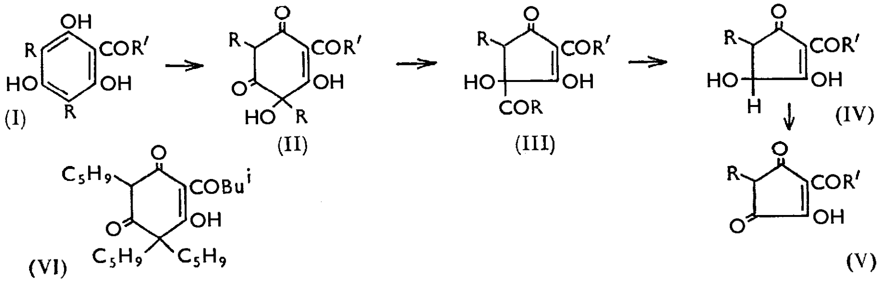
32. *Chemistry of Hop Constituents. Part XIV.** *2,4-Diacetyl-3,4-dihydroxy-5-methylcyclopent-2-enone, An Analogue of Isohumulone A.*

By P. Margaret Brown and G. A. Howard.

4-Acetyl-5,6-dihydroxy-2,6-dimethylcyclohex-4-ene-1,3-dione, an analogue of the hop bitter substance humulone, is isomerised by hot dilute aqueous sodium carbonate to 2,4-diacetyl-3,4-dihydroxy-5-methylcyclopent-2-enone. This cyclopentenone, an analogue of isohumulone A, is hydrolysed by aqueous sodium hydroxide to 2-acetyl-3,4-dihydroxy-5-methylcyclopent-2-enone and oxidised by air in the presence of lead acetate to 4-acetyl-5-hydroxy-2-methylcyclopent-4-ene-1,3-dione. The analogue lacks the bitter taste and the considerable bacteriostatic power which distinguish isohumulone A.

Transformation of humulone (II; $R = C_5H_9$, $R' = Bu^i$) into isohumulone A (III; $R = C_5H_9$, $R' = Bu^i$) is important in brewing since the latter and its congeners are largely responsible for the bitter flavour and bacteriostatic power imparted by hops to beer. Although analogues of humulone have been synthesized none of isohumulone A has been prepared (other than those which are derived from hops as such).



The simplest known analogue (II; $R = R' = Me$) of humulone, obtained by Campbell and Coppinger[1] by aerial oxidation of dimethylphloroacetophenone (I; $R = R' = Me$) in the presence of lead acetate, was selected as a suitable starting point for such a synthesis. We find that in this oxidation never more than 0·35 mol. of oxygen is absorbed and much of the starting material can be recovered and used again. The product differed from humulone in having no bitter taste and only low bacteriostatic activity against *Lactobacilli* isolated from infected beer.

Boiling 0·1N-sodium carbonate, which converts humulone into isohumulone A,[2] converted the analogue (II; $R = R' = Me$) into a crystalline substance which resembled isohumulone A[3] in giving a red colour with methanolic ferric chloride while its equivalent weight and ultraviolet and infrared absorption were consistent with its having the structure (III; $R = R' = Me$). Like that of isohumulone A, its ultraviolet light absorption in aqueous solution at all pH's above 1 is similar to that in alkaline ethanol. Analysis fails to distinguish between the compounds (III; $R = R' = Me$) and (IV; $R = R' = Me$), so close characterisation was needed. Although the only crystalline derivatives of isohumulone A are the metal salts,[4] the new compound readily formed crystalline anils the analyses of which left no doubt that our ketone was the desired analogue (III; $R = R' = Me$) of isohumulone A. Hydrolysis by dilute sodium hydroxide afforded the crystalline analogue

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* Part XIII, *J.*, 1959, 545.

[1] Campbell and Coppinger, *J. Amer. Chem. Soc.*, 1951, **73**, 1849.

[2] Howard, *J. Inst. Brewing*, in the press.

[3] Howard and Tatchell, *ibid.*, 1957, 142.

[4] Hudson and Rudin, *ibid.*, in the press.

(IV; R = R′ = Me) of humulinic acid (IV; R = C_5H_9, R′ = Bu^i), which was also characterised by the preparation of anils. As with isohumulone A, potentiometric titration of the compound (III; R = R′ = Me) with methanolic lead acetate failed to reveal the formation of a lead salt although, after titration, the solution deposited a precipitate. Removal of lead from the deposit afforded a crystalline substance which from its ultraviolet spectrum and ferric chloride reaction appeared to have structure (V; R = R′ = Me). This unexpected reaction recalls the ready oxidation [5] of isohumulone A itself by bismuth acetate to the compound (V; R = C_5H_9, R′ = Bu^i) and provides further evidence of the ring structure and ease of oxidation of isohumulone A and its analogues. Isohumulone A and the other iso-compounds present in beer give blue colours with the Folin–Denis and the Folin–Ciocalteu reagent which have been proposed as a method for their determination,[6,7] but the analogue (III; R = R′ = Me) gives no colour with these reagents. The crystalline isohumulones,[8] tetrahydroisohumulone, and tetrahydroisocohumulone [5] also give no reaction with the Folin–Ciocalteu reagent and this reaction is thus apparently confined to certain iso-compounds of the type found in beer. The reasons for this specificity remain unknown.

The isohumulone analogue (III; R = R′ = Me) differed from the bitter substances found in beer also in that it had no bitter taste and its bacteriostatic power was low. The latter finding recalls Riedl's observations [9] that the bacteriostatic power of analogues of lupulone (VI) falls as the size of the acyl side-chain is reduced.

EXPERIMENTAL

4-*Acetyl*-5,6-*dihydroxy*-2,6-*dimethylcyclohex*-4-*ene*-1,3-*dione* (II; R = R′ = Me).—A solution of dimethylphloroacetophenone (12·5 g.) and lead acetate trihydrate (24 g.) in methanol (400 ml.) was shaken with oxygen. In 4 min., when 500 ml. of oxygen had been taken up, the rate of uptake had diminished and the precipitate (21 g.) was filtered off and decomposed with hydrogen sulphide in methanol to give a product (5·5 g.). Concentration of the filtrate from the oxidation gave unchanged starting material (4·5 g.; m. p. 228—230°) which was shaken in methanolic lead acetate (8 g.) until uptake of oxygen (170 ml.) had ceased (30 min.). Filtration gave the lead salt (6 g.) from which further product (2·2 g.) was obtained. Dilution with water of the filtrate from the oxidation gave unchanged starting material (2 g.). The total crude product (7·9 g., 69%), m. p. 156°, was recrystallised from water to give the pure product (6·5 g.), m. p. 157·5—159° [Found: C, 56·9; H, 5·95; active H, 1·28%; *M*, by potentiometric titration against methanolic lead acetate (4% w/v), 220. Calc. for $C_{10}H_{12}O_5$: C, 56·6; H, 5·7; 3 active H, 1·41%; *M*, 212], having $\lambda_{max.}$ 230, 240, 283, and 315 mμ (ε 10,800, 10,200, 9650, and 6400 respectively) in acidic ethanol and 257 and 310 mμ (ε 19,400 and 12,800 respectively) in alkaline ethanol.

The compound gave a blue colour with the Folin–Denis reagent but failed to reduce ammoniacal silver nitrate.

2,4-*Diacetyl*-3,4-*dihydroxy*-5-*methyl*cyclo*pent*-2-*enone* (III; R = R′ = Me).—A solution of the preceding product (2·2 g.) and sodium carbonate (5·2 g.) in water (100 ml.) was raised to the b. p. in 2 min., boiled for 2 min., cooled rapidly, and acidified at 0°. The solution was saturated with sodium chloride and extracted with ethyl acetate, and the extract washed with brine and evaporated. The solid residue crystallised from water, giving the *product* as rosettes of needles, m. p. 118—119° (Found: C, 56·8; H, 5·9; active H, 0·92%; equiv., 212, 204, 203. $C_{10}H_{12}O_5$ requires C, 56·6; H, 5·7; 2 active H, 0·92%; equiv., 212). The compound sublimed readily *in vacuo*, gave a red colour with methanolic ferric chloride, was acidic to litmus, failed to reduce Fehling's solution, the Folin–Denis reagent, or ammoniacal silver nitrate, and had $\lambda_{max.}$ 227 and 265 mμ (ε 10,900 and 9100 respectively) in acidic ethanol and 253 and 267 (infl.) mμ (ε 19,200 and 18,100 respectively) in alkaline ethanol. In 0·2M-sodium phosphate buffers it had $\lambda_{max.}$ 253 and 270 (infl.) mμ (ε 15,200 and 12,200 respectively) at pH 1·0 and $\lambda_{max.}$ 253 and 270 mμ (ε 18,800 and 14,900 respectively) at pH 2·4 and 4·4. It afforded in the usual way an

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[5] Brown, Howard, and Tatchell, *J.*, 1959, 545.
[6] Hall, *Proc. Europ. Brew. Conven.*, 1957, 314.
[7] Owades, Jakovac, and Brenner, *Amer. Brewer*, 1959, No. 2, p. 42.
[8] Howard, Slater, and Tatchell, *J. Inst. Brewing*, 1957, 237.
[9] Riedl, *Annalen*, 1953, **585**, 38.

anil, m. p. 168° (from methanol) (Found: C, 66·2; H, 5·9; N, 4·9. $C_{16}H_{17}O_4N$ requires C, 66·9; H, 5·9; N, 4·9%), $\lambda_{max.}$ 245 and 315 mμ (ε 12,700 and 22,000 respectively) in ethanol and 270 mμ (ε 20,300) in alkaline ethanol, a p-*chloroanil*, m. p. 155° (from ethanol) (Found: C, 59·8; H, 4·5; N, 4·0; Cl, 10·9. $C_{16}H_{14}O_4NCl$ requires C, 59·7; H, 5·0; N, 4·4; Cl, 11·0%), and a 2,4-*dichloroanil*, m. p. 168° (from ethanol) (Found: C, 53·9; H, 4·4; N, 4·2; Cl, 19·7. $C_{16}H_{15}O_4NCl_2$ requires C, 53·9; H, 4·2; N, 3·9; Cl, 19·9%).

2-*Acetyl*-3,4-*dihydroxy*-5-*methyl*cyclo*pent*-2-*enone* (IV; R = R′ = Me).—The compound (III; R = R′ = Me) (500 mg.) in ethanol (7·5 ml.) and aqueous N-sodium hydroxide (25 ml.) was boiled under reflux for 4 hr. and then poured on ice. The solution was acidified at 0° and extracted with ethyl acetate, and the extract washed with brine, dried ($MgSO_4$), and evaporated to a solid (326 mg.; m. p. 92—94°) which was sublimed at 100°/2 mm., giving the *product*, m. p. 111—112° (unchanged on recrystallisation from water) (Found: C, 56·4; H, 6·3. $C_8H_{10}O_4$ requires C, 56·4; H, 5·8%). The compound gave an orange colour with methanolic ferric chloride and afforded in the usual way an *anil*, m. p. 205° (from ethanol) (Found: C, 68·0; H, 6·1; N, 5·8. $C_{14}H_{15}O_3N$ requires C, 68·6; H, 6·1; N, 5·7%), a p-*chloroanil*, m. p. 177° (from ethanol) (Found: C, 59·7; H, 5·0; N, 5·1; Cl, 13·2. $C_{14}H_{14}O_3NCl$ requires C, 60·1; H, 5·0; N, 5·0; Cl, 12·7%), and a 2,4-*dichloroanil*, m. p. 204° (from ethanol) (Found: C, 53·2; H, 4·1; N, 4·6; Cl, 22·7. $C_{14}H_{13}O_3NCl_2$ requires C, 53·5; H, 4·1; N, 4·5; Cl, 22·6%).

4-*Acetyl*-5-*hydroxy*-2-*methylcyclopent*-4-*ene*-1,3-*dione* (V; R = R′ = Me).—A solution of the compound (IV; R = R′ = Me) (43 mg.) in methanol (5 ml.) was titrated conductimetrically with methanolic lead acetate (415 mg. of trihydrate in 10 ml. of methanol). No break was observed in the titration curve and after 4 ml. of the solution had been added the mixture was left for 10 min. The orange-brown precipitate (19 mg.) was filtered off, suspended in methanol, and treated with hydrogen sulphide. Lead sulphide was filtered off and the filtrate evaporated to dryness. The residue crystallized from water, sublimed at 180°, and then recrystallized from water to give a yellow *product*, m. p. 208—210° (Found: C, 57·4; H, 5·0. $C_8H_8O_4$ requires C, 57·2; H, 4·8%) which gave a green colour with methanolic ferric chloride and had $\lambda_{max.}$ 253 and 275 (infl.) mμ (ε 21,900 and 16,300 respectively) in acidic ethanol and 272 and 300 mμ (ε 26,000 and 22,300 respectively) in alkaline ethanol.

Bacteriostatic Activity.—Compounds were tested against a *Lactobacillus* (isolated from infected beer) by serial dilution in the liquid version of the medium L described by Williamson.[10] Isohumulone A was active at 50 p.p.m., effected almost complete inhibition at 75 p.p.m., and completely prevented growth at 100 p.p.m. Neither compound (II) nor (III; R = R′ = Me) affected growth at concentrations of 100 p.p.m.

Infrared Spectra.—Absorption maxima for Nujol mulls, determined with a Perkin–Elmer spectrophotometer model 137, were as follows.

Dimethylphloroglucinol, 2·78w, infl., 2·85s, 6·11m, 6·20m, 6·61m, 7·72m, 7·92s, 8·32w, 9·20s, 9·80m, 11·60m, 12·30m μ.

Dimethylphloroacetophenone, 2·85s, 6·20s, 6·35w, 6·67w, 7·03w, 7·35m, 7·78m, 8·15s, 8·50m, 8·95s, 9·70m, 11·0s, 11·45m, 12·02m, and 14·10m μ.

The analogue (II; R = R′ = Me) of humulone, 2·98s, 3·18m, 6·02s, 6·10m, 6·55m, 7·20 (infl.), 7·28m, 7.35m, 7·85s, 8·38m, 8·91m, 9·48m, 9·70m, 10·02m, 10·75m, 11·08m, 11·82m, and 12·78m μ.

The analogue (III; R = R′ = Me) of isohumulone, 2·95m, 5·88s, 5·98s, 6·2 to 6·45s, 8·02w, 8·13m, 8·47m, 8·70s, 9·30m, 9·47m, 9·75m, 10·20m, 10·70m, 11·14m, 11·59w, 11·92m, and 13·03m μ.

The analogue (IV; R = R′ = Me) of humulinic acid, 2·86m, 5·80s, 6·10s, 6·30s, 7·65w, 8·0w, 8·17w, 8·40m, 8·94m, 9·08w, 9·45m, 10·41m, 11·15m and 12·13m μ.

The anil of compound (III; R = R′ = Me), 2·85m, 3·06w, 5·74m, 5·87w, 6·10s, 6·30 and 6·38s, 7·65m, 9·21m, 9·61m, 10·80m, 11·0m, 11·5m, 13·10w, and 14·23m μ.

The anil of compound (IV; R = R′ = Me), 2·83m, 3·05w, 5·74w, 5·87m, 6·12s, 6·38s, 7·92m, 8·26m, 8·97m, 9·45m, 10·26m, 10·70w, 11·05w, 11·80m, 13·20m and 14·17s μ.

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[10] Williamson, *J. Inst. Brewing*, 1959, 154.