic fraction was distilled, to give only *iso*valeric acid, b. p. 166° (Found: equiv., 99·4. Calc. for $C_4H_9{\cdot}CO_2H$: equiv., 102) (*p*-bromophenacyl ester, m. p. 67° undepressed by admixture with an authentic specimen). The neutral fraction from the hydrolysis was distilled and the fraction, b. p. 106° (0·3 g.), collected. Higher-boiling fractions gave no recognisable derivatives. The fraction, b. p. 106°, gave a 2 : 4-dinitrophenylhydrazone, m. p. 87°, undepressed by admixture with that of authentic 6-methylhept-5-en-2-one (Found: C, 54·6, H, 5·8; N, 18·3. Calc. for $C_{14}H_{18}O_4N_4$: C, 54·9; H, 5·9; N, 18·3%). A portion of the material, b. p. 106°, was hydrogenated in methanol by use of Adams's platinum oxide; the product gave a 2 : 4-dinitrophenylhydrazone, m. p. 78°, undepressed by admixture with an authentic specimen of 6-methylheptan-2-one 2 : 4-dinitrophenylhydrazone.

Dihydrohumulinic Acid.—Humulinic acid (8·5 g.) in methanol (300 c.c.) in the presence of Adams's platinum oxide absorbed 700 c.c. of hydrogen in 2 hours. The solution was concentrated and the product allowed to crystallize. Recrystallization from ethanol and then from *cyclo*hexane gave dihydrohumulinic acid (7·6 g., 90%), m. p. 126° (Found: C, 66·8; H, 8·9; active H, 0·40%; equiv., 268. Calc. for $C_{15}H_{24}O_4$: C, 67·2; H, 9·0; 1 active H, 0·37%; equiv., 268). Wöllmer (*loc. cit.*) gives m. p. 125—126°. Ultra-violet absorption spectrum in ethanol: Max., 259 and 267 mμ ($\varepsilon = 9500$ and 10,500 respectively). Dihydrohumulinic acid was soluble in sodium hydrogen carbonate solution and gave a yellow colour with alcoholic ferric chloride. It failed to reduce Fehling's solution or ammoniacal silver nitrate and was recovered unchanged after attempts to prepare its phenylurethane or to oxidise it with alkaline hydrogen peroxide. Dihydrohumulinic acid consumed 0·98 equiv. of periodate with the formation of 1·94 equivs. of acid.

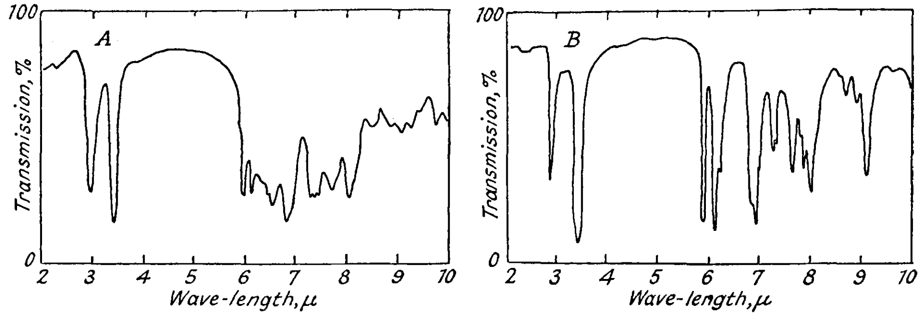
The *oxime*, prepared in the usual way and recrystallized from methanol and from ether–*cyclo*hexane, had m. p. 125° (Found: C, 63·6; H, 8·6; N, 5·0. Calc. for $C_{15}H_{25}O_4N$: C, 63·6; H, 8·8; N, 5·0%). The ultra-violet absorption spectrum in ethanol was very similar to that of humulinic acid oxime: Max., 251 and 303; Min., 272 mμ ($\varepsilon = 16,400$, 17,200, and 6700 respectively).

Hydrobromite Oxidation of Dihydrohumulinic Acid.—(*a*) To a cooled solution of dihydrohumulinic acid (2·5 g.) in N-sodium hydroxide (100 c.c.) was added, dropwise, with shaking, a freshly saturated solution of bromine in water (330 c.c.). Excess of bromine was removed by sodium hydrogen sulphite. The solution was extracted with ether; evaporation of the washed dried extract gave bromoform, b. p. 146° (0·928 g., 44%), which gave phenyl *iso*cyanide with aniline and aqueous sodium hydroxide. The alkaline reaction mixture was acidified and the acids extracted with ether. The washed and dried extract was evaporated and distilled, giving *iso*valeric acid, b. p. 160° (0·52 g., 59%), which gave a *p*-bromophenacyl ester, m. p. 68°, undepressed by admixture with an authentic specimen.

(*b*) In another experiment dihydrohumulinic acid (5 g.) absorbed the equivalent of 6 atoms of bromine. The bromoform was removed by light petroleum, and the aqueous alkaline solution acidified and then extracted with ethyl acetate. The extract was washed with water, dried (Na_2SO_4), and evaporated, to give a residue (5 g.) which was diluted with light petroleum (50 c.c.) and kept at 0°. A sticky solid, m. p. 94—100° (0·162 g.), separated and was filtered off.

A further crop was obtained by evaporation of the mother-liquors *in vacuo,* dissolution of the residue in benzene (5 c.c.), addition of light petroleum to incipient turbidity, and cooling. The combined crops were boiled with benzene (2 c.c.) to remove oil, and the insoluble material (m. p. 122°; 76 mg.) was filtered off and recrystallized by dissolution in acetone (3 drops) and addition of light petroleum. The product (49 mg.) had m. p. 124° (Found : C, 52·9; H, 7·8; active H, 0·73. $C_9H_{16}O_5$ requires C, 52·9; H, 7·8; 2 active H, 0·98%). The compound was acid to litmus, effervesced with sodium hydrogen carbonate, and evolved carbon monoxide and sulphur dioxide when treated with concentrated sulphuric acid.

Clemmensen Reduction of Dihydrohumulinic Acid.—To a solution of dihydrohumulinic acid (4·2 g.) in a mixture of ethanol (50 c.c.) and concentrated hydrochloric acid (10 c.c.) was added zinc amalgam (20 g.; cf. Fieser *et al., J. Amer. Chem. Soc.,* 1948, **70**, 3203). Dry hydrogen chloride was passed into the mixture for 10 minutes; the solution then no longer gave a colour with ferric chloride after neutralization with sodium hydrogen carbonate. The reaction mixture was diluted with water and extracted with ether. The ethereal solution was washed with water and extracted with saturated aqueous sodium hydrogen carbonate solution, then with 2N-sodium hydroxide, and finally washed with water and dried. Evaporation gave a mixture of high-boiling ketones. Neither these ketones nor their 2 : 4-dinitrophenylhydrazones have been studied further. The sodium hydroxide extract was acidified and extracted with ether. The ethereal extract was washed with water, dried, and evaporated, to give a colourless solid, m. p. 138°. Recrystallization from 70% aqueous methanol gave deoxydihydrohumulinic acid (2·1 g., 60%), m. p. 141° (Found : C, 74·9, 74·9; H, 11·1, 10·9%; equiv., 222. Calc. for $C_{15}H_{26}O_2$: C, 75·7; H, 10·9%; equiv., 238. Calc. for $C_{14}H_{24}O_2$: C, 75·7; H, 10·7%; equiv., 224). Wieland and Martz (*loc. cit.*) give m. p. 146°. Deoxydihydrohumulinic acid did not form a 2 : 4-dinitrophenylhydrazone or an azine with *o*-phenylenediamine. It had p*K* 6·9 in 50% aqueous acetone. The compound was insoluble in aqueous sodium hydrogen carbonate solution, did not give a colour with alcoholic ferric chloride solution, and decolourised a solution of bromine in carbon tetrachloride. Deoxydihydrohumulinic acid was completely stable to Fehling's solution, ammoniacal silver nitrate, alkaline hydrogen peroxide, boiling 10N-sodium hydroxide, and boiling ethanolic hydrochloric acid (3 vols. of ethanol to 2 of concentrated hydrochloric acid). It failed to form a benzylidene derivative with alkaline benzaldehyde, unchanged starting material being recovered; it was not hydrogenated in ethanol in the presence of Adams's platinum oxide and was stable to periodate. The compound reduced aqueous alkaline permanganate solution and was oxidised by hypobromite. Dihydrohumulinic acid was recovered unchanged after treatment with gaseous hydrogen chloride for 3 hours in an ethanol solution containing hydrochloric acid, zinc chloride, and mercuric chloride.



A, Humulone. B, Dihydrohumulinic acid.

Attempted Clemmensen Reduction of 3 : 5-*Diphenyl*cyclo*pentane*-1 : 2 : 4-*trione.*—To a solution of this trione (2 g.) (Claisen, *Annalen,* 1895, **284**, 250) in ethanol (50 c.c.) and concentrated hydrochloric acid (20 c.c.) was added zinc amalgam (20 g.), and dry hydrogen chloride was passed into the mixture rapidly for 3 hours. The reaction mixture was then worked up as in the dihydrohumulinic acid reduction. The portion soluble in sodium hydroxide solution gave unchanged starting material (1·9 g.), m. p. and mixed m. p. 193°.

Infra-red Spectra.—The infra-red spectra of humulone and dihydrohumulinic acids are compatible with the structures suggested (see Fig.).

The spectrum of humulone in Nujol mull showed a band at 3300 cm.$^{-1}$ attributed to a non-hydrogen-bonded hydroxyl. The asymmetry of this peak suggests that a hydrogen-bonded enolic hydroxyl group is also present in the molecule. Carbonyl bands are present at 1626 and 1663 cm.$^{-1}$, the latter being due, probably to a conjugated carbonyl group and the former to a group which is hydrogen-bonded so as to form a six-membered ring. The band at 836 cm.$^{-1}$ is typical of a triply substituted C=C group. This evidence is consistent with the enolic forms of (V) or (XII) containing internal hydrogen bonding. The spectrum is very similar in its essentials to that of lupulone (preceding paper).

The infra-red spectrum of dihydrohumulinic acid shows a very sharp hydroxyl band at 3420 cm.$^{-1}$; the slight asymmetry of the band suggests the presence of a hydrogen-bonded hydroxyl group. The carbonyl bands at 1690 and 1629 cm.$^{-1}$ are attributed to one hydrogen-bonded and one unbonded group; the dimethyl doublet at 1365 cm.$^{-1}$ is very marked. These data are consistent with structures like (IV) or (X); the sharpness of the hydroxyl band is noteworthy.

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