# Oxidation of Deoxyhumulones with Air. Evidence of Divergent and Sequential Products from Deoxyhumulones

By **Patrick V. R. Shannon*** and **Gareth D. John**, Chemistry Department, University College, Cardiff CF1 1XL

Air oxidations of the model deoxyhumulones 2',4',6'-trihydroxy-3'-methyl-5'-(3-methylbut-2-enyl)acetophenone (6), 2',4',6'-trihydroxy-3'-isopentyl-5'-(3-methylbut-2-enyl)acetophenone (12), and 2',4',6'-trihydroxy-3',5'-di-(3-methylbut-2-enyl)acetophenone (7) (deoxyacetohumulone) have shown that besides the corresponding humulones, 8-acetyl-2,2,6-trimethylchroman-3,5,7-triol (10), 8-acetyl-2,2-dimethyl-6-isopentylchroman-3,5,7-triol (18), and 8-acetyl-2,2-dimethyl-6(3-methylbut-2-enyl)chroman-3,5,7-triol (20) respectively are formed competitively. For the model deoxyhumulones (6) and (12) which give rise to two isomeric humulones, in each case one of the latter compounds undergoes further oxidation discriminately, whilst deoxyacetohumulone (7) gives a low yield of the isohumulones (25) and evidence for formation of the epoxyisohumulone (23) is presented. The hydroxychroman (20) oxidises slowly in air to 2,3,7,8-tetrahydro-5-acetyl-7,7-dimethyl-2-(1-methyl-1-hydroxyethyl)furo[2,3-*f*]benzopyran-4,8-diol (24).

SINCE the deoxyhumulones (1) became readily available [1] our interest has increased in the chemical counterpart of their evident biochemical oxidation in hops [2-4] to humulones (2) and other compounds; (—) humulone (3) is formed in the growing hop cone [3] whilst (racemic) humulones (2) can be obtained in up to 30% yield under the most favourable conditions when deoxyhumulones (1) are treated with oxygen. The low yields of humulones (2) *in vitro* are significant for commercial chemical production whilst any subsequent oxidation of the humulones has important consequences (*a*) during the storage of hops and (*b*) during the wort-boiling stage in brewing. In this paper we present evidence for both divergent and sequential products during the aerial oxidation of deoxyhumulones (1) *via* the humulones (2).

It is known [4] that substantial differences in yield exist between the humulones and their saturated side-chain analogues when obtained by aerial oxidation of deoxyhumulones and tetrahydrodeoxyhumulones (4) respectively, and more recent work [5] has established under standard conditions, the variation in yields of 'humulones' isolated by lead(II) 'salt' precipitation from three structurally different deoxyhumulones (5), (6), and (7) with an increasing degree of unsaturation. The yields, derived from the precipitated lead salts were 56, 42, and 31% respectively. However, examination of the mother liquors from (5) and (7) revealed both unchanged deoxyhumulones and incompletely precipitated humulones so that rigid interpretation of this trend would be unjustified. Nonetheless, it is generally agreed that the lower yields obtained from the naturally occurring fully unsaturated compounds (1) arise because of oxidative attack at the alkenyl side-chains besides the desired oxidation at the C-3 and C-5 positions of the aromatic nucleus. In principle, side-chain oxidations can occur either (A) before or (B) after nuclear oxidation; either event would lower the yield of humulones (2).

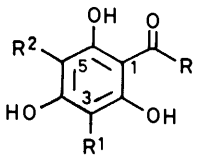
In earlier work [4] a wider study showed that kinetic competition for oxygen between the deoxyhumulones (7) and (5) gave a slightly greater reaction rate for the unsaturated compound (7), which could be taken as tentative support for (A). It was also shown [4] that for the model synthetic 'deoxyhumulone' (6), aerial oxidation in the presence of lead(II) acetate (which generally precipitates the humulones as their lead salts) gave, after precipitation and regeneration of the humulones, a significant, reproducible excess of one (8) of the two possible expected isomeric humulones (8) and (9). This result cannot be a consequence of (A) but would be explained by (B) if, in the incompletely precipitated compounds, the dimethyllayl side-chain of (9) was attacked preferentially to that in (8) by oxygen. It is noteworthy that (9) possesses a 'doubly allylic' methylene group, as in the natural humulones (2), whilst (8) does not. Differing solubilities of the lead(II) salts of (8) and (9), or other effects, might, however, also explain this result.

Some indications, therefore, exist that the reaction of deoxyhumulones with air follows a divergent pathway to either side-chain-oxidised aromatic compounds or humulones, and that the latter, perhaps because of definable structural features, oxidise further.

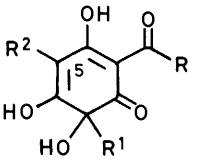
Previously,[4] the deoxyhumulone (6) had given on aerial oxidation, unequal amounts of the two precipitated lead(II) humulinates (8) and (9) and although (6) was not an ideal model, we began by re-examining its oxidation in air but without the presence of lead(II) acetate.

The reaction was followed by n.m.r. and t.l.c. and after 12 days, when starting material had disappeared, a complex mixture of products was evident. Within 3 h a low-field OH signal at $\tau$ 3.98 and $OCMe_2$ singlets at 8.73, 8.68, and 8.65 could be seen. After 28 h a triplet at $\tau$ 6.32 was recognisable as was later, a further low-field signal at $\tau$ —3.94. It was apparent from the shift of the low-field signals that initial oxidation was occurring to give *aromatic* derivatives other than the expected humulones (8) and (9), potentially recognisable from

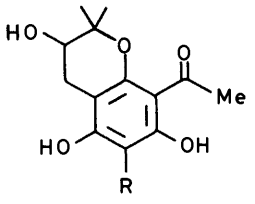
[1] E. Collins and P. V. R. Shannon *J.C.S. Perkin I*, 1973, 419.
[2] R. O. V. Lloyd, P. V. R. Shannon, and S. J. Shaw, *J. Inst. Brewing*, 1969, **75**, 32.
[3] P. V. R. Shannon, R. O. V. Lloyd, and D. M. Cahill, *J. Inst. Brewing*, 1969, 75, 376.
[4] E. Collins, G. D. John, and P. V. R. Shannon, *J.C.S. Perkin I*, 1975, 96.
[5] E. Collins, Ph.D. Thesis, University of Wales, 1974.



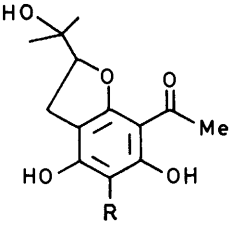
(1) $R^1 = R^2 = CH_2CH{:}CMe_2$
(4) $R^1 = R^2 = CH_2CH_2CHMe_2$
(5) R = Me, $R^1 = R^2 = CH_2CH_2CHMe_2$
(6) R = $R^1$ = Me, $R^2 = CH_2CH{:}CMe_2$
(7) R = Me $R^1 = R^2 = CH_2CH{:}CMe_2$
(12) R = Me, $R^1 = CH_2CH_2CHMe_2$, $R^2 = CH_2CH{:}CMe_2$
(14) R = Me, $R^1 = CH_2CH_2CHMe_2$, $R^2$ = H



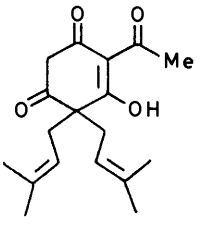
(2) $R^1 = R^2 = CH_2CH{:}CMe_2$
(3) R = $CH_2CHMe_2$, $R^1 = \beta CH_2CH{:}CMe_2$, $R^2 = CH_2CH{:}CMe_2$
(8) R = $R^2$ = Me, $R^1 = CH_2CH{:}CMe_2$
(9) R = $R^1$ = Me, $R^2 = CH_2CH{:}CMe_2$
(16) R = Me, $R^1 = CH_2CH{:}CMe_2$, $R^2 = CH_2CH_2CHMe_2$
(17) R = Me, $R^1 = CH_2CH_2CHMe_2$, $R^2 = CH_2CH{:}CMe_2$
(19) R = Me, $R^1 = R^2 = CH_2CH{:}CMe_2$



(10) R = Me
(18) R = $CH_2CH_2CHMe_2$
(20) R = $CH_2CH{:}CMe_2$



(11) R = Me
(21) R = $CH_2CH{:}CMe_2$



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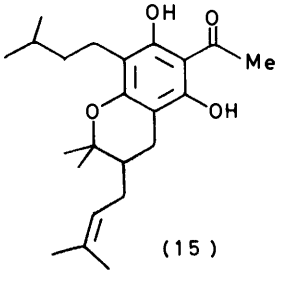
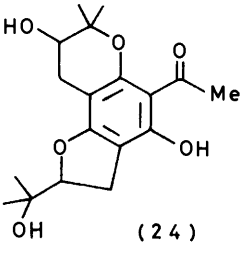
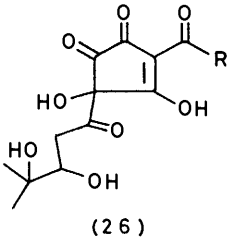


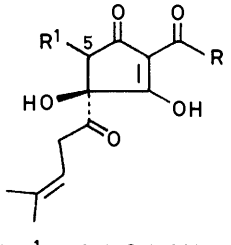
(15)



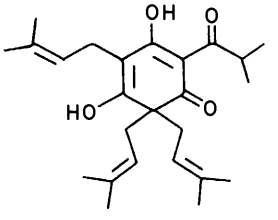
(24)



(26)
(27) R = $CH_2CHMe_2$



(22) $R^1 = CH_2CH{:}CMe_2$
(23) R = Me, $R^1 = CH_2CH(O)CMe_2$
(25) R = Me, $R^1 = CH_2CH{:}CMe_2$
(31) R = $CH_2CHMe_2$, $R^1 = Me_2C(OH)CH(OH)CH_2$



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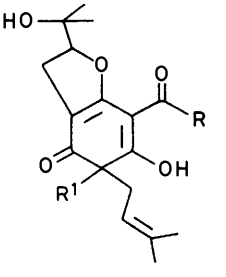


(29) R = $CHMe_2$, $R^1 = CH_2CH{:}CMe_2$
(30) R = $CH_2CHMe_2$, $R^1$ = OH

known [4] assignments. The latter two compounds were in fact detected in the mixture ($\tau$ −8.6 and −8.5) later (*ca.* 50 h) in the oxidation, the higher-field signal, from (8) being about three times the intensity of that from (9). The ratio of (8) : (9) was greater than that obtained from the precipitated lead(II) salts and supported the possibility that one humulone (9) was being selectively oxidised in solution. Chromatography of the mixture afforded only two reasonably pure compounds, in low yields. The first was identified by comparison with the literature [4] as the humulone (8). The second, which appeared to be a phloroacetophenone with a free *para*-OH group ($\lambda_{max.}$ 290, $\lambda_{max.}$ alkaline ethanol 336 nm) showed the addition of 1 oxygen atom ($M^+$ 266) and in the n.m.r. spectrum evidence of Ar–Me (singlet $\tau$ 8.04) and Ar·CO·Me (singlet $\tau$ 7.44) groups. A singlet at $\tau$ 8.60 and the absence of a signal at *ca.* $\tau$ 5.18 [6] indicated the hydroxychroman structure (10), not the alternative dihydrobenzofuran ring system (11).

Other components were isolated, but proved on further examination to be either unstable or to be mixtures, but on the basis of these preliminary results it seemed likely that for the model compound (6) oxygen does attack the side-chain double-bond and can precede oxidation at the aromatic nucleus.

For more thorough investigation, a closer model to the natural series of deoxyhumulones, dimethylallylisopentylphloracetophenone (12), with better solubility in organic solvents than (6), was required. Hydrogenolysis of the trione (13), a major by-product in the synthesis of acetodeoxyhumulone (7) [1] gave isopentylphloracetophenone (14), which was ' prenylated ' using 3-hydroxy-3-methylbut-2-ene and boron trifluoride dietherate.[1] A minor aromatic by-product of this synthesis was isolated whose spectroscopic properties (see Experimental section) lead unambiguously to structure (15) analogous to minor products formed in the prenylation of phloroacetophenone.[1] The major, required phenol (12), was obtained crystalline after chromatography and was characterised by its spectroscopic properties. Aerial oxidation of (12) was first carried out in the presence of lead(II) acetate in order to obtain specimens of both the anticipated humulones (16) and (17) needed for subsequent spectral comparisons. As before, the precipitated lead(II) humulinates were centrifuged and washed repeatedly before quantitative regeneration of the humulones with dilute acid. The ratio of (16) and (17) obtained (100 : 63 respectively, 100 : 63 in a repeat experiment) was derivable, as for the simpler model compounds, from the n.m.r. spectrum of the mixture, shown in part in the Figure. By analogy with the first pair, the isomer with the ' doubly allylic ' methylene group (17) was the minor one since the corresponding doublet at $\tau$ 6.90 ($J$ 7 Hz), repeatedly integrated for less than the sum of the vinylic hydrogen atoms at $\tau$ 5.0. Quantitative estimation of the ratio of isomers was best made from their separate low-field signals at $\tau$ −9.06 and −9.02. Of particular note is the separation of the isopropyl doublets at $\tau$ 9.0 and 9.15.

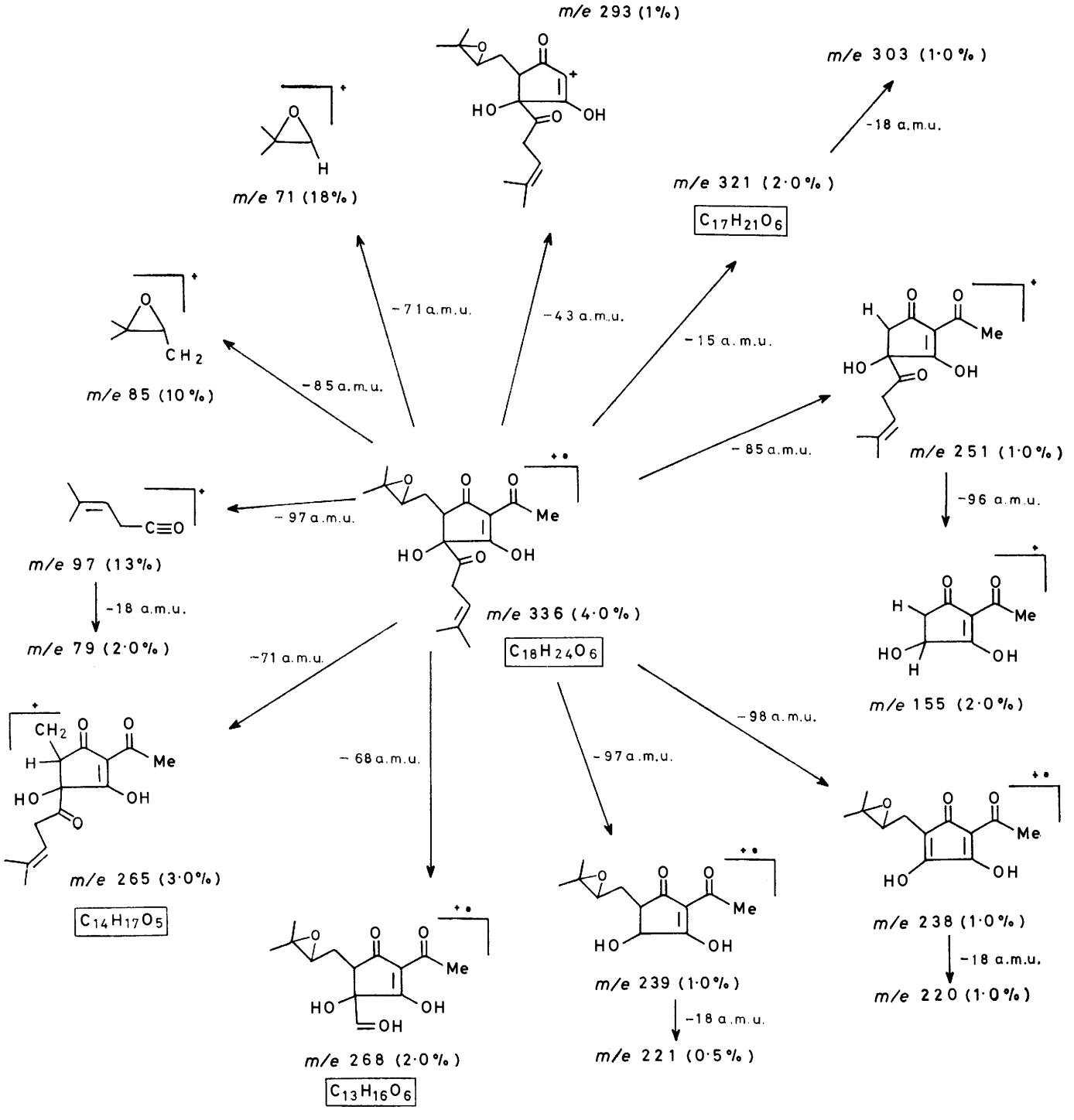
Again there was an indication that, accepting that the pair of humulones (16) and (17) would not be necessarily identically or completely insoluble as their lead(II) salts, one isomer, (17), was being selectively oxidised whilst still in solution. The slightly different ratios of the humulone pairs, 100 : 74 for (8) : (9) and 100 : 64 for (16) : (17) may be due to different solubilities of the lead salts of each pair. To confirm these indications a solution of the free humulones (16) and (17) was allowed to oxidise slowly in air in deuteriochloroform and the mixture monitored by $^1$H n.m.r. spectroscopy. After 600 h little change was seen, but on admitting a slow stream of air, breakdown was soon evident; after a total of 630 h the least intense of the two low-field $^1$H n.m.r. signals, corresponding to (17), had decreased to 30% of the major one. Chromatography of the mixture afforded a compound whose t.l.c., u.v., and $^1$H n.m.r. spectra showed it unambiguously to be (16).

In a final experiment, the deoxyhumulone (12) was kept in deuteriochloroform and allowed to oxidise whilst again using t.l.c. and n.m.r. to follow the reaction. After 140 h measurable signals began to appear in the humulone region of the low-field $^1$H n.m.r. spectrum and thereafter the ratio of the humulones (16) and (17) remained sensibly constant at 100 : 33. In addition, during the oxidation an extra low-field enol signal at $\tau$ −4.32 developed. This, together with high-field signals, *ca* $\tau$ 8.60 discernible with difficulty, suggested the presence of a hydroxychroman as for the first model. Chromatography gave one humulone (16) as an oil, and also the anticipated hydroxychroman (18) which was obtained as 6 mg of a brown solid. Its mass spectrum confirmed the molecular formula as $C_{18}H_{26}O_5$ and the u.v. spectrum showed $\lambda_{max.}$ 293 with a bathochromic alkaline shift of 43 nm characteristic of a phloroacylphenone with unsubstituted *para*-hydroxy-group. The associated hydroxydihydropyran ring system could be inferred from $^1$H n.m.r. signals at $\tau$ 8.74 (1 H, s, OH), 8.60 (6 H, s, $OCMe_2$), 7.17 (2 H, d, $J$ 6 Hz, ·CH(OH)-$CH_2$Ar), and 6.14 (1 H, m, C*H*OH). Further, on treatment of (12) with *m*-chloroperbenzoic acid and *p*-toluenesulphonic acid in chloroform (reagents known to give the hydroxychroman in analogous systems [6]) the hydroxychroman (18) was obtained, identical in m.p., mixed m.p., t.l.c. and spectroscopic properties with the compound from aerial oxidation. The humulone (16) was also obtained but in lower yield than in aerial oxidation.

The final experiments were carried out on deoxyacetohumulone (7), a near-perfect model for the natural compounds and differing from them only in that the spectroscopically simple acetyl side-chain replaces the natural isovaleryl and isobutyryl groups. Previous competitive oxidation rate studies [4] suggested that this variation would not affect significantly the results observed.

A freshly crystallised sample of (7) in deuteriochloroform was kept for several months. The mother liquor

[6] (*a*) W. Steck, *Canad. J. Chem.*, 1971, **49**, 2298; (*b*) *ibid*, p. 1198.




SCHEME 1 Fragmentation pattern of the epoxyisohumulone (23) on electron-impact mass spectrometry

from the crystallisation, examined by t.l.c., showed only (7), and was evaporated to a yellow oil and kept at 20 °C for eight weeks during which substantial oxidation occurred. T.l.c. revealed the presence of three major oxidation products. On careful chromatography, acetohumulone (19) was identified unambiguously, identical in all respects with a sample prepared *via* its insoluble lead(II) salt. The second compound, also obtained as an oil, displayed spectroscopic properties (see Experimental section) leading only to the hydroxychroman structure (20). In particular the absence of an ion at *m/e* 59 a.m.u. in the mass spectrum [7] and of a triplet, *J* 9 Hz, at *ca.* τ 5.2 [8] in the $^1$H n.m.r. spectrum excluded the possible alternative 2-(1-hydroxy-1-methylethyl)dihydrofuran ring system as in (21).

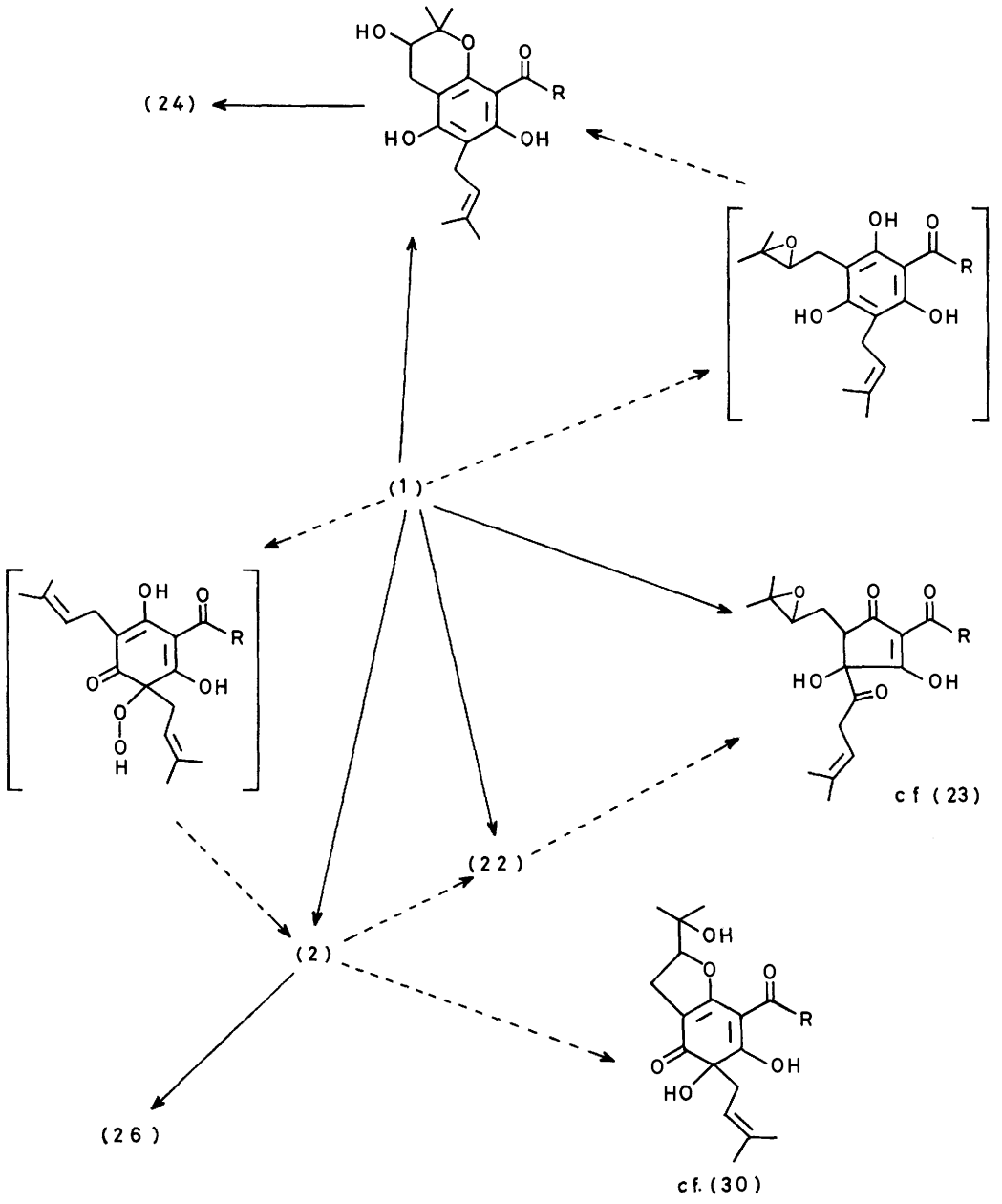
Other minor inseparable products of intermediate polarity were also obtained which were most probably phloroacetophenones with mixed dihydrofuran and dihydropyran ring systems, but elution of the most polar components from the column gave a brown solid whose u.v. spectrum [$\lambda_{max.}$ (acid EtOH) 228sh and 278 nm, $\lambda_{max.}$ (alkaline EtOH) 254 and 271sh nm] was very similar to that of the isohumulones (22) [9] and indicated a 5-membered β-triketone nucleus. Measurement of the $^1$H n.m.r. spectrum was not possible due to insolubility. The field desorption mass-spectrum showed ($M^+$ 336 a.m.u.) the addition of two oxygen atoms to deoxyacetohumulone. The low-resolution electron-impact mass spectrum was similar in many respects to that of isohumulone and other related 5-membered ring compounds [10] in which prominent fragmentations from the molecular ion include the isohexenoyl side-chain ($M^+$ — 96 and *m/e* 97) and the dimethylallyl side-chain ($M^+$ — 68, $M^+$ — 69, and *m/e* 69). However in the present compound predominant ions at *m/e* 97, 85, and 71 were seen, but no ion at *m/e* 69. Those at *m/e* 85 and 71 may be rationalised by the presence of an epoxide function on the dimethylallyl side-chain at C-5 leading to the tentative structure (23). The mass spectral fragmentation pattern may be rationalised as in Scheme 1 in which molecular formulae quoted have been determined by accurate mass measurement.

The results obtained by n.m.r. and t.l.c. monitored aerial oxidation of the freshly crystallised sample of deoxyacetohumulone (7) proved to be broadly comparable. After 6 days the virtually simultaneous appearance of two low-field OH signals (τ −4.10 and −8.60) corresponded to the hydroxychroman (20) and the humulone (19). The oxidation was followed for 11 months during which time the quantities of (19) and (20) increased to a steady level. Ultimately chromatography of the complex mixture gave fully characterised samples of (19) and (20). Both compounds appeared to have oxidised further. In fact the purified hydroxychroman (20) after several months in air at 0 °C clearly oxidised to a new product whose structure was unambiguously shown from its spectroscopic properties to be (24). In particular, the $^1$H n.m.r. spectrum of this crystalline tricyclic oxidation product showed the replacement of the dimethylallyl proton signals present in (20) by those characteristic of the 2-(1-hydroxy-1-methylethyl)dihydrofuran ring system. Not surprisingly, the derivative (24) did not undergo any further aerial oxidation and it thus proves to be an 'end-point' of one aerial oxidative pathway from acetodeoxyhumulone (7).

In a second experiment on the oxidation of the mother liquors from crystallisation of acetodeoxyhumulone, it was hoped to obtain more of the epoxide (23). In the event, aerial oxidation for a 10-week period, followed by chromatography, afforded in low yield (*ca.* 5%) acetohumulone (19) with much smaller amounts (*ca.* 1%) of the tricyclic compound (24). Significantly there was also isolated a very small amount (*ca.* 1%) of a mixture of *cis*- and *trans*-acetoisohumulones (25) which were identified by comparison of their u.v., n.m.r., and E.I. mass-spectra and t.l.c. properties with those of authentic specimens prepared from acetohumulone (19). The isolation of the mixture (25) from the aerial oxidation, even though in small yield, supports the identification of the epoxide (23).

These experiments prove that deoxyhumulones in general undergo oxidation in air both at the double-bond, where presumably epoxide formation precedes intramolecular cyclisation to hydroxychromans, (*cf.* ref. 6), and at the aromatic nucleus to give humulones, which may well be formed *via* a hydroperoxide (*cf.* ref. 11). In each instance the products oxidise further by divergent pathways as indicated in Scheme 2. The 'aromatic' branch of the scheme can be regarded as established. In the case of the humulones (2) the experiments described above with model compounds seem to indicate that the C-5 side-chain is a primary point of attack by oxygen. Such a process presumably could lead to breakdown products such as (26), already isolated in the example (27) [12] from aerial oxidation of the humulone (3). Epoxides of type (23) may arise by an alternative mechanism involving direct attack at the double-bond (*cf.* ref. 13); presumably this occurs *after* ring contraction to isohumulone (22; R = $CH_2CNMe_2$) since aerial oxidation of colupulone (28) and peracid oxidation of the humulone (3) give the intramolecularly cyclised stable 2-(1-hydroxy-1-methylethyl) derivatives (29) [14] and (30) [15] respectively, whereas peracid oxidation of the isohumulone (12; R = $CH_2CHMe_2$) affords the glycol (31).[15] The possible aerial oxidation of the

[7] S. J. Shaw and P. V. R. Shannon, *Org. Mass Spectrom.*, 1970, **3**, 941.
[8] 'High Resolution N.M.R. Catalogue' Spectrum 310, Varian Associates, Palo Alto, California, 1962.
[9] R. Stevens, *Chem. Rev.*, 1967, **67**, 19.
[10] S. J. Shaw, P. V. R. Shannon, and E. Collins, *Org. Mass Spectrom.*, 1972, **6**, 873.
[11] J. Sigg-Gratter and J. Wild, Swiss Pat. 1,458,343, 1976.
[12] P. R. Ashurst and J. A. Elvidge, *J. Chem. Soc.*, (*C*)., 1966, 675.
[13] S. J. Moss and H. Steiner, *J. Chem. Soc.*, 1965, 2372.
[14] D. M. Cahill and P. V. R. Shannon, *J. Chem. Soc.* (*C*), 1969, 938.
[15] A-M. Davis and P. V. R. Shannon, unpublished observations.




SCHEME 2 Some established (——►) and possible (·····►) interrelationships between deoxyhumulones (1) and their aerial-oxidation products

humulone (3) to the dihydrofuran (30) would also at least in part explain the selective oxidation of (17) in admixture with (16). The details of the further oxidation of the humulones (2) however require further studies.

## EXPERIMENTAL

For general experimental conditions see ref. 16. U.v. and n.m.r. spectra were measured in ethanol and deuteriochloroform respectively unless stated otherwise. Mass spectra are electron impact initiated except where stated. M.p.s were corrected. Silicic acid used was Mallinckrodt 100 mesh A.R. Elemental analyses were generally not carried out for compounds which were obtained as small quantities of oils, or which were unstable to air. Where accurate mass measurement of molecular ions was used to deduce molecular formulae, the t.l.c. characteristics and $^1$H n.m.r. spectra indicated a pure compound. Where spectral data (u.v., n.m.r., or mass) have been deposited as a Supplementary publication* (SUP No. 22143, 11 pp.) the compound name is asterisked.

*Oxidation of 2′,4′,6′-Trihydroxy-3′-methyl-5′-(3-methylbut-2-enyl)acetophenone* (6).—The above phenol (6) (74 mg,

* For details of the Supplementary Publications Scheme, see Notice to Authors No.7, *J.C.S. Perkin I*, 1976, Index issue.

[16] E. Collins and P. V. R. Shannon, *J.C.S. Perkin I*, 1974, 944.

m.p. 114—116 °C) in deuteriochloroform (0.5 ml) was left to oxidise slowly at 20 °C. Progress of the reaction was followed by $^1$H n.m.r. spectroscopy and t.l.c. and the disappearance of the deoxyhumulone (6) appeared complete after 12 days. The approximate relative heights of the low-field signals as a function of time were as follows:

| $t$/h | τ −8.6 | −8.5 | −3.98 | −3.94 |
|---|---|---|---|---|
| 3 | | | 2 | |
| 21 | | | 2 | |
| 46 | 1 | 3 | 3 | 2 |
| 70 | 3 | 9 | 5 | 5 |
| 142 | 3 | 8 | 5 | 6 |
| 166 | 6 | 14 | 6 | 9 |
| 190 | 7 | 17 | 7 | 11 |
| 215 | 7 | 17 | 5 | 11 |
| 240 | 9 | 25 | 7 | 15 |
| 290 | 8 | 24 | 6 | 13 |

After 290 h the solvent was removed under reduced pressure to leave a brown oil (70 mg) which was chromatographed on silicic acid (column 17 × 2.5 cm). Elution with ethyl acetate–light petroleum (1 : 9) gave an oil (16 mg) giving a single spot on t.l.c., $R_F$ 0.72 (hexane–ethyl formate, 1 : 1), and identified as the humulone (8) *.[4] Elution with ethyl acetate–light petroleum (1 : 14) gave a component ($R_F$ 0.65) (7 mg) as an oil which decomposed on storage overnight. Ethyl acetate–light petroleum (3 : 7) afforded the hydroxychroman (10) * as an oil (5 mg) ($R_F$ 0.55). Crystallisation from chloroform–light petroleum (b.p. 30—40 °C) gave a solid (1 mg, m.p. 181—184.5 °C) showing identical t.l.c. characteristics to the oil. Elution with ethyl acetate–light petroleum (1 : 1) gave a single component of $R_F$ 0.49, but u.v. and $^1$H n.m.r. analysis showed this to be a complex mixture.

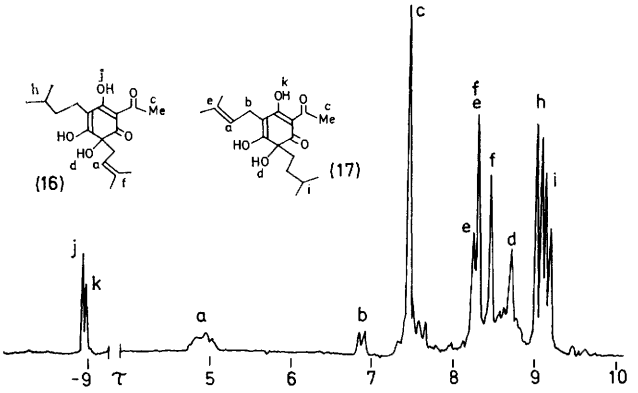
*Dimethylallylation of Isopentylphloracetophenone* (14).—Isopentylphloracetophenone (14) [1] (2.80 g, m.p. 183—184 °C) in dry dioxan (25 ml) was stirred and treated dropwise, with boron trifluoride diethetate (redistilled, 1 ml) and then 2-methylbut-3-en-2-ol (1.03 g) in dry dioxan (25 ml). The mixture was stirred for 9 h at 20 °C. Ether (100 ml) was then added and the solution washed with water (2 × 100 ml) and sodium carbonate solution (1%, 3 × 100 ml). The ether layer was dried ($MgSO_4 \cdot H_2O$) and the solvent evaporated under reduced pressure to yield an oil (1.00 g) which was chromatographed on silicic acid (column 24 × 3 cm).

Elution with ether–light petroleum (1 : 24) gave *6-acetyl-8-isopentyl-2,2-dimethyl-3-(3-methylbut-2-enyl)chroman-5,7-diol* (15) * as a yellow oil (68 mg) (Found: *M*, 374.243 9. $C_{23}H_{34}O_4$ requires *M*, 374.245 7).

Elution with ether–light petroleum (1 : 9) gave a solid; crystallisation (×2) from light petroleum (b.p. 30—40 °C) gave *2′4′,6′-trihydroxy-3′-isopentyl-5′-(3-methylbut-2-enyl)-acetophenone* (12) * (482 mg), m.p. 92.5—93.5 °C (Found: C, 70.4; H, 8.8. $C_{18}H_{26}O_4$ requires C, 70.6; H, 8.55%).

*Acetylation.*—The above phenol (12) (50 mg), in dry pyridine (1.4 ml) was treated with acetic anhydride (2.64 ml) and kept for 3 days at 20 °C. After work-up and chromatography the *triacetate* of (12) * was obtained as an oil (25 mg) (Found: *M*, 432.217 0. $C_{24}H_{32}O_7$ requires *M*, 432.213 9).

*Oxidation of 2′,4′,6′-Trihydroxy-3′-isopentyl-5′-(3-methylbut-2-enyl)acetophenone* (12).—(a) *With lead*(II) *acetate trihydrate.* The phenol (12) (200 mg) and lead(II) acetate trihydrate (250 mg) in AnalaR methanol (4.0 ml) were treated with a slow stream of air for 45 h. The solvent level was kept constant during this time. The yellow precipitate formed was collected by centrifugation, washed twice with a little cold methanol, and dried with nitrogen (170 mg). The mother liquor was kept at 0 °C overnight after which time a further 20 mg of precipitate were collected. The total lead salt (190 mg) was shaken with hydrochloric acid (60 ml; 0.5M) and ether (60 ml), until the lead salt was decomposed. The ether layer was separated, washed with saturated sodium chloride, solution and dried ($MgSO_4 \cdot H_2O$). Evaporation of the solvent under reduced pressure gave the humulones (16) and (17) as a yellow gum (96 mg), $\lambda_{max.}$ 238, 284, 324, and 350sh nm; $\lambda_{max.}$ (alkaline ethanol) 258, 315, and 350sh nm. The $^1$H n.m.r. spectrum, shown in the Figure enabled the ratio of humulones




100 MHz $^1$H n.m.r spectrum of the humulones (16) and (17)

(16 and (17) to be calculated as 100 : 63. A repeat experiment gave an identical ratio.

(b) *Oxidation without lead*(II) *acetate.* The phenol (12) (90 mg) in deuteriochloroform (0.3 ml) in an n.m.r. sample tube was allowed to oxidise at 20 °C. The $^1$H n.m.r. spectrum of the solution was measured at intervals over 284 h after which time virtually all the phenol had oxidised. The reaction was also monitored by t.l.c. No measurable signals were seen in the ' humulone ' region (τ −8 to −10) of the $^1$H n.m.r. spectrum until after *ca.* 140 h after which the ratio of the chelated OH signals of (16) and (17) was as shown below:

| $t$/h | Ratio (5b) : (6b) | $t$/h | Ratio (5b) : (6b) |
|---|---|---|---|
| 140 | 100 : 34 | 187 | 100 : 36 |
| 142 | 100 : 35 | 194 | 100 : 34 |
| 163 | 100 : 32 | 211 | 100 : 34 |
| 170 | 100 : 33 | 284 | 100 : 33 |

After all the deoxyhumulone (12) had disappeared (284 h) the mixture was taken and the solvent removed to yield a dark brown gum (90 mg) which was chromatographed on silicic acid (column 19 × 2.5 cm). A pale brown spot corresponding to the anticipated hydroxychroman (18) was observed on the plate of lower $R_F$ than the humulone (16) spot. Elution with redistilled ethyl acetate–light petroleum (1 : 24) (200 ml) gave the *humulone* (16) * as an oil (42 mg) which gave a single spot (blue-black with ferric chloride), $R_F$ 0.80 in hexane–n-butyl formate–formic acid (10 : 10 : 1) (Found: *M*, 322.175 6. $C_{18}H_{26}O_5$ requires *M*, 322.178 0).

Elution with ethyl acetate–light petroleum (1 : 4) gave an oil (23 mg) which gave a spot, $R_F$ 0.57, initially pale brown with ferric chloride but turning strong orange-brown with

time. Crystallisation from chloroform and light petroleum (b.p. 30—40 °C) gave the hydroxychroman (18) * as a powder (6 mg), m.p. 156—158 °C (Found: *M*, 322.178 0. $C_{18}H_{26}O_5$ requires *M*, 322.178 0).

(c) *Oxidation with* m-*chloroperbenzoic acid.* The phenol (12) (100 mg), *m*-chloroperbenzoic acid (100 mg), and *p*-toluenesulphonic acid (4 mg) in chloroform (12 ml) were stirred for 1 h. T.l.c. then showed the formation of 2 main components having orange and blue-black spots with iron(III) chloride. The mixture was washed with dilute aqueous sodium hydrogencarbonate (2 × 25 ml) and water (2 × 25 ml) before evaporation of the chloroform under reduced pressure. The residual brown oil (108 mg) was chromatographed on silicic acid (column 19 × 2.5 cm). Elution with ethyl acetate–light petroleum (1 : 9) gave the oily humulone (16) (18 mg) whose u.v. and $^1$H n.m.r. spectra and $R_F$ were identical in all respects to the specimen isolated in (b) above.

Elution with ethyl acetate–light petroleum (1 : 4) afforded the hydroxychroman (18) (20 mg), m.p. 155—158 °C, mixed m.p. 155—158 °C and identical in spectroscopic data to the chromanol isolated in (b).

*Autoxidation of Deoxyacetohumulone* (7).—Deoxyacetohumulone was synthesised as described previously.[1] (a) Freshly crystallised material (74 mg, m.p. 78—79 °C) was dissolved in deuteriochloroform (0.3 ml) in an n.m.r. tube and kept at 20 °C over 11 months; the progress of any reaction was followed by t.l.c. and $^1$H n.m.r. spectroscopy (see below).

(b) The mother liquors from the recrystallisation were evaporated under reduced pressure to a yellow oil (300 mg), t.l.c. examination of which showed only deoxyhumulone to be present. The oil was kept at 20 °C in air for 8 weeks during which time t.l.c. showed the formation of at least 3 oxidation products. Chromatography of the oily mixture on silicic acid (column 28 × 2.5 cm) and elution with ethyl acetate–light petroleum (redistilled) (2 : 23) afforded *acetohumulone* (19) * as an oil (68 mg), $R_F$ 0.70 in hexane–ethyl formate (1 : 1) (Found: *M*, 320.160 8. $C_{18}H_{24}O_5$ requires *M*, 320.162 4).

Elution with ethyl acetate–light petroleum (1 : 4) gave the *hydroxychroman* (20) * as an oil (35 mg), $R_F$ 0.51 (Found: *M*, 320.160 0. $C_{18}H_{24}O_5$ requires *M*, 320.162 3).

Elution with ethyl acetate–light petroleum (3 : 7) gave a component ($R_F$ 0.46). This oil (20 mg) on u.v. and $^1$H n.m.r. analysis appeared to be a mixture of dihydrofuran and dihydropyrans.

The remaining components were washed off the column with methanol and evaporation of the solvent left an oil (122 mg). Extraction with ether (2 × 100 ml) left the epoxide (23) * as a light brown solid (21 mg), m.p. 137—142°, $R_F$ 0.15 (Found: *M*, 336.157 1. $C_{18}H_{24}O_6$ requires *M*, 336.157 3).

*Freshly crystallised sample* (*a*). The deoxyhumulone (7), 74 mg, in deuteriochloroform (0.3 ml) was kept at 20 °C and $^1$H n.m.r. spectra were run at intervals over the following year. The low-field signal at $\tau$ −8.7 due to the humulone (19) was detectable after 66 h; that at −4.2 due to the hydroxychroman (20) was seen after 113 h. Thereafter the two peaks reached a steady level, that due to the humulone remaining in slight excess.

After 11 months, the solvent was evaporated under reduced pressure and the residual oil (72 mg) chromatographed on silicic acid (column 17 × 2.5 cm). Elution with ethyl acetate–light petroleum (redistilled) (2 : 23) afforded unchanged deoxyhumulone (7) (4 mg) identified by its $R_F$ and u.v. spectrum and acetohumulone (19) (11 mg), identified by comparison of its $R_F$ value and u.v. and n.m.r. spectra. Elution with ethyl acetate–light petroleum (1 : 4) gave the hydroxychroman (20) as an oil (9 mg) which was identified by its $R_F$ value and u.v. and $^1$H n.m.r. spectra. The remaining components of the mixture were washed off the column with methanol and yielded an oil (37 mg), whose t.l.c. characteristics showed it to be a complex mixture.

*Aerial Oxidation of the Hydroxychroman* (20).—The oily hydroxychroman (20) (pure by t.l.c., $^1$H n.m.r.) (32 mg) was kept in air at 0 °C for 18 months. T.l.c. analysis then showed total oxidation to two components. Ether (3 ml) was added and the resulting precipitate filtered off. This solid (11 mg) was recrystallised from chloroform–light petroleum (b.p. 30—40 °C) to give the furobenzopyran (24) as an off-white powder (6 mg), m.p. 196—198 °C, identical in all respects ($^1$H n.m.r., t.l.c., u.v.) with sample (m.p. 197—199.5 °C) (see following paper) obtained from the peracid oxidation of deoxyacetohumulone (mixed m.p. 195—199°).

*Aerial Oxidation of Deoxyacetohumulone to* (±)-cis- (25; $R^1 = \beta$) *and* (±)-trans- (25; $R' = \alpha$) *Acetoisohumulone.*—Removal of solvent from the mother liquors of recrystallisation of deoxyacetohumulone (7) (see above) gave a yellow oil (1.1 g) which showed only (7) on t.l.c. The oil was kept in air at 20 °C for 10 weeks before chromatography on silicic acid. Acetohumulone (19) (62 mg) and the tricyclic compound (24) (7.5 mg, m.p. 193—195 °C) were obtained as above; both specimens were identified by comparison of their physical and spectroscopic properties with previously characterised samples.

Elution with ethyl acetate–light petroleum (2 : 3) gave a mixture of (±) *cis*- and *trans*-acetoisohumulones (25) as an oil (11 mg), $R_F$ 0.15 (hexane–ethyl formate (1 : 1), $\lambda_{max.}$ 250 and 270sh nm; $\lambda_{max.}$ (acid EtOH) 228 and 275 nm; $\lambda_{max.}$ (alkaline EtOH) 251 and 269 nm; field desorption mass spectrum $M^+$ 320 a.m.u. (100%). The t.l.c. (in various systems) and $^1$H n.m.r. and E.I. mass spectra showed a mixture of the *cis*- and *trans*-isomers by comparison with the pure synthetic isomers (see below).

*Preparation of* (±)-cis- (25; $R^1 = \beta$) *and* (±)-trans- (25, $R^1 = \alpha$) *Acetoisohumulones.*—Deoxyacetohumulone (7) (1.50 g), m.p. 78—79 °C, and lead diacetate trihydrate (1.86 g) were dissolved in AnalaR methanol. A steady stream of air was bubbled through the mixture at 20 °C for 4 days. The yellow precipitate was filtered off and washed with a little cold methanol. This lead salt (1.0 g) was shaken with ether (200 ml) and sulphuric acid (4N; 4 × 100 ml), the salt being broken down slowly over ½ h. The ether layer was washed with saturated sodium chloride solution (2 × 100 ml) and water (3 × 100 ml), and dried ($MgSO_4 \cdot H_2O$). Evaporation of the solvent under reduced pressure gave (±)-acetohumulone (19) as a yellow oil (502 mg), $\lambda_{max.}$ (acid EtOH) 234, 283, 321sh, and 350sh nm; $\lambda_{max.}$ (alkaline EtOH) 254 and 320sh nm.

The above (±)-acetohumulone (19) (502 mg) was added in AnalaR methanol (8 ml) to boiling aqueous sodium carbonate (0.1M; 250 ml) under nitrogen. The mixture was refluxed for 20 min, then poured on to crushed ice (100 g) and concentrated hydrochloric acid (6.0 ml). The white precipitate formed was extracted into ether (3 × 200 ml) and chloroform (1 × 100 ml), and dried ($MgSO_4 \cdot H_2O$). The ether was evaporated under reduced pressure to leave

a yellow oil (430 mg), $\lambda_{max.}$ 229, 254, and 265sh nm which, was chromatographed on silicic acid (28 × 2.5 cm). Elution with ethyl acetate–light petroleum (2 : 23) afforded two fractions.

Fraction A was eluted between 210 and 350 ml as an oil (112 mg), and fraction B between 420 and 460 ml as a solid (65 mg). T.l.c. in hexane–ethyl formate (1 : 1) gave separate spots, $R_F$ = 0.17 (A) and 0.14 (B). Fraction A could not be crystallised, but was pure by t.l.c. and identified as (±)-cis-*acetoisohumulone* (25; $R^1 = \beta$) *, a pale yellow oil (Found: $M^+$, 320.160 8. $C_{18}H_{24}O_5$ requires $M$, 320.162 4).

Fraction B was recrystallised from light petroleum (b.p. 30—40 °C) at low temperature to yield (±)-trans-*isoacetohumulone* (25; $R^1 = \alpha$) * as an off-white solid (15 mg), m.p. 61.5—62 °C (Found: $M^+$, 320.160 4. $C_{18}H_{24}O_5$ requires $M$, 320.162 4).

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