224. *Chemistry of Hop Constituents. Part XXIV.*[1] *The Structure of the Isohumulinones.*

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Two isohumulinones have been isolated and their structures elucidated. Evidence is presented that the new isomer possesses a novel heterocyclic system as in (IV).

HUMULINONE (I; R = Bu^i), an oxidation product of the hop resin humulone (II), was reported [2] to occur naturally in the hop, but Verzele and Govaert [3] believed that it was an artefact produced by the method of isolation from the hop, and by chromatography on silica gel they separated three components. Two substances eluted by benzene were shown to be humulinone and cohumulinone (I; R = Pr^i). The third component, eluted from the column with ether, was thought to be adhumulinone (I; R = Bu^s) until the latter was prepared.[4] Recrystallisation of the third material, shown to be a mixture by oxidation with alkaline hydrogen peroxide, gave a pure compound isomeric with humulinone and termed isohumulinone. Treatment of a benzene solution of pure humulinone with silica gel afforded the same compound, and in a similar manner isocohumulinone was obtained from cohumulinone.

Initial examination of isohumulinone, now called isohumulinone A, showed that it contained one active hydrogen less than the parent humulinone, suggesting cyclisation of one of the unsaturated side-chains. The rearrangement of hop resins to yield pyran

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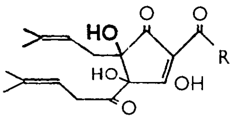
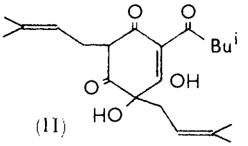
[1] Part XXIII, Burton, Elvidge, and Stevens, preceding Paper.
[2] Cook and Harris, *J.*, 1950, 1873.
[3] Verzele and Govaert, *J.*, 1952, 3313.
[4] Cook, Howard, and Slater, *J. Inst. Brewing*, 1956, **62**, 220.

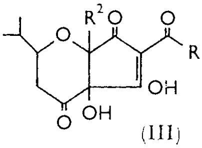
systems has been reported.[5] Reagents such as hydrogen chloride in methanol, formic acid in benzene, and perchloric acid in glacial acetic acid were used to obtain isohumulinone, the last reagent giving a 50—60% yield in contrast to the 4—5% obtained by the action of silica gel on a solution of humulinone in benzene. Isohumulinone A and the pyrans obtained from colupulone give an intense red coloration with concentrated sulphuric acid. A similar colour produced by humulinone is possibly due to *in situ* formation of isohumulinone A. Further evidence of cyclisation was provided by hydrogenation of isohumulinone A under conditions favouring reduction of the olefinic double bonds; only a dihydro-derivative was obtained in contrast to the tetrahydro-derivative produced from humulinone. As expected, no isomeric substances were obtained by the action of any of the above reagents on tetrahydrohumulinone.



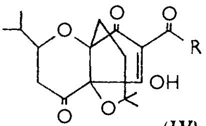
(I)



(II)



(III)



(IV)

Alkaline hydrolysis of humulinone gave rise to 4-methylpent-3-enoic acid. This acid was not, however, obtained on alkaline hydrolysis of isohumulinone A, which was recovered unchanged together with about 10—20% of a further isomer which has been called isohumulinone B. Moreover, treatment of dihydroisohumulinone A under the same conditions did not yield 4-methylpentanoic acid, and again the starting material was recovered. It is thus concluded that the formation of isohumulinone A involves cyclisation of the 4-methylpentenoyl side-chain of humulinone and that dihydroisohumulinone A is to be formulated as (III; $R^1 = Bu^i$, $R^2 = CH_2{\cdot}CH_2{\cdot}CHMe_2$).

Isohumulinone B could not be hydrogenated under conditions favouring reduction of olefinic double bonds. It had presumably arisen by cyclisation of the $CH_2{\cdot}CH{:}CMe_2$ group in isohumulinone A. Isohumulinone B was also obtained in varying yields when humulinone was isomerised using benzene and formic acid.

From the above evidence it is suggested that isohumulinone A has the structure (III; $R^1 = Bu^i$, $R^2 = CH_2{\cdot}CH{:}CMe_2$), and that isohumulinone B is (IV; $R = Bu^i$).

Optical properties, and proton magnetic resonance spectra obtained at 60 Mc./sec., are in agreement with these structures. Examination of isohumulinone A by the latter technique was only of limited value owing to sparing solubility in suitable solvents, but it showed that the proportion of methyl-group protons bonded to saturated carbon atoms was that which would be expected following cyclisation of one unsaturated side-chain. Infrared spectra indicated a marked decrease in the intensity of the hydroxyl absorption in passing from humulinone to isohumulinone B, the presence of a chelated ketone in all the compounds, and the presence of a cyclic ether system in both isohumulinones.

The ultraviolet spectrum of humulinone is due to the presence of the triketone system $CO{\cdot}CH(CO{\cdot}CH_2{\cdot}CHMe_2){\cdot}CO$, and it is noteworthy that the spectra of isohumulinones A and B under both acid and alkaline conditions exhibit features very similar to those of humulinone, thus confirming the presence of this system in the isohumulinones.

The possibility of the formation of other cyclic systems [1] cannot, in spite of the foregoing evidence, be completely excluded.

Cohumulinone was prepared from cohumulone, which had been obtained on a preparative scale for the first time by the use of a reversed-phase chromatographic column. This was also isomerised, and both isocohumulinone A (III; $R^1 = Pr^i$, $R^2 = CH_2{\cdot}CH{:}CMe_2$) and isocohumulinone B (IV; $R = Pr^i$) were isolated and characterised.

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[5] Howard, Pollock, and Tatchell, *J.*, **1955**, 174; Arnold, Hastings, and Walker, *Chem. and Ind.*, 1955, 323; Brown, Burton, and Stevens, *Tetrahedron Letters*, **1963**, 289.

EXPERIMENTAL

Light petroleum had boiling point 40—60° unless otherwise stated.

Humulone and Cohumulone.—Mixed lead salt of cohumulone and humulone (15 g.) was finely ground and suspended in glacial acetic acid (150 ml.). After 2 hr. sulphuric acid (*d* 1·84; 3 ml.) was added followed by water (100 ml.), and the mixture was extracted with light petroleum, washed with water, dried ($MgSO_4$), and evaporated, to yield a resin (6·2 g.) containing humulone and cohumulone. The resin was chromatographed on a reversed-phase column using chloroform (375 g.) suspended on hydrophobic kieselguhr (300 g.) as the stationary phase and formamide, pH 6·0 buffer, and acetone (5 : 5 : 1, equilibrated) as the mobile phase. The kieselguhr was made hydrophobic by suspending it in a 1% solution of dichlorodimethylsilane in light petroleum for 0·5 hr.

The first eluate (1·5 l.) was discarded, and the second (1·0 l.) diluted with hydrochloric acid (~0·1N; 1·0 l.) and extracted with ether, washed with water, dried ($MgSO_4$), and evaporated, to give cohumulone as a pale yellow resin (2·7 g.). The third eluate (1·2 l.) was similarly treated and afforded humulone (2·3 g.) as a yellow amorphous solid, m. p. 60—61°.

Humulinone.—Humulone (2·0 g.) in ether (50 ml.) containing cumene hydroperoxide (2·0 ml.) was allowed to stand over saturated aqueous sodium hydrogen carbonate (70 ml.) for 72 hr. Colourless needles of sodium humulinone (1·55 g.), m. p. 223—224°, were filtered off and acidified, and the free resin recovered as a yellow oil (1·3 g.) which, when dissolved in light petroleum (b. p. 60—80°), deposited colourless needles of humulinone (1·1 g.), m. p. 74·5—75°.

Alkaline Hydrolysis of Humulinone.—Humulinone (1·0 g.) was refluxed for 2 hr. in aqueous sodium hydroxide solution (25 ml.; 0·1N). Volatile substances were removed by steam-distillation, the volume being kept constant by the addition of distilled water. After 0·5 hr. the residue was acidified (HCl) and again steam-distilled. The acid distillate was extracted with ether, and the extract washed with water, dried ($MgSO_4$), and evaporated, to yield an oil (150 mg.) from which 4-methylpent-3-enoic acid was isolated as its *p*-bromophenacyl ester, m. p. and mixed m. p. 97—98°. The acid was further characterised by gas chromatography.

Similar attempts were made to hydrolyse isohumulinone A, dihydroisohumulinone A, and isohumulinone B, but in all cases the starting substance was recovered. In the case of isohumulinone A, partial isomerisation to the further isomer occurred.

Isohumulinone A.—(*a*) A solution of humulinone (1·0 g.) in benzene (15 ml.) and formic acid (98—100%; 15 ml.) was refluxed for 1 hr., cooled, diluted with ether (50 ml.), washed with water, and evaporated, to yield an orange oil. After addition of light petroleum isohumulinone A crystallised as colourless needles, m. p. 197—198° (0·75 g.) (lit.,[2] 195°) (Found: C, 66·6; H, 7·9. Calc. for $C_{21}H_{30}O_6$: C, 66·65; H, 8·0%), $\lambda_{max.}$ (H_2SO_4; *d* 1·84) 366 mμ ($E^{1\%}_{1\,cm.}$ 1130).

(*b*) A solution of humulinone (1·0 g.) in glacial acetic acid (25 ml.) containing perchloric acid (72%; 0·5 ml.) was stored for 2 hr. at room temperature before being diluted with water (100 ml.) and extracted with ether. The extract was washed with water, saturated aqueous sodium hydrogen carbonate, and water, dried ($MgSO_4$), and evaporated. The residue was triturated with ether and filtered, the filtrate being evaporated to leave a resin which was redissolved in light petroleum. From this solution isohumulinone A crystallised as colourless needles.

(*c*) A solution of humulinone (0·5 g.) in methanol (20 ml.) containing hydrochloric acid (*d* 1·18; 5 ml.) was refluxed for 0·5 hr. Working-up in the usual manner afforded a resin from which no products could be characterised.

Isohumulinone B.—The mother-liquors from isohumulinone A obtained by benzene–formic acid isomerisation of humulinone afforded, on further concentration, *isohumulinone B*, m. p. 152° (0·22 g.) (Found: C, 66·6; H, 8·0. $C_{21}H_{30}O_6$ requires C, 66·6; H, 8·0%), $\lambda_{max.}$ (ethanol) 250, 273 mμ ($E^{1\%}_{1\,cm.}$ 318, 289), $\lambda_{max.}$ (alkaline ethanol) 253, 269 mμ ($E^{1\%}_{1\,cm.}$ 449, 397), $\lambda_{max.}$ (sulphuric acid; *d* 1·84) 366 mμ ($E^{1\%}_{1\,cm.}$ 533), $\nu_{max.}$ (Nujol) 3400s, 2850vs, 1710s, 1630s, 1580s, 1460vs, 1380s, 1270m, 1190s, 1090m, 1080m, 1020w, 985m, 930m, 820m, 715w cm.$^{-1}$.

Hydrogenation of Isohumulinone A.—Isohumulinone A (302 mg.) in glacial acetic acid (20 ml.) was hydrogenated in the presence of Adams catalyst (50 mg.). Hydrogen absorption (19·0 ml.) (1 double bond requires 17·8 ml.) was complete in 3 hr. After removal of solvent and catalyst the residue gave *dihydroisohumulinone*, pale orange crystals, m. p. 164—165° (from light petroleum) (210 mg.) (Found: C, 66·3; H, 8·3. $C_{21}H_{32}O_6$ requires C, 66·3; H, 8·4%), $\lambda_{max.}$ (ethanol) 252, 282sh mμ ($E^{1\%}_{1\,cm.}$ 286, 161), $\lambda_{max.}$ (alkaline ethanol) 260sh, 274 mμ ($E^{1\%}_{1\,cm.}$ 350, 425), $\nu_{max.}$ (KBr) 3450vs, 1710s, 1620vs, 1580vs, 1410s, 1330s, 1290sh, 1240m, 1170m, 1135m, 1080m, 1030m, 990w, 915—865w, 820w cm.$^{-1}$. After similar treatment, isohumulinone B was recovered unchanged.

Isocohumulinones.—In an analogous manner, cohumulinone gave *isocohumulinone* A, m. p. 232° (Found: C, 66·0; H, 7·75. $C_{20}H_{28}O_6$ requires C, 65·9; H, 7·75%), $\lambda_{max.}$ (ethanol) 230, 252, 272 mμ ($E^{1\%}_{1\,cm.}$ 345, 309, 313), $\lambda_{max.}$ (alkaline ethanol) 252, 271sh mμ ($E^{1\%}_{1\,cm.}$ 668, 578), $\lambda_{max.}$ (sulphuric acid) 366 mμ ($E^{1\%}_{1\,cm.}$ 1,180), which on further treatment gave *isocohumulinone B*, m. p. 171—173° (Found: C, 66·2; H, 8·1%) $\lambda_{max.}$ (ethanol) 253, 268 mμ ($E^{1\%}_{1\,cm.}$ 310, 305), $\lambda_{max.}$ (alkaline ethanol) 253, 269 mμ ($E^{1\%}_{1\,cm.}$ 571, 505), $\lambda_{max.}$ (sulphuric acid) 366 mμ ($E^{1\%}_{1\,cm.}$ 760).

The authors thank Dr. A. H. Cook, F.R.S., for his advice and encouragement, and Professor E. R. Andrew and Dr. S. Clough for the p.m.r. spectra.

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