185. *Chemistry of Hop Constituents. Part XVIII.*[1] *Hulupinic Acid.*

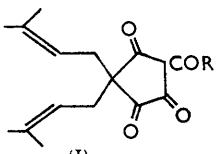
By J. S. Burton and R. Stevens.
(*With an Addendum.* By J. A. Elvidge).

Cohulupone and hulupone are oxidized to hulupinic acid, which has the structure 4,5-dihydroxy-2,2-di-(3-methylbut-2-enyl)cyclopent-4-ene-1,3-dione (II).

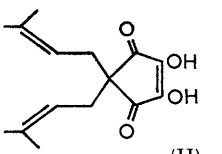
Among the oxidation products of the hop β-acids (lupulones) the hulupones have now been well characterized.[1-3] When colupulone is boiled in wort [3b] or in aqueous solution it is partially converted into cohulupone (I; R = Pr^i) but when the conversion was followed for 9 hours by paper chromatography it was found that cohulupone failed to

[1] Part XVII, Burton and Stevens, *J.*, **1963**, 4382.
[2] (*a*) Stevens and Wright, *J.*, **1963**, 1763; (*b*) Wright, *J.*, **1963**, 1769.
[3] (*a*) Spetsig and Steninger, *J. Inst. Brewing*, 1960, **65**, 413; (*b*) Stevens and Wright, *ibid.*, 1961, **67**, 496; (*c*) Lloyd, Proc. Eur. Brewing Conv., Vienna, 1961, Elsevier, p. 112.

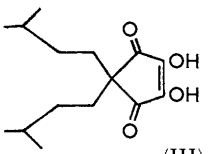
accumulate but was oxidized further. Owing to the limiting solubility of colupulone in water the reaction could only be carried out in dilute solution, and no pure product could be isolated. The autoxidation of cohulupone was therefore investigated in alcoholic solutions. Thus, when a stream of oxygen was passed through a boiling solution of cohulupone in ethanol it was shown by paper chromatography that all the cohulupone was oxidized after 3 days. From this reaction a crystalline product was isolated for which we suggest the name hulupinic acid and, on the basis of the evidence here presented, assign the structure 4,5-dihydroxy-2,2-di-(3-methylbut-2-enyl)cyclopent-4-ene-1,3-dione (II).



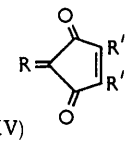
(I)



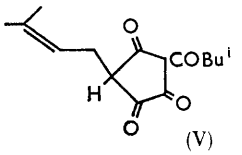
(II)



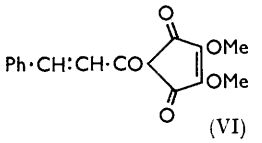
(III)



(IV)



(V)



(VI)

Hulupinic acid, which lacked the bitter properties of the hulupones, analysed satisfactorily for $C_{15}H_{20}O_4$ showing the apparent loss of a C_4 fragment. Since the same product was obtained when oxidation was carried out in methanol and propan-2-ol, an alcoholic residue cannot have added to the molecule. The ultraviolet light absorption spectrum was similar to that of cohulupone but all the maxima were displaced to longer wavelengths. The infrared spectrum showed strong absorption at 3300 cm.$^{-1}$, associated with bonded hydroxyl, and carbonyl absorptions at 1750, 1685, and 1660 cm.$^{-1}$.

Hulupinic acid was soluble in sodium hydrogen carbonate solution giving an intense yellow solution. Its salts, however, in contrast to those of the hulupones, were not soluble in ether. With ammonia it gave an amorphous monoammonium salt. Methylation of hulupinic acid with methyl iodide and potassium carbonate in acetone or with ethereal diazomethane gave an oily dimethyl ether which had the same ultraviolet light absorption ($\lambda_{max.}$ 293 mμ) in acid and alkaline solution.

Hydrogenation of hulupinic acid gave a tetrahydro-derivative (III) which had similar ultraviolet light absorption to the parent compound and which on further oxidation with alkaline hydrogen peroxide afforded di-isopentylmalonic acid. No isobutyric acid could be detected among the oxidation products in contrast to the oxidation of tetrahydrocohulupone.[2a] The same tetrahydrohulupinic acid was obtained when tetrahydrocohulupone [2a] underwent autoxidation in ethanolic solution. Hydrogenation of the dimethyl ether of hulupinic acid afforded the corresponding tetrahydro-derivative. Hulupinic acid was unchanged on attempted reduction with sodium borohydride but lithium aluminium hydride, as with cohulupone,[1] caused drastic modification of the molecule. The sum of the degradative evidence is that the acyl side-chain present in cohulupone has been replaced by a hydroxyl group in hulupinic acid, and this view is supported by the fact that hulupone (I; R = Bu^i) itself [2b] gave rise to the same hulupinic acid.

The triketone (V), related to hulupone, was unchanged by attempts to oxidize it in a similar manner to that employed to prepare hulupinic acid.

Hulupinic acid is therefore regarded as having structure (II), the enediol structure being preferred to the ketol on the basis of spectroscopic data. No exact model existed for

comparison of the ultraviolet light absorption spectrum. The parent system, cyclopent-4-ene-1,3-dione (IV; R = H_2, R′ = H) has recently been prepared [4a] and its 2-benzylidene derivative [4b] (IV; R = Ph·CH=, R′ = H) together with the open-chain analogue, dihydroxyfumaric acid,[5] provided the closest analogies. More recently, however, spectroscopic data for croconic acid (IV; R = O, R′ = OH) have become available.[6] This compound has $\lambda_{max.}$ 231 and 298 (log ε 4·13 and 4·45) in acidic solution comparable with hulupinic acid ($\lambda_{max.}$ 301 mμ [log ε 4·01]). Similarities may also be noted between the structure of the dimethyl ether of hulupinic acid and linderone [7] (VI).

The mechanism of the oxidation remains unknown. In contrast, alkaline hydrolysis of the related isohumulinic acid (V; with alkene side-chain saturated) affords 5-isopentylcyclopentane-1,2,4-trione but the unsaturated compound (V) is more resistant to hydrolysis.[8] It is perhaps noteworthy that when the ethanolic reaction mixture was concentrated and examined by gas chromatography no peak with the retention time of ethyl isovalerate was observed but a peak was present with a slightly higher retention time, which, although not characterized further, was regarded as a decomposition or rearrangement product of ethyl peroxyisovalerate.

Addendum

Final confirmation of structure (II) came from a study of the nuclear magnetic resonance spectrum of a 10% solution in deuterochloroform. This showed signals, at 60 Mc./sec., corresponding to 2 pairs of methyl groups (τ 8·44, 8·38; each signal being broadened J approx. 1c./sec., by an olefinic proton), a pair of equivalent methylene groups (τ 7·28; each signal being split as a doublet, J = 8·0 c./sec., by an olefinic proton), 2 equivalent olefinic protons (τ 5·16; each signal being split as a triplet, J = 8·0 c./sec., by the adjacent methylene), and a broad (1·2 p.p.m.) band centred at τ 2·6, corresponding to 2 exchangeable hydroxyl protons. The proton resonance spectrum is therefore in agreement with structure II for hulupinic acid, although it is not possible to distinguish between this and the isomeric dienol form. The diketone–ketol tautomers, however, are ruled out by the absence from the spectrum of a signal attributable to a proton in the environment ·CO·CH(O·)· and the fact that there are *two* enolic protons. The alternative dienol form of (II) would be a *vic*-diketone and would be expected to be yellow and absorb light weakly in the visible region of the spectrum: hence this is rejected in favour of structure (II). In this connection it should be mentioned that although hulupone (I) is written here in the triketone form it is undoubtedly enolized, and the major enolic form(s) can be distinguished by nuclear resonance spectroscopy.[9]

Experimental

Light petroleum refers to the fraction of b. p. 40—60°. Paper chromatography was carried out on Whatman's DE-20 paper in the acetate form and 2% glacial acetic acid in methanol as solvent.[10] Using this system the following R_F values were found: colupulone, 0·55; cohulupone, 0·01; and hulupinic acid, 0·32.

Hulupinic Acid (II).—(*a*) Cohulupone [2b] (I; R = Pr^i) (2·07 g.) in ethanol (400 ml.) was heated under reflux while a stream of oxygen was passed through the solution. After 3 days the solvent was removed in a vacuum and the residual mass of crystals washed with light

[4] (*a*) DePuy and Zawaski, *J. Amer. Chem. Soc.*, 1959, **81**, 4920; (*b*) DePuy and Wells, *ibid.*, 1960, **82**, 2909.
[5] Goodwin and Witkop, *J. Amer. Chem. Soc.*, 1954, **76**, 5599.
[6] Fatiadi, Isbell, and Sager, *J. Res. Nat. Bur. Stand.*, 1963, **67**, *A*, 153.
[7] Kiang, Lee, and Sim, *J.*, 1962, 4338.
[8] Harris, Howard, and Pollock, *J.*, 1952, 1906.
[9] Forsen, Nilsson, Elvidge, Burton, and Stevens, *Acta Chem. Scand.*, forthcoming publication.
[10] Roberts, *J. Inst. Brewing*, 1962, **68**, 302.

petroleum (3 × 20 ml.) and recrystallized from ether–light petroleum and aqueous methanol. *Hulupinic acid* (II) formed needles (0·48 g.), m. p. 167—168° (Found: C, 68·4, 68·05; H, 7·5, 7·55%; *M* (ebullioscopic), 249. $C_{15}H_{20}O_4$ requires C, 68·2; H, 7·6%; *M*, 264); $\lambda_{max.}$ 301 mμ (log ε 4·01) in acidified ethanol and $\lambda_{max.}$ 261 and 393 mμ (log ε 4·16 and 4·09) in alkaline ethanol; $\nu_{max.}$ (KBr disc) 3300, 3000, 1750, 1685, 1660, 1452, 1382, 1320, 1280, 1220, 1190, 1110, 1078, 1056, 1026, 978, 895, 879, 847, 790, 771, 751, and 700 cm.$^{-1}$. Similar results were obtained when the oxidation was carried out in methanol and propan-2-ol.

(*b*) In a similar manner hulupone [2b] (I; R = Bu^i) (0·47 g.) was oxidized to hulupinic acid (0·10 g.), m. p. and mixed m. p. 167—168°.

4,5-Dimethoxy-2,2-di-(3-methylbut-2-enyl)cyclopent-4-ene-1,3-dione.—(*a*) Hulupinic acid (82 mg.) in dry acetone (10 ml.) was heated under reflux with methyl iodide (0·14 g.) and anhydrous potassium carbonate (0·14 g.) for 24 hr. After dilution with water and acidification, the reaction mixture was extracted with ether, and the extract washed, dried, and evaporated. The residual oil was distilled, b. p. 115—120°/8 × 10^{-4} mm., to afford the *dimethyl ether* (Found: C, 69·8; H, 8·5. $C_{17}H_{24}O_4$ requires C, 69·9; H, 8·2; OMe, 21·2%); $\lambda_{max.}$ 293 mμ (log ε 3·92) $\lambda_{min.}$ 248 mμ in both acidic and basic ethanol; $\nu_{max.}$ (as a film) 3000, 1755, 1700, 1635, 1485, 1445sh, 1382, 1340, 1300sh, 1218, 1144, 1120, 1030, 950sh, 934, 898, 862, and 837 cm.$^{-1}$.

(*b*) Hulupinic acid (0·21 g.) in ether (5 ml.) was added to an excess of ethereal diazomethane. The mixture was set aside for 6 days and the solvent removed. The residual oil was distilled, b. p. 125—130° (bath)/8 × 10^{-4} mm., giving the dimethyl ether which had an infrared spectrum identical with that of the sample obtained above (Found: OMe, 23·1%).

Tetrahydrohulupinic Acid (III).—(*a*) Hulupinic acid (0·17 g.) in methanol (30 ml.) was hydrogenated in the presence of Adams catalyst, until no more hydrogen was absorbed (31 ml.; 2·0 double bonds). After removal of catalyst and solvent the residue was recrystallized from aqueous methanol to afford *tetrahydrohulupinic acid* (0·11 g.), m. p. 171—172° (Found: C, 67·3; H, 8·8. $C_{15}H_{24}O_4$ requires C, 67·2; H, 8·95%); $\lambda_{max.}$ 300 mμ (log ε 4·04) in acidified ethanol and $\lambda_{max.}$ 260 and 395 mμ (log ε 4·15 and 4·08) in alkaline ethanol; $\nu_{max.}$ (KBr disc) 3300, 3000, 1670, 1640, 1382, 1368, 1332, 1295, 1240, 1176, 1110, 1097, 1022, 984, 917, 776, 740, and 700 cm.$^{-1}$.

(*b*) Tetrahydrocohulpone [2a] (1·86 g.) in ethanol (400 ml.) was oxidized as above to afford tetrahydrohulupinic acid (0·12 g.) with m. p. and mixed m. p. 170—171°.

4,5-Dimethoxy-2,2-di-isopentylcyclopent-4-ene-1,3-dione.—The dimethyl ether of hulupinic acid (0·14 g.) in methanol (15 ml.) was hydrogenated in the presence of Adams catalyst. After removal of catalyst and solvent the *dimethyl ether* of tetrahydrohulupinic acid distilled, b. p. 120—125° (bath)/6 × 10^{-4} mm. (Found: C, 68·9; H, 9·8. $C_{17}H_{28}O_4$ requires C, 68·9; H, 9·5%); $\lambda_{max.}$ 293 mμ (log ε 3·82) in both acidic and basic ethanol.

Oxidation of Tetrahydrohulupinic Acid.—The acid (0·11 g.) in 2N-sodium hydroxide (2 ml.) was set aside with hydrogen peroxide (1·5 ml.), for 3 days. Acidification afforded di-isopentylmalonic acid which, after recrystallization from benzene–light petroleum, had m. p. and mixed m. p. 152—154°. Isobutyric acid could not be isolated from the mother-liquors.

Attempted Reduction with Sodium Borohydride.—Hulupinic acid (0·19 g.) in methanol (6 ml.) was added with stirring to a solution of sodium borohydride (0·19 g.) in water (7 ml.). A vigorous reaction occurred and the mixture was stirred for a further 2½ hr.; working up in the usual way, however, afforded unchanged hulupinic acid (0·15 g.), m. p. and mixed m. p. 167—168°.

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Brewing Industry Research Foundation,
Nutfield, Surrey.
(J. A. E.) Imperial College of Science and Technology,
London S.W.7.

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