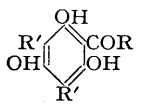
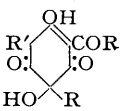
## 350. *The Chemistry of Hop Constituents. Part II.* Oxidation Products of Lupulone.*

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

A new oxidative degradation of lupulone is described, and structures are suggested for the new degradation products.

Lupulone, one of the bitter resins of the hop, was shown by Wöllmer (*Ber.*, 1916, **49**, 750; 1925, **58**, 672) to undergo hydrogenolysis, yielding 2-methylbutane and a compound believed to be represented by (I), which was then oxidised to "tetrahydrohumulone" (II). Alkaline hydrolysis of (II) gave 4-methylpentanoic acid † and dihydrohumulinic acid (III); the structure of (III) was considered incontrovertible. This degradation, considered with the lack of optical activity of lupulone, its empirical formula ($C_{26}H_{38}O_4$), and the similarity between "tetrahydrohumulone" and humulone, led Wöllmer (*loc. cit.*) to propose structure (IV) for lupulone. This structure has recently received further support by a synthesis of lupulone by trialkylation of 2 : 4 : 6-trihydroxy*iso*valerophenone with 1-bromo-3-methylbut-2-ene (Riedl, *Brauwissenschaft*, 1951, 133); although this greatly limits the possibilities it is not entirely unambiguous, as *O*-alkylation might well occur under the conditions used.


(I)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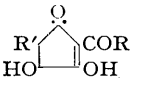
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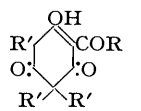

(IV)

R = $CH_2$·$CHMe_2$; R′ = $CH_2$·CH:$CMe_2$.

The present paper presents a new degradation of lupulone involving changes which do not involve the elimination of 2-methylbutane such as occurs during hydrogenolysis. Walker (*J. Inst. Brewing*, 1924, **30**, 712) has reported the stability of lupulone to aqueous alkali. It has now been shown that hydrogen peroxide in alkaline medium induces a rapid degradation in the cold, to a carboxylic acid, lupuloxinic acid, $C_{22}H_{32}O_5$, and *iso*-butyric acid. Lupuloxinic acid behaves as a strong dibasic acid ($pK_1$, 3·9; $pK_2$, 9·7) which causes effervescence in aqueous sodium hydrogen carbonate. It contains two active hydrogen atoms, shows an absorption maximum at 252 mμ (ε = 10,000 in ethanol), and gives no colour with ferric chloride solution. Lupuloxinic acid reduces one equivalent of periodate very rapidly and is unsaturated (tetranitromethane). By analogy with lupulone, it was thought that lupuloxinic acid might undergo loss of a $C_5$ fragment on hydrogenation;

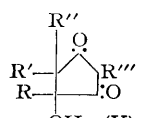
* Part I, *J.*, 1950, 1873.

† Geneva nomenclature ($CO_2H$ = 1).

this was not the case, however, hexahydrolupuloxinic acid, $C_{22}H_{38}O_5$, being the main product of hydrogenation over palladium chloride. Together with hexahydrolupuloxinic acid, an enolic fraction was present, from which a crystalline substance, hexahydrolupulenol, $C_{21}H_{38}O_3$, was isolated. The latter was obtained as the main product when Adams's catalyst in dioxan–acetic acid was used. Hexahydrolupulenol contains two active hydrogen atoms and behaves as a weak monobasic acid. It gives a purple colour with ferric chloride solution, shows characteristic ultra-violet absorption at 250 (maximum) and 256 mμ (inflexion; $\varepsilon = 14{,}000$ and 12,000 respectively in ethanol), and rapidly reduces one equivalent of periodate. It forms a phenylurethane, which is, however, obtained only in poor yield. These properties indicate clearly that the relation of hexahydrolupulenol to hexahydrolupuloxinic acid is that of a β-diketone to the corresponding ββ′-diketocarboxylic acid. That lupuloxinic acid indeed possesses the latter structure is apparent from its very smooth decarboxylation in toluene, to yield lupulenol, $C_{21}H_{32}O_3$, the properties of which are very similar to those of its hexahydro-derivative. Thus lupulenol contains two active hydrogen atoms, behaves as a monobasic acid of p$K$ 6·4, and gives a purple colour with ferric chloride solution. It possesses an absorption maximum at 253 mμ ($\varepsilon = 12{,}340$) and rapidly reduces one equivalent of periodate. Lupulenol is unsaturated (tetranitromethane), and on catalytic hydrogenation over Adams's catalyst absorbs the equivalent of six atoms of hydrogen, affording hexahydrolupulenol in excellent yield.

Periodate oxidation of lupulenol gives a resinous enolic acid which, on hydrolysis with sodium hydroxide, yields, as the major product, a compound, $C_{18}H_{30}O$. Although, by reason of its method of preparation and its lack of active hydrogen, this compound must necessarily be a ketone, it fails to react with 2 : 4-dinitrophenylhydrazine. It is unsaturated, and on hydrogenation yields its hexahydro-derivative, $C_{18}H_{36}O$, as an oil which also fails to form ketonic derivatives. Vigorous oxidation of this hexahydro-derivative gave 4-methylpentanoic acid, thus establishing the presence of the 4-methylpentanoyl or 5-methylhexanoyl group.

Since lupulenol and its hexahydro-derivative each contain three oxygen atoms, two of which are accommodated in a β-diketone system, and since each compound contains two active hydrogen atoms and reduces one equivalent of periodate, an α-ketol grouping must be present. Further, as both acids are stable to Fehling's solution, a tertiary hydroxyl group is indicated and, moreover, the ketol must be part of a ring system, otherwise its periodate oxidation would give rise to a simple ketone as a primary product. These facts are completely accommodated only by (V); alternative expressions are excluded as they would lead, on oxidation and hydrolysis, to stable diketones. The ketone, $C_{18}H_{30}O$, obtained from lupulenol must therefore be represented by R·CO·CHR′R″ where the most probable formulation for R is $CMe_2{:}CH{\cdot}CH_2{\cdot}CH_2$ and for R′ and R″ is $CMe_2{:}CH{\cdot}CH_2$. Lupulenol and its hexahydro-derivative thus become respectively (V; R = $CMe_2{:}CH{\cdot}CH_2{\cdot}CH_2$; R′ = R″ = $CMe_2{:}CH{\cdot}CH_2$; R‴ = H) and (V; R = $CHMe_2{\cdot}[CH_2]_3$; R′ = R″ = $CHMe_2{\cdot}[CH_2]_2$; R‴ = H). These formulations are compatible



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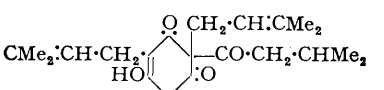

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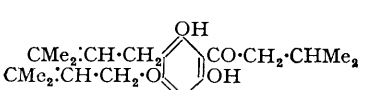


(VII)

with the formation of a monophenylurethane by hexahydrolupulenol. The similarity of the ultra-violet absorption spectra of lupulenol and hexahydrolupulenol shows that the double bonds of the former which are susceptible to hydrogenation are not conjugated; incidentally, the close similarity between the absorption of these compounds and that of 4-hydroxy-2-methyl*cyclo*pentane-1 : 3-dione (max., 249 mμ; $\varepsilon = 16{,}000$; Bastron, Davis, and Butz, *J. Org. Chem.*, 1943, **8**, 515), 2-methyl*cyclo*pentane-1 : 3-dione (max., 250 mμ; $\varepsilon = 18{,}000$; *idem, ibid.*), and 2-ethyl-4-*n*-propyl*cyclo*pentane-1 : 3-dione (max., 252 mμ; $\varepsilon = 14{,}450$; Blout, Eager, and Silverman, *J. Amer. Chem. Soc.*, 1946, **68**, 568) strongly supports the structures postulated. It follows from the foregoing that lupuloxinic acid must have the structure (V; R = $CMe_2{:}CH{\cdot}CH_2{\cdot}CH_2$; R′ = R″ =

$CMe_2{:}CH{\cdot}CH_2$; R''' = $CO_2H$), and that therefore for lupulenol R''' in formula (V) must be H.

Only the structures (V) now postulated for lupulenol and lupuloxinic acid satisfactorily explain all the properties of the compounds. It must be admitted, none the less, that a clear understanding of the mechanism whereby lupulone becomes transformed into lupuloxinic acid is not yet possible. The recent synthesis by Riedl (*loc. cit.*) was accepted by him as confirming the structure (IV) postulated by Wöllmer (*loc. cit.*). It should be pointed out, however, that the synthesis could equally well lead to (VI) or (VII), of which the latter seems the more acceptable, since it alone accommodates the hydrogenolysis of lupulone as a rational property of its structure.

## Experimental

*Lupulone and Humulone.*—(a) *From hops.* The method of Lewis *et al.* (*J. Clin. Invest.*, 1949, **28**, 916) was adapted for the extraction of lupulone from hops. Ground, commercially dried cones of Bullion hops (4·91 kg.) were packed in columns (5 in. diameter × 9 in. high) and extracted by percolation with light petroleum (b. p. 40—80°; 10 l.). The percolates were dried ($CaCl_2$) and evaporated under nitrogen *in vacuo* at 40°, to leave a syrup (870 g.) from which lupulone crystallized on cooling to 0°. Recrystallization from light petroleum gave lupulone (75 g., 1·5% based on hops), m. p. 92—94° (Found : C, 75·0, 75·4, 75·6; H, 9·1, 9·4, 9·2; active H, 0·49%; I.V., 126·7; equiv. by titration, 404, 411. Calc. for $C_{26}H_{38}O_4$: C, 75·3; H, 9·2; 2 active H, 0·48%; I.V. for 2 double bonds, 123·7; equiv., 414). The mother-liquors from the initial crystallization of the lupulone were diluted with methanol (1 l.), the solution set aside for 30 minutes, and the upper layer decanted from black resin. A solution of lead acetate (238 g.) in a mixture of methanol (250 c.c.) and water (140 c.c.) was added, and the precipitate filtered off on kieselguhr (Hyflo Supercel) and washed with methanol. The air-dried humulone lead salt weighed 483 g.

(b) *From lupulin.* A suspension of Horst's Fancy Oregon lupulin (500 g.) in methanol (2 l.) was set aside under nitrogen overnight. The suspension was then filtered and the residue was washed thoroughly with methanol (500 c.c.) by resuspension. To the filtrate was added a solution of lead acetate (157 g.) in a mixture of water (96 c.c.) and methanol (864 c.c.). The humulone lead salt was filtered off, washed with methanol, and dried in air (yield, 217 g.). The filtrate and washings were combined and diluted with light petroleum (2 l.), followed by 1% aqueous sodium chloride (4 l.). The upper layer was removed and the aqueous layer extracted with light petroleum (1 l.). The combined light petroleum extracts were washed with 1% aqueous sodium chloride, dried ($Na_2SO_4$), and evaporated under nitrogen *in vacuo* at 40°, leaving a dark oil (119 g.). The oil was dissolved in light petroleum (100 c.c.) and the solution set aside at 0° while lupulone (35 g.), m. p. 86—91°, separated. A second crop (9·6 g.) was obtained by concentration of the mother-liquors. The combined crops were recrystallized from 90% aqueous methanol, giving lupulone (45 g.), m. p. 89°.

*Lupuloxinic Acid.*—To a solution of lupulone (10 g.) in 10% aqueous sodium hydroxide (500 c.c.) at room temperature was added 30% hydrogen peroxide (40 c.c.), and the solution was kept at room temperature for 5 hours. The gel resulting was stirred into iced 10% sulphuric acid (1 l.) and the mixture was cooled to 0° overnight. The *lupuloxinic acid* was filtered off and the filtrate was reserved. The product was dissolved in ethanol (30 c.c.), and treated with water (70 c.c.). When separation was complete, the product was filtered off, dried *in vacuo* over calcium chloride, and thoroughly stirred with light petroleum (b. p. 40—60°) to remove yellow material; *lupuloxinic acid* (5·0 g.), m. p. 107° (decomp.), remained. For analysis the material was recrystallized from aqueous methanol, forming colourless plates, m. p. 107—108° [Found : C, 70·2, 70·6; H, 8·6, 8·4; active H, 0·62%; *M* (Rast), 348; equiv. by titration, 192, 384. $C_{22}H_{32}O_5$ requires C, 70·2; H, 8·5; 2 active H, 0·54%; *M*, 378; equiv. (dibasic acid), 189, 378]. Lupuloxinic acid was soluble, with effervescence, in aqueous sodium hydrogen carbonate, to give a colourless solution. It did not yield a crystalline *p*-bromophenacyl ester under the usual conditions.

The filtrate from the reaction mixture was extracted with ether (5 × 100 c.c.). The combined extracts were shaken with three portions of saturated aqueous sodium hydrogen carbonate, and the aqueous layers were acidified, saturated with sodium chloride, and extracted with ether (5 × 20 c.c.). The combined extracts were dried ($Na_2SO_4$) and evaporated. The residue was distilled, affording *iso*butyric acid (0·660 g.), b. p. 152—154°, characterized as *p*-bromophenacyl *iso*butyrate, m. p. 76°.

*Hydrogenation of Lupuloxinic Acid.*—(i) *In the presence of palladium chloride.* Lupuloxinic acid (3·0 g.) in methanol (70 c.c.) containing 5% aqueous palladium chloride (2 c.c.) was shaken with hydrogen. The rapid uptake was of 570 c.c., after which hydrogenation became very slow. The catalyst was filtered off, and the filtrate diluted with water (300 c.c.) and extracted thoroughly with ether. The combined ethereal extracts were washed with water and then extracted thoroughly with saturated aqueous sodium hydrogen carbonate. Acidification of the aqueous layer yielded an oil which soon crystallized. The product was recrystallized from aqueous methanol, affording *hexahydrolupuloxinic acid* (1·38 g.), m. p. 154—164°. By recrystallization from aqueous methanol, a small amount of the pure acid, m. p. 156—157° (decomp.), was obtained (Found : C, 69·3; H, 9·6; active H, 0·63. $C_{22}H_{38}O_5$ requires C, 69·1; H, 9·9; 2 active H, 0·53%). The acid gave no colour with ferric chloride.

The ethereal extracts, having been extracted with sodium hydrogen carbonate, were extracted with 2N-sodium hydroxide (2 × 100 c.c.). The combined sodium hydroxide extracts were acidified with hydrochloric acid, to give a sticky semicrystalline precipitate which, on recrystallization from aqueous methanol, yielded *hexahydrolupulenol* (0·68 g.), m. p. 178° [Found : C, 74·9, 74·9; H, 11·2, 11·2; active H, 0·55%; *M* (Rast), 334; equiv. by titration, 332. $C_{21}H_{38}O_3$ requires C, 74·6; H, 11·3; 2 active H, 0·60%; *M*, 338; equiv. (for monobasic acid), 338]. Hexahydrolupulenol was soluble in 2N-sodium hydroxide, and was not affected by aqueous sodium hydrogen carbonate. Potentiometric titration showed the compound to have p*K* 6·3, and to be monobasic. Heating hexahydrolupulenol in refluxing toluene with phenyl *iso*cyanate for 1 hour gave the *phenylurethane*, in poor yield, as colourless rhombs (from aqueous methanol), m. p. 148° (Found : N, 3·2. $C_{28}H_{43}O_4N$ requires N, 3·1%). The enol (0·5 g.) was recovered unchanged after being heated under reflux with a solution of 12N-hydrochloric acid (1 c.c.) in methanol (20 c.c.).

(ii) *In the presence of platinum oxide.* Lupuloxinic acid (900 mg.) in dioxan (30 c.c.) and glacial acetic acid (20 c.c.) was hydrogenated at 22° in the presence of Adams's platinum oxide. Uptake of hydrogen became very slow after 137 c.c. had been absorbed (theor. for 3 double bonds, 160·5 c.c.). When a further quantity of Adams's catalyst was added, rapid uptake of hydrogen occurred during 5 minutes, after which hydrogenation became very slow. The total uptake was 200 c.c. The catalyst was removed, and the filtrate evaporated. The crystalline residue was recrystallized from aqueous methanol, affording hexahydrolupulenol (506 mg.), m. p. and mixed m. p. 178°.

*Lupulenol.*—A suspension of lupuloxinic acid (2·0 g.) in toluene (15 c.c.) was heated slowly to boiling. At just over 100°, carbon dioxide was evolved (baryta test). After 5 minutes' refluxing the yellow solution was cooled to 0°, seeded, and stored overnight at 0°. *Lupulenol* (1·7 g.) was filtered off, washed with light petroleum, and dried. The m. p. was 118°, unchanged by crystallization from aqueous methanol (needles) [Found : C, 76·1; H, 9·4; active H, 0·52%; *M* (Rast), 297; equiv. by titration, 337. $C_{21}H_{32}O_3$ requires C, 75·9; H, 9·7; 2 active H, 0·60%; *M*, 332; equiv., 332]. Lupulenol was insoluble in aqueous sodium hydrogen carbonate. It dissolved in 2N-aqueous sodium hydroxide and was precipitated unchanged on acidification. In 50% aqueous methanol the acid had p*K* 6·4. Treating lupulenol (0·2 g.) with 12N-hydrochloric acid (5 c.c.) in methanol (20 c.c.) under reflux gave a yellow solution : the product could not be crystallised.

*Hexahydrolupulenol.*—Lupulenol (5 g.) in methanol (50 c.c.), hydrogenated over Adams's platinum oxide catalyst, absorbed 104 c.c. of hydrogen (theor. for 3 double bonds, 101 c.c.). The catalyst was removed and the methanol was evaporated *in vacuo*. The residue was crystallized from aqueous methanol, giving hexahydrolupulenol (5 g.), m. p. and mixed m. p. 178°.

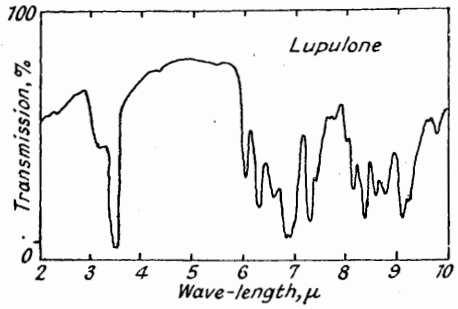
*Periodate Oxidation of Lupulenol.*—A solution of lupulenol (2·6 g.) in ethanol (65 c.c.) gave a clear solution in aqueous sodium metaperiodate (1·8 g. in 26 c.c.). After 5 seconds sodium iodate began to separate. After 1 hour the sodium iodate (1·4 g.) was filtered off and washed with ethanol. The filtrates were diluted with water (400 c.c.), acidified with 2N-hydrochloric acid (20 c.c.), and extracted with ether (300 and 150 c.c.). The combined extracts were washed with water, dried ($Na_2SO_4$), and evaporated, to leave a yellow oil. This was treated with 2N-sodium hydroxide (100 c.c.) under reflux for 6 hours. The insoluble material was removed with ether and the ethereal solution was washed with water, dried ($Na_2SO_4$), and evaporated. The yellow oil which remained was distilled at 196°/40 mm. as a pale yellow oil (0·5 g.). Redistillation at 196°/40 mm. gave a colourless oil, $n_D^{12}$ 1·4832 [Found : C, 82·4; H, 11·7; active H, 0·07%; *M* (Rast), 218. $C_{18}H_{30}O$ requires C, 82·4; H, 11·4%; *M*, 262].

*Hydrogenation of the Compound,* $C_{18}H_{30}O$.—The compound (1·2 g.), when hydrogenated in

methanol (20 c.c.) over Adams's platinum oxide, absorbed 320 c.c. of hydrogen (theor. for three double bonds, 312 c.c.). The *hexahydro*-derivative, isolated by means of ether, was a colourless oil (1·0 g.), b. p. 166°/20 mm., $n_D^{12}$ 1·4506, $d_{12}^{12}$ 0·845 (Found: C, 80·2; H, 13·3. $C_{18}H_{36}O$ requires C, 80·6; H, 13·5%).

*Periodate Oxidation of Hexahydrolupulenol.*—Hexahydrolupulenol (4 g.) in ethanol (100 c.c.) was mixed with aqueous sodium metaperiodate (2·7 g. in 40 c.c.). After 1 hour the sodium iodate (2·3 g.) was filtered off and washed with ethanol. The fitrate was worked up in the usual way, to yield a yellow resin which was heated under reflux with 2N-sodium hydroxide for 6 hours. The oil was removed with ether, dried, and isolated. The product was distilled at 168°/20 mm., affording the compound, $C_{18}H_{36}O$ (1·2 g.), $n_D^{20}$ 1·4445, $d_{12}^{20}$ 0·845.

*Oxidation of the Compound,* $C_{18}H_{36}O$.—The compound (0·8 g.) was heated with refluxing concentrated nitric acid (40 c.c.) for 2 hours. The solution was diluted with water (250 c.c.), saturated with salt, and extracted with ether (3 × 50 c.c.). The combind ethereal extracts were extracted with saturated aqueous sodium hydrogen carbonate (3 × 20 c.c.). The combined extracts were acidified with 12N-hydrochloric acid, saturated with sodium chloride, and extracted with ether (3 × 50 c.c.). The ethereal solutions were dried ($CaCl_2$) and evaporated, leaving an acid which was distilled at *ca.* 166°/760 mm. (80 mg.). The product was converted into the *p*-bromophenacyl ester, which crystallized as plates, m. p. 67°, from methanol. Mixtures with the following *p*-bromophenacyl esters had the m. p.s shown (m. p. of authentic material in parentheses): *iso*butyrate (77°), 54°; *iso*valerate (68°), 57°; 4-methylpentanoate (76°), 73°.




*Infra-red Spectra.*—The infra-red absorption spectrum of lupulone in Nujol mull (see Fig.) shows a band at 3130 cm.$^{-1}$, ascribed to a hydrogen-bonded OH group. Carbonyl bands occur at 1588 and 1666 cm.$^{-1}$, the former being attributed to a hydrogen-bonded carbonyl group and the latter to a non-bonded, conjugated carbonyl group. A band at 1100 cm.$^{-1}$ cannot be positively identified, although it is not inconsistent with the presence of an ether grouping. The triply substituted C⁼C group is identified as that giving rise to the band at 827 cm.$^{-1}$.

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THE BREWING INDUSTRY RESEARCH FOUNDATION,
NUTFIELD, SURREY.

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