New Rearrangement and Oxidation Products from Humulone

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(−)-Humulone, in boiling water in the presence of air, was converted into tricyclic and bicyclic compounds resulting from both isomerisation and oxidation. Oxidation of (−)-humulone with *m*-chloroperbenzoic acid afforded hydroxydihydropyrano- and 2-(1-hydroxy-1-methylethyl)dihydrofurano-derivatives. Similar oxidations of photoisohumulone gave isohumulone glycols or bicyclic products, depending upon the conditions.

IT has been known for some time that humulone (1) and its analogues undergo complex rearrangements and oxidation processes under mild conditions. The products vary considerably in structure; for example humulone, in mildly acidic boiling aqueous solutions gives derivatives as diverse as tricyclodehydroisohumulone (2) [1] or the tetronic acid (3),[2] whilst oxidation by air is reported to give the cyclopentenedione (4).[3] Some of these compounds, especially those containing the five-membered-ring β-tricarbonyl nucleus such as (2) and (4), like the isohumulones (5) and (6), are bitter tasting. Their production by aerial oxidation in stored hops and their survival or further structural elaboration by rearrangement and oxidation during wort boiling are believed to contribute substantially to the bittering power of hops in the brewing process. During wort boiling, humulone (1) rearranges rather inefficiently to the isohumulones (5) and (6), but other transformations have been identified. For example the subsequent loss of the 3-methylbutanoyl side-chain is known [4] and the conversion of humulone into the glycol (7) has been reported.[5] The latter product was not completely identified, but since it appeared to fit into a hypothetical sequence between humulone (1) and the ketone (4), perhaps *via* the isohumulone glycol (8), a re-examination of the fate of humulone when boiled in water was undertaken.

Natural (−)-humulone (1) was kept in boiling water, open to the air, for 2.5 h; most of the humulone was then found by t.l.c. to have disappeared. In several preparations the same products appeared to be formed but their ratios varied; they were extracted and chromatographed on silicic acid.

The least-polar compound was a tasteless crystalline solid, obtained in up to 13% yield. The field desorption (f.d.) mass spectrum, (M^+ 362), the (unusually abundant) molecular ion observed in electron impact (e.i.) mass spectrometry, and elemental analysis showed it to be an isomer of humulone $C_{21}H_{30}O_5$. Moreover the u.v. spectrum (see later) suggested the presence of a humulone-like six-membered-ring nucleus which was confirmed by the ^{1}H n.m.r. spectrum, which showed a pair of low-field signals at τ −9.0 and −8.4. This duplication of signals (ratio *ca.* 100 : 15, respectively) was also observed in the methyl and methylene resonances of the 3-methylbutanoyl side-chain, and this suggested two alternative chelation states.

The vinylic methyl resonances of humulone (1) were absent, but were replaced by two singlets at τ 8.52 (3 H) and 8.65 (9 H), together indicative of two O–CMe₂ groups. These features, together with two triplets at τ 7.64 and 8.27 pointed strongly to the tautomeric dihydropyran part-structure (9). The u.v. spectrum [$\lambda_{max.}$ (EtOH) 245, 285, and 320 nm, $\lambda_{max.}$ (alkaline EtOH) 250, 287, and 325sh nm] was very similar to that of the two dihydropyrans (10) and (11),[6] but the alkaline solution spectrum closely resembled only that for the former ($\lambda_{max.}$ 245, 273, and 330sh nm), which confirmed the orientation of the dihydropyran ring as shown in (9). The alternative cyclisation mode [as for (11)] would give only a single low-field enolic proton signal.

The remaining methylene signals in the ^{1}H n.m.r. spectrum (apart from that of the 3-methylbutanoyl side chain) appeared as a complex multiplet (*ca.* 4 H) at τ 7.72—8.14 as expected of the β-protons of tetrahydrofuran (τ 8.12). On treatment with D₂O, only the low-field OH signals were affected, confirming the absence of the tertiary OH group present in humulone (1). These facts lead only to the tetrahydrofuranyl tricyclic structure (12). The major tautomer, by analogy with humulone,[7] is assumed to be (12b), and the absolute configuration [7] is as shown since the optical activity of compound (13) (see later) shows that the C-3 chiral centre in humulone does not racemise under the conditions of the isomerisation.

From subsequent reactions, a compound of higher polarity than the tricyclic product (12) was isolated as an oil (2.2—13%). The f.d. mass spectrum (M^+ 362, 100%) showed that this compound was also isomeric with humulone but, unlike (12), the e.i. spectrum did not give a molecular ion; the highest mass fragment appeared at m/z 294.1460 ($C_{16}H_{22}O_5$), corresponding to the loss of a dimethylallyl side-chain from a quaternary carbon atom with hydrogen rearrangement. The u.v. spectrum of a solution in alkaline methanol ($\lambda_{max.}$ 250, 284, and 325sh nm) was almost identical with that of compound (12), and partially obscured multiplets at τ *ca.* 7.5 and 8.2 and a singlet (6 H) at τ 8.60 in the ^{1}H n.m.r. spectrum again pointed to the same dihydropyran system. The spectrum also showed clearly an unchanged C-3 dimethylallyl side-chain with the usual vinyl hydrogen and methyl signals at τ 5.03 (1 H, t, *J* 8 Hz) and 8.33 and 8.47 (2 s, each 3 H). These features, together with the expected low-field signal at τ −8.9 (1 H) were con-
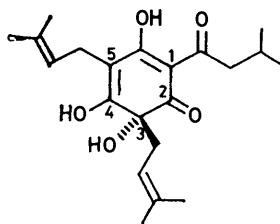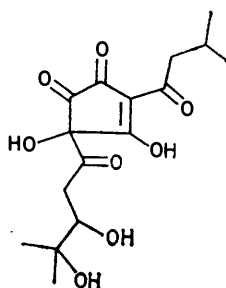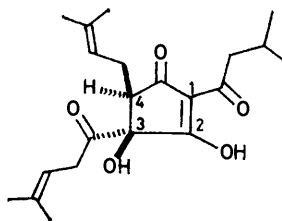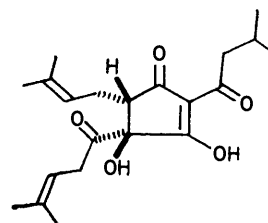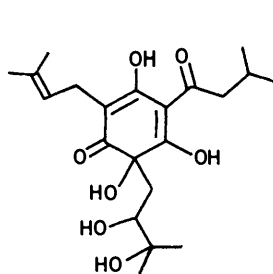
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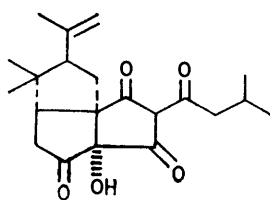
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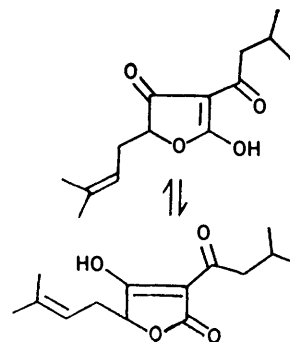
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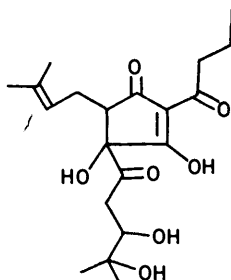
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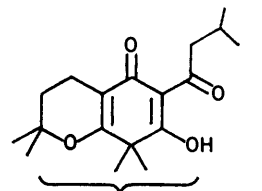
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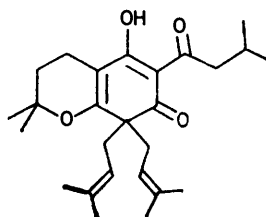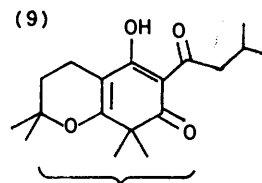
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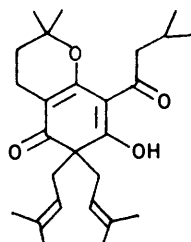
(11)

sistent with the incompletely cyclised dihydropyran structure (13). Chemical confirmation was obtained by hydrogenolysis (*cf.* ref. 9) over palladium chloride to the quinol (14). The orientation of the dihydropyran ring in the quinol and in the original compound (13) was confirmed by measurement of the u.v. spectrum in slightly acidic, neutral, and then alkaline ethanol in the presence of an excess of sodium borohydride. Under these conditions no oxidation to the corresponding

(a)   (12)   (b)

(13)   (14)

quinone could take place and the alkaline shift was only 13 nm, in keeping with the absence of a free hydroxy-group *para* to the ketone and in contrast to the behaviour of the quinol (19) (see later). Finally, suppor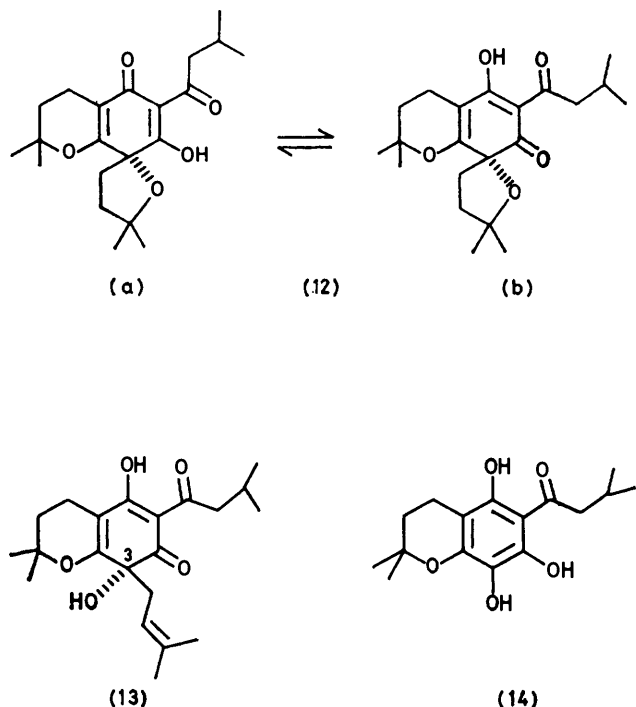t for the relationship between compounds (12) and (13) was obtained when the alcohol (13), after treatment with boiling dilute acid, gave several products (t.l.c.) of which one was the tricyclic derivative (12).

In all experiments with humulone in boiling water a new product was observed of R_F value lower than that of the isohumulones (5) and (6). When the products from several experiments were combined it proved possible to isolate a colourless solid after chromatography of the chloroform extract. Recrystallisation gave a pure sample of tricyclodehydroisohumulone (2), identical with a sample prepared by oxidation of (−)-humulone with lead tetra-acetate[1]; the probable mechanism for its formation *via* radical intermediates has already been discussed.[1]

The fourth product, isolated as an oil (3.7%) in a reasonably pure state, proved too unstable in air for its structure to be established beyond doubt. The [1]H n.m.r. spectrum, however, obtained immediately after column chromatography, indicated that the compound was pure.

The signals due to the 3-methylbutanoyl and iso-hexenoyl side-chains were clearly seen (see Table), but there was no very low-field OH signal typical of a six-membered-ring nucleus; instead a broad OH signal (1 H) at τ −2.8, characteristic of the enolised cyclopentatrione nucleus of type (5), was visible. The signals normally associated with the C-4 side-chain in the isohumulone (5), however, were replaced by two triplets (*J* 7 Hz) àt τ 8.07 (2 H) and 6.72 (1 H, and two singlets at 8.76 and 8.80. These could be assigned to the hydroxy-dihydropyran ring system as shown in structure (15). The signal due to the C-4 methine proton in (15) could be recognised as a partially obscured triplet (1 H) at τ 7.23.
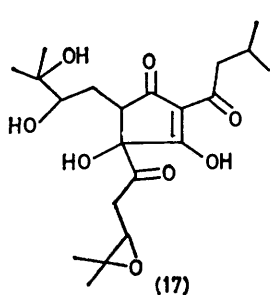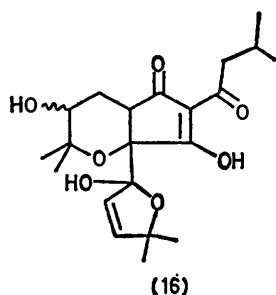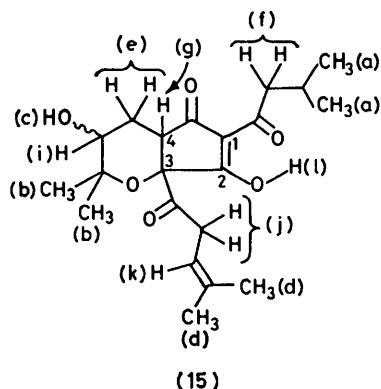
[1]H N.m.r. assignments for structure (15)

Chemical shift (τ)	Intensity	Multiplicity	*J*/Hz	Assignment
9.05	6 H	d	6.5	(a)
8.80	3 H	s		(b)
8.76	3 H	s		(b)
8.65		s		(c)
8.41	3 H	s		(d)
8.30	3 H	s		(d)
8.07	2 H	t	7	(e)
7.27	2 H	d	7	(f)
7.23	1 H	t	7	(g)
6.72	1 H	t	7	(i)
6.41	2 H	m		(j)
4.76	1 H	t	7	(k)
−2.80	1 H	s (br)		(l)

Unfortunately, before mass-spectrometric or other measurements were obtained on this compound it was transformed in air into a new derivative of the same R_F value, the f.d. mass spectrum of which showed a molecular ion of 394 a.m.u. This corresponded to the addition of a further atom of oxygen to structure (15). The new compound, which itself proved unstable, nonetheless gave a clear [1]H n.m.r. spectrum, which revealed, as the only evident impurity, a small amount of the precursor (15). The most obvious difference in the spectrum from that of (15) was the replacement ot the signals of the isohexenoyl side-chain in (15) by two singlets at τ 8.58 and 8.63, together with a broad *two*-proton olefinic signal at τ 2.93. The explanation of these changes is speculative, but it is possible that the transformation product has a structure such as (16) which arises from oxidation and spontaneous cyclisation of the C-3 side-chain in (15). The proposal of structure (15) must remain tentative because of lack of data, but it is advanced here partly because of its relationship to the similarly transient isomer (25) (see later).

The difficulties experienced in isolating some of the components of the water-soluble reaction products from humulone prompted a different approach to the reported glycol (7) and its probable transformation products, *e.g.* the glycol (8). In previous work [1,10,11] it has often proved possible to prepare oxidised derivatives of hop constituents by chemical means which have subsequently facilitated their isolation from mixtures obtained by aerial oxidations. We first therefore examined the reaction of (−)-humulone with *m*-chloroperbenzoic acid.

Peroxy-acids are known to attack C=C bonds readily;[12] moreover, monoperphthalic acid has already been shown to convert humulone (1) into the ring-contracted epoxyglycol (17).[10]

(15)

These two last signals are characteristic of the 2-(1-hydroxy-1-methylethyl)dihydrofuran ring system shown in structure (18). In the e.i. mass spectrum an intense ion at m/z 59 (70%) could be seen, as predicted for fragmentation of the 1-hydroxy-1-methylethyl substituent.[13]

The remaining doublet at τ 7.26 in the ¹H n.m.r. spectrum, assignable to the CH_2 group of the 3-methylbutanoyl chain in (18), and the vinyl methyl signals at τ 8.36 and 8.5, like the O–CMe_2 signals, were split. This effect may be explained by the tautomeric equilibrium (18a) ⇌ (18b) shown, and was substantiated by the intensity (0.5 H) of the low-field enolic proton signal, showing the proportions of the two forms to be 1 : 1. If the dihydrofuran ring system had been formed by cyclisation onto the alternative 4-OH group in (18), a low-field enol proton signal of total intensity 1 H would have resulted.

The orientation shown in structure (18) was proved by hydrogenolysis over palladium chloride to give, in high

(16)   (17)
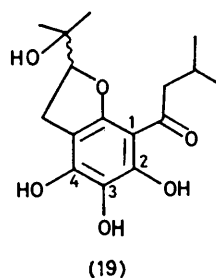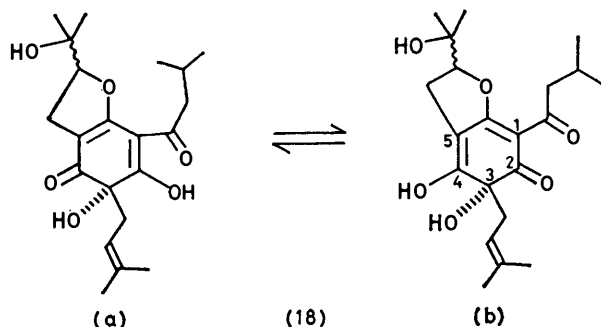

(a)   (18)   (b)

(19)

(−)-Humulone (1), on treatment with m-chloroperbenzoic acid (2 mol equiv.) at 20 °C in dry ether, overnight, gave, besides unchanged humulone, a mixture of products which was subjected to column chromatography. The major component was an optically active oil (20—27%) [M^+ (f.d.) 378]. Accurate mass measurement showed the molecular formula to be $C_{21}H_{30}O_6$ and the two most intense ions in the e.i. spectrum were at m/z 310, corresponding to loss of a quaternary dimethylallyl substituent with hydrogen rearrangement [8] as in humulone (1), and m/z 69, from direct cleavage. The u.v. spectrum indicated the retention of the humulone ring nucleus, which was also evident from a low-field signal (0.5 H) at τ −6.15 in the ¹H n.m.r. spectrum. In the high-field region the signals from one of the pairs of vinyl methyl groups in humulone (1) were replaced by two split signals at τ 8.68 and 8.75, which indicated a new O–CMe_2 group. Between τ 7.0 and 7.6 a six-proton multiplet was seen which, when the solvent was changed to [²H₆]acetone, was resolved into three doublets (each 2 H). The doublet at highest field (τ 7.48) could be assigned to the CH_2 protons of the unchanged C-3 dimethylallyl side-chain; furthermore, irradiation at the frequency of this signal caused decoupling of the associated olefinic signal at τ 5.0. Irradiation at the frequency of the lowest field doublet (τ 7.14) caused collapse of a sharp triplet (J 9 Hz) at τ 5.21 to a singlet.

yield, the crystalline quinol (19), M^+ 310. The alkaline shift of the main maximum in the u.v. spectrum of (19), measured in the presence of an excess of sodium borohydride as for the quinol (14), was 37 nm. In the ¹H n.m.r. spectrum of the quinol (19), the doublet (τ 6.92, J 9 Hz) and the triplet (τ 5.29, J 9 Hz) of the dihydrofuran ring protons could be seen clearly. Finally, the quinol was optically inactive, showing the absence of any stereoselectivity in the cyclisation of the presumed epoxide to the parent compound (18).

A further oily optically active component, of very similar polarity to (18), was obtained from the chromatography. Like (18) it showed a weak molecular ion at

m/z 378, recognised by the loss of 68 a.m.u. to give a major ion at *m/z* 310.1406 ($C_{16}H_{22}O_6$). The u.v. characteristics and the low-field enolic proton (0.4 H) regions of the ^{1}H n.m.r. spectrum resembled those for (18), suggesting an identical six-membered-ring nucleus and tautomeric equilibrium. The dihydrofuran ring signals of (18) however were replaced by a multiplet (1 H) at τ 6.21 and a complex multiplet centred on τ 7.47. By irradiation at the frequency of the latter signal (over the range τ 7.51 to 7.45) it was possible to cause collapse at both the multiplet at τ 6.21 and the olefinic triplet at τ 5.03 to broad singlets. The lack of an intense ion at *m/z* 59 in the mass spectrum and the absence of the doublet and triplet signals (*J* 9 Hz) characteristic of the dihydrofuran ring system in (18) pointed to the isomeric structures (20). The complex multiplet at τ 7.47 already referred to can then be assigned to the two methylene groups (c) and (d) of the hydroxychroman (20), and the multiplet at τ 6.21 to the α-carbinyl proton (e).

Hydrogenolysis of (20), as for (18), gave the optically inactive phenol (21) showing M^+(f.d.) 310. In its ^{1}H

n.m.r. spectrum, the expected [14] multiplet and triplet of the ABX system of the hydroxypyran ring system were clearly evident, and the alkaline shift in the u.v. maximum, measured as above, was 38 nm, confirming the ring orientation shown for the hydroxydihydropyran ring.

Since, under our conditions, peroxy-acid oxidation of the side chains of humulone evidently led to immediate cyclisation of the intermediate epoxides, we next tried the oxidation of the isohumulone system directly. In order to try to reduce the number of possible stereo-isomers, photoisohumulone of known configuration (6) was prepared by photoisomerisation [15] of (−)-humulone and crystallised to constant m.p. (60—61 °C). On treatment with *m*-chloroperbenzoic acid (2 mol equiv.) at 20 °C in dry ether overnight, photoisohumulone gave a mixture containing unchanged starting material, *i.e.* retaining the configuration (6), and several other products. Two of these, (22) and (23), were obtained pure after extraction with sodium carbonate solution and column chromatography on silicic acid.

The glycol (22) showed in its e.i. mass spectrum a molecular ion at *m/z* 396.2145 ($C_{21}H_{32}O_7$); the u.v. spectrum confirmed the retention of the isohumulone nucleus. In the ^{1}H n.m.r. spectrum, the vinylic methyl resonances were reduced in intensity by half, and were replaced by new signals at τ 8.76 and 8.84. These resonances, together with multiplets at τ 7.85 (2 H) and 6.27 (1 H), are assigned to the 2,3-dihydroxy-3-methyl-butanyl side-chain. That the C-3 isohexenoyl group was intact was evident from the characteristic methylene doublet at τ 6.53 and from the mass spectrum. This showed the separate loss of two molecules of water from the molecular ion (*m/z* 378 and 360) and the loss of 96 a.m.u. (loss of $COCH_2CH=CMe_2$ with hydrogen transfer) from M^+, $M^+ − H_2O$, and $M^+ − 2H_2O$.

The configurations of the C-3 and C-4 chiral centres are assumed to be unchanged from those in photoiso-humulone (6), since the latter was recovered unchanged from the peroxy-acid reaction mixture after chromato-graphy; presumably two diastereoisomers result from the oxidation to the glycol.

The epoxy-glycol (23) was obtained as a crystalline solid, m.p. 65—70 °C. The ^{1}H n.m.r. signals were rather broad, but no vinylic methyl signals were seen and the intensity of the signals of the O-CMe_2 groups was doubled relative to compound (22); the signal at τ 6.53 due to the CH_2 group of the isohexenoyl side-chain in (6) had, as expected, shifted. In the e.i. mass spectrum a prominent ion at *m/z* 113 established the position of the epoxide group on the C-3 side-chain, and this was con-firmed by the conversion by oxidation with peroxy-acid of the glycol (22) into (23) as shown by comparison of the i.r. and e.i. mass spectra. It seems clear that the epoxy-glycol (23) is the same as, or a mixture of stereoisomers of, the compound (17), m.p. 65—68 °C prepared earlier by Connett and Elvidge from humulone.[10]

The production of the glycol (22) and the epoxy-glycol (23) with *m*-chloroperbenzoic acid may be the result of

(20)

(21)

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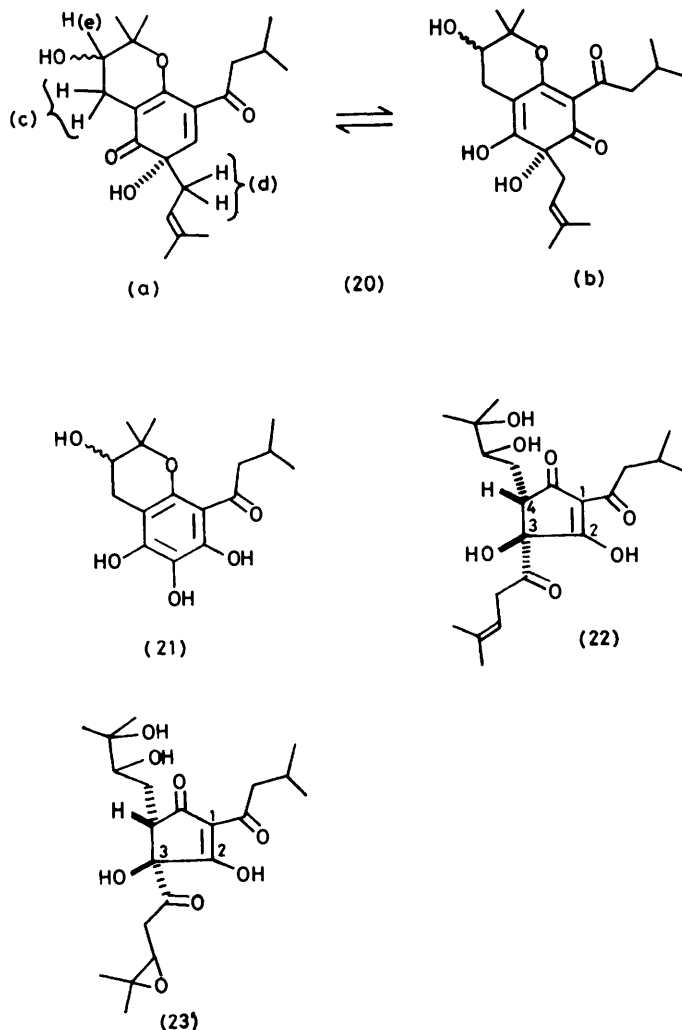
the aqueous work-up procedure. Several attempts were made to isolate the mono-epoxide (24), and although small quantities of products which might have been (24) were isolated, they were always unstable. In one reaction, however, in which photoisohumulone (6) was treated with 1 mol equiv. of peroxy-acid, a compound of R_F below that of isohumulone was isolated. E.i. mass spectrometry showed a molecular ion at m/z 378. An ion at m/z 71 indicated an epoxidised side-chain, but the lack of any significant ion at m/z 113 and the presence

(24)   (25)
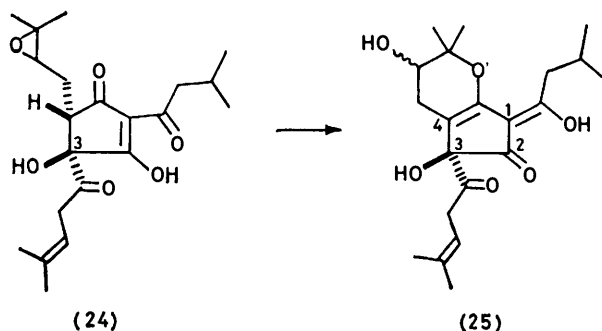
of the base peak at m/z 281, corresponding to cleavage of the unchanged 3-isohexenoyl side-chain, indicated that the C-4 side chain of (6) had been epoxidised. Unfortunately, before any further information could be obtained this product changed into a new compound of very similar R_F value. This latter compound was more efficiently obtained, with the absence of any glycols, when the peroxy-acid oxidation was carried out with 2 mol equiv. of isohumulone and if water was omitted from the work-up. The u.v. spectrum was different from that of isohumulone, but could only be that of a five-membered-ring structure. The e.i. mass spectrum showed a molecular ion at m/z 378, confirming that still only one atom of oxygen had been added to isohumulone (6). In the ^{1}H n.m.r. spectrum, the existence of the C-3 iso-hexenoyl side-chain was confirmed by a triplet at τ 4.78 and a doublet at 6.48, and irradiation at the frequency of the former signal caused collapse of the doublet to a broad singlet. The oxygenation is therefore associated with the C-4 dimethylallyl side-chain and a multiplet at τ 7.96 and a multiplet at τ 5.6—6.1 could be explained by a fused hydroxypyran ring system. Irradiation at τ 5.6—6.1 caused a marked sharpening of the associated multiplet at 7.96.

These facts can best be explained (but tentatively) by the structure (25), which is an isomer of the proposed structure (15) derived from the humulone–water reaction described above. The abnormally high τ value (τ 7.5) of the doublet signal for the CH_2 group of the 3-methyl-butanoyl side-chain compared with the more usual values of τ 7.27 and 7.30 for structures (15) and (5), respectively, is in keeping with the absence of the 5-oxo-group in structure (25). Like its isomer (15), compound (25) was found to oxidise spontaneously in air. Immediate hydrogenation afforded a mixture whose f.d. mass

spectrum showed two predominant ions, at m/z 380 [from (25)] and 396 (from its oxidation product).

The isomer (15) formed during treatment of humulone in boiling water presumably arises *via* epoxidation of the C-4 side chain of *cis*-isohumulone (5), itself derived from prior isomerisation of humulone. The alternative orientation of the product (25) and the apparent absence of (15) when photoisohumulone (6) is treated with peroxy-acid are due presumably to the specific configuration of (6), and hence that of the epoxide (24), in which attack on the epoxy-group by the 3β-OH group is sterically precluded.

To summarise, the transformations of humulone in air and water are complex. Cyclisation of the side chains to compounds such as (12) appears to give relatively stable products. Direct oxidation of the C-5 side-chain double bonds, presumably *via* epoxide intermediates, gives rapidly formed dihydrofuran and dihydropyran ring systems. Ring contraction of the humulone nucleus [*e.g.* (20) ——► (25)] may then occur, but oxidation of the preformed isohumulones (5) and (6) may give glycols such as (22) and (23) or may result in cyclisation to give products such as (15) and (25) which are susceptible to further oxidation in air. It seems that, from the products identified so far, there is a tendency for the C-5 side-chain of humulone (or C-4 in isohumulone) to be oxidised before the alternative dimethylallyl substituent. Independent evidence for this process in humulone will be presented in a future publication.

EXPERIMENTAL

Mass spectra were obtained with a Varian CH5-D instrument (a) by electron impact (e.i.) with a direct insertion probe at 70 eV and 50 μA, or (b) by field desorption (f.d.) at a wire current of 15—20 μA. I.r. spectra were measured on a Unicam SP 200 grating spectrophotometer; u.v. spectra were determined on a Unicam SP 800 instrument. N.m.r. spectra were determined on a Perkin–Elmer R 32 (90 MHz) spectrometer for solutions in $CDCl_3$ unless stated otherwise. T.l.c. was performed on Whatman silica gel 50F T.L.C. Light petroleum refers to the fraction boiling at 40—60 °C. Silicic acid used for column chromatography was Mallinck-rodt 100 mesh AR. Elemental analyses were not obtained for compounds isolated as very small amounts of oil, or which were unstable in air. Where accurate mass measurement of molecular ions was used to infer molecular formulae, the t.l.c. characteristics and ^{1}H n.m.r. spectrum both indicated a pure compound.

Products from Humulone (1) *and Boiling Water.*—In a typical experiment, (−)-humulone (200 mg) in methanol (10 ml) was added to boiling water (1 250 ml) and the solution was boiled for 2 h. It was then acidified and extracted first into iso-octane (2 × 250 ml) (this extract was dried over $MgSO_4$ and evaporated to dryness) and secondly into chloroform (2 × 250 ml) [this extract was also dried ($MgSO_4$) and evaporated to dryness]. The residual oils from the two and extracts were chromatographed separately on silicic acid, eluting with ethyl acetate (re-distilled) and light petroleum mixtures. The t.l.c. system

used for the non-polar products in the iso-octane extract was 12.5% ethyl acetate in light petroleum.

(a) *Iso-octane extract.* Elution with ethyl acetate–light petroleum (1 : 19) afforded the *dihydropyran* (12), which on recrystallisation from light petroleum (b.p. <40 °C) gave colourless crystals, m.p. 96—98 °C (5.7%), $\lambda_{max.}$ (acidic methanol) 352sh (ε 5 965), 320 (9 450), 285 (9 140), 245 (10 980), and 234sh nm (10 050), $\lambda_{max.}$ (alkaline methanol) 325sh (ε 6 280), 287 (9 730), and 250 nm (18 370), τ 9.01 (6 H, d, J 6.5 Hz, COCH_2CHMe_2), 8.65 (9 H, s, 3 × OCMe), 8.52 (3 H, s, OCMe), 8.27 (2 H, t, J 6.5 Hz, OCMe$_2$CH_2 of dihydropyran ring), 7.64 (2 H, t, J 6.5 Hz, C=CCH_2), 7.72—8.14 (4 H, m, CH_2CH_2 of tetrahydrofuran ring), 7.14 (2 H, d, J 7 Hz, CH_2CO), −8.4 (0.15 H, br, s, OH), and −9.0 (0.85 H, br, s, OH); m/z (f.d.) 362 (100%, M^+), (e.i.) 362 (69%, M^+), 347 (48), 304 (55), 289 (77), 209 (100), 85 (20), 69 (50), and 57 (50) (Found: C, 69.1; H, 8.5. $C_{21}H_{30}O_5$ requires C, 69.2; H, 8.24%). Elution with ethyl acetate–light petroleum (1 : 19) afforded the *dihydropyran* (13) as an oil (2—13%), $\lambda_{max.}$ (acidic methanol) 350sh (ε 3 590), 322 (5 260), 282 (6 220), and 24 nm (7 120), $\lambda_{max.}$ (alkaline methanol) 325sh (3 770), 284 (6 220), and 250 nm (10 170), τ 9.02 and 8.99 (6 H, 2d, each J 6.5 Hz, COCH_2CHMe_2), 8.60 (6 H, s, OCMe_2), 8.47 and 8.33 (6 H, 2s, 2 × C=CMe), 8.20 (partially obscured m, CH_2CH_2OCMe$_2$), 7.3—7.7 (2 partially obscured m, CH$_2$CH$_2$OCMe$_2$, and C=CCH_2), 7.45 (br, s, OH), 7.17 (2 H, d, J 7 Hz, CH_2CO), 6.14 (1 H, s, OH), 5.03 (1 H, br, t, J 7 Hz, C=CH), and −8.9 (1 H, s, chelated OH); m/z (f.d.) 362 (100%, M^+), (e.i.) 294 (10%, M − 68), 259 (14), 147 (33), 129 (100), 113 (43), 71 (40), 70 (40), 69 (19), 58 (79), and 56 (34) (Found: M − 68, 294.1468. $C_{16}H_{22}O_5$ requires 294.147).

Hydrogenation of the dihydropyran (13). The dihydropyran (13) (17.5 mg) in methanol (1.5 ml) was hydrogenated over palladium chloride (3.5 mg) for 75 min; water was added, and the mixture extracted with ether. The ether layer was washed with water, dried (MgSO$_4$), and evaporated under reduced pressure to yield the quinol (14), as an oil (14 mg, 96%), $\lambda_{max.}$ (ethanol pH 9.5 + NaBH$_4$) 305 and 250 nm, $\lambda_{max.}$ (ethanol pH 7.0 + NaBH$_4$) 293 and 239sh nm, τ 9.05 (6 H, d, J 6.5 Hz, COCH_2CHMe_2), 8.67 (6 H, s, OCMe_2), 8.23 (2 H, t, J 7 Hz, ArCH$_2$CH_2), 7.78 (1 H, m, J 6.5 Hz CH$_2$CHMe$_2$), 7.40 (2 H, t, J 7 Hz, ArCH_2CH$_2$), 7.06 (2 H, d, J 7 Hz, COCH_2), 5.10 (1 H, br, s, OH), 3.85 (1 H, s, OH), and −3.62 (1 H, s, chelated OH); m/z (e.i.) 294 (78%, M^+), 292 (40), 277 (65), 265 (82), 264 (40), 239 (44), 238 (74), 237 (81), 236 (51), 235 (54), 223 (72), 221 (60), 209 (90), 208 (58), 207 (62), 195 (24), 193 (33), 182 (100), 181 (95), 180 (72), 167 (30), 165 (26), 152 (52), 151 (42), 85 (35), 69 (66), and 57 (65).

Conversion of the dihydropyran (13) *into the tetrahydrofuran* (12). The dihydropyran (13) (2 mg) was treated with water (5 ml) to which 1 drop of dilute hydrochloric acid had been added, and the solution was heated under reflux for 2 h. After cooling and work-up *via* chloroform, t.l.c. indicated the presence of compound (12).

(b) *Chromatography of the chloroform extract.* The chloroform extract of the reaction mixture from (−)-humulone (several reactions) was chromatographed on silicic acid; elution with ethyl acetate–light petroleum (1 : 3) gave tricyclodehydroisohumulone (2) as a colourless solid (3.7%). Recrystallisation from light petroleum (b.p. 100—120 °C) gave a sample of m.p. 160—162°, τ 9.46 (3 H, s, Me), 9.03 (6 H, d, J 6 Hz, COCH_2CHMe_2), 8.93 (3 H, s, Me), 8.22 (3 H, s, MeC=C), 8.15—7.33 (9 H, complex multiplets), 7.20 (2 H,

d, J 6.5 Hz, COCH_2CH), 5.22 (1 H, s,br, H–C=C), and 5.00 (1 H, s, br, H–C=C), m/z (f.d.) 360 (100%, M^+). The sample had mixed m.p. 160—163° with an authentic sample of tricyclodehydroisohumulone, m.p. 163° (kindly supplied by Dr. D. R. J. Laws), and showed the same t.l.c. characteristics. Elution with ethyl acetate–light petroleum (3 : 97) gave the hydroxypyran (15) as an oil (3.7%); for τ (see Table); m/z (f.d.) 378 (100%, M^+). In air this compound was oxidised to a new product of the same R_F value, tentatively assigned structure (16), $\lambda_{max.}$ (acidic methanol) 274sh and 235 nm, $\lambda_{max.}$ (alkaline methanol) 274sh and 243 nn, τ 9.05 (6 H, d, J 6.5 Hz, COCH_2CHMe_2), 8.83 (3 H, s, OCMe), 8.79 (3 H, s, OCMe), 8.63 (3 H, s, OCMe), 8.58 (3 H, s, OCMe), 8.07 (m, CHOH·CH_2), 7.27 (2 H, d, J 7 Hz, COCH_2), 7.25—7.0 (partially obscured multiplet, COCH-CH$_2$), 6.49 (1 H, t, J 6.5 Hz, CHOH), and 2.93 (2 H, br, s, $^-$CH=CH–); m/z (f.d.) 394 (100%, M^+).

Oxidation of (−)-*Humulone with* m-*Chloroperbenzoic Acid.*—m-Chloroperbenzoic acid (425 mg) in anhydrous ether (10 ml) was added to a solution of (−)-humulone (420 mg) in anhydrous ether (20 ml) and the mixture, protected with a drying tube, was stirred at 20 °C for 28 h. The ether was evaporated off under reduced pressure and the remaining gum, dissolved in the minimum quantity of ethyl acetate–light ptroleum (1 : 4), was chromatographed on silicic acid (85 g column 200 × 30 mm). Elution with ethyl acetate–light petroleum (1 : 4) yielded a mixture of unchanged humulone and m-chlorobenzoic acid. Elution with ethyl acetate–light petroleum (1 : 2) followed by the same solvents in the ratio (3 : 2) gave several very minor products which it was not possible to characterise. Elution with ethyl acetate–light petroleum (7 : 3) gave the *hydroxydihydropyran* (20) (74 mg, 17%) as an oil contaminated with impurities visible by t.l.c. Preparative t.l.c. [formic acid–ethyl formate–hexane (1 : 8 : 12)] afforded a pure sample (12 mg), R_F 0.36, $[\alpha]_D^{20}$ +124° (c 0.76 in methanol), $\lambda_{max.}$ (acidic methanol) 314, 250, and 223 nm, $\lambda_{max.}$ (alkaline methanol) 395, 308, and 247 nm; τ 9.03 (6 H, d, J 7 Hz, COCH_2CHMe_2) 8.66, 8.64, 8.58, and 8.56 (6 H, 4s, OCMe_2), 8.52 and 8.37 (6 H, 2s, CH=CMe_2), 7.82 (1 H, m, CH$_2$-CHMe$_2$), 7.33—7.55 (4 H, m, CH_2CH=CMe$_2$ and CH_2-CHOH), 7.27 and 7.24 (2 H, 2d, each J 7 Hz, COCH_2CHMe_2), 6.21 (1 H, m, CHOH), 5.03 (1 H, t, J 7.5 Hz, CH$_2$CH=CMe$_2$), and −7.24 (0.4 H, s, chelated OH), m/z (e.i.) 378 (0.6%, M^+), 310 (62), 277 (19), 239 (100), 238 (32), 223 (25), 221 (21), 182 (38), 85 (17), 69 (49), and 57 (24) (Found: M − 68, 310.1406. $C_{16}H_{22}O_6$ requires 310.1416).

Elution with ethyl acetate–light petroleum (7 : 3) gave the *dihydrofuran* (18) as an oil (83 mg, 20%), R_F 0.30 in the above system, $[\alpha]_D^{20}$ +79° (c 1.13 in methanol), $\lambda_{max.}$ (acidic methanol) 327 (ε 6 140) and 255 nm (6 150), $\lambda_{max.}$ (alkaline methanol), 365 (ε 5 300), 308 (5 800), and 246 nm. τ[(CD$_3$)$_2$CO] 9.02 (6 H, d, J 7 Hz, CH$_2$CHMe_2), 8.78, 8.73, 8.72, and 8.66 (6 H, 4s, OCMe_2), 8.51 and 8.53 (3 H, 2 overlapping s, C=CMe), 8.39 and 8.42 (3 H, two overlapping s, C=CMe), 7.48 (2 H, d, J 7 Hz, CH_2CH=CMe$_2$), 7.2 (2 H, d, J 7 Hz, COCH_2), 7.16 (2 H, d, J 9 Hz, OCH–CH_2 of dihydrofuran ring), 6.6 (2 H, vbr, s, 2 × OH), 5.13 (1 H, partially overlapping t, J 9 Hz, OCH–CH$_2$ of dihydrofuran ring), and 5.0 (1 H, overlapping broad t, J 7 Hz, CH=CMe$_2$) [signals in CDCl$_3$ were essentially the same but the multiplet at τ 7.86 (1 H, COCH$_2$CHMe$_2$) was visible]; m/z (f.d.) 378 (100%, M^+), (e.i.) 378 (19%, M^+), 310 (100), 277 (86), 252 (40), 238 (87), 85 (82), 69 (90), and 59 (70) (Found: M^+, 378.2012. $C_{21}H_{30}O_6$ requires M, 378.2042).

Hydrogenolysis of the Hydroxydihydropyran (20).—The dihydropyran (20) (8.3 mg) in methanol–water (10 : 1) (0.5 ml) was hydrogenated over palladium chloride (1.5 mg) for 1 h; water (5 ml) was added and the solution extracted with ether (10 ml). After washing with water (5 ml) and drying (MgSO₄), the ether was removed under reduced pressure to give an oil (4.1 mg, 60%). Crystallisation from chloroform–light petroleum afforded yellow-green crystals of the quinol (21), m.p. 188—194 °C, $\lambda_{max.}$ (alkaline ethanol + NaBH₄) 337 and 247 nm; $\lambda_{max.}$ (acidic ethanol) 299 and 239 nm; τ[CDCl₃–(CD₃)₂CO] 9.05 (d, *J* 7 Hz, CH₂CH*Me₂*), 8.76 (s, OCMe), 8.64 (s, OCMe), 7.2—7.5 (m, ArC*H₂*), 7.1 (d, *J* 7 Hz, COC*H₂*), 6.20 (m, C*H*OH), and 4.0 (s, chelated OH); *m/z* (f.d.) 310 (100%, *M⁺*), e.i. 311 (11%), 310 (48, *M⁺*), 277 (19), 240 (16), 239 (100), 238 (31), 223 (29), 221 (26), 183 (14), 182 (63), 181 (28), 179 (12), 154 (12), 83 (11), 71 (15), 69 (10), 67 (11), 57 (15), and 55 (15).

Hydrogenolysis of the Dihydrofuran (18).—The dihydro-furan (18) (65 mg) in methanol–water (10 : 1) (3 ml) was hydrogenated over palladium chloride (13.5 mg) until hydrogen uptake was complete (40 min). Water (10 ml) was added and the product extracted into ether (20 ml). Normal work-up afforded a yellow solid (45 mg, 84%). Crystallisation from chloroform gave the *quinol* (19), m.p. 152—156 °C, $\lambda_{max.}$ (alkaline ethanol + NaBH₄) 331 (ε 12 600) and 247 nm (4 700), $\lambda_{max.}$ (acidic ethanol) 290 (ε 6 000) and 240sh nm; τ 9.04 (6 H, d, *J* 6.5 Hz, COCH₂-CH*Me₂*), 8.76 (3 H, s, OCMe), 8.63 (3 H, s, OCMe), 8.45 (1 H, br, s, CHO*H*), 7.99 (1 H, m, *J* 6.5 Hz, COCH₂C*H*Me₂), 7.16 (2 H, m, COC*H₂*), 6.92 (2 H, d, *J* 9 Hz, ArC*H₂*), 5.29 (1 H, t, *J* 9 Hz, ArCH₂C*H*O), 4.70 (1 H, s, ArO*H*), 3.83 (1 H, s, ArO*H*), and −3.42 (1 H, s, chelated OH); *m/z* (f.d.) 310 (100%, *M⁺*), (e.i.) 310 (10, *M⁺*), 277 (12), 239 (8), 84 (100), and 86 (65) (Found: *M⁺*, 310.1415. C₁₆H₃₂O₆ re-quires *M*, 310.1416).

Oxidation of Photoisohumulone (6) *with* m-*Chloroper-benzoic Acid.*—Photoisohumulone¹⁵ (6) (399.6 mg; m.p. 60—61 °C) in ether (20 ml) was treated with *m*-chloroper-benzoic acid (400.5 mg) in ether (10 ml); and the solution kept at 20 °C for 18 h. It was then poured into water and the mixture extracted with sodium hydrogen carbonate (1%; 6 × 20 ml). The extract was extracted in turn with ether, and the latter extract dried (MgSO₄) and evaporated under reduced pressure to give a yellow oil which was chromatographed on silicic acid (column 50 × 3 cm). Elution was begun with light petroleum, with increasing amounts of ethyl acetate. Elution with ethyl acetate–light petroleum (4 : 1) afforded the *glycol* (22) (27 mg, 1.6%) as an oil giving a black spot (FeCl₃) on t.l.c.; $\lambda_{max.}$ (acidic methanol) 279 and 225 nm, $\lambda_{max.}$ (alkaline methanol) 275sh and 253 nm; τ 9.02 (6 H, d, *J* 6.5 Hz, COCH₂CH*Me₂*), 8.87, 8.82, 8.79, and 8.75 (6 H, 4s, 2 × OC*Me*), 8.44, 8.38, 8.30, and 8.24 (6 H, 4s, C=CMe₂), 7.85 (3 H, m, C*H₂*CHOH and COCH₂C*H*), 7.24 (complex multiplet, COCH₂ and CHOHCH₂C*H*), 6.53 (2 H, d, *J* 7 Hz, COC*H₂*CH=C), 6.27 (1 H, m, C*H*OH), 4.74 (1 H, br, t, *J* 7 Hz, C=CH), and 4.0 (4 H, br, s, 4 × OH); *m/z* (f.d.) 396 (100%, *M⁺*), (e.i.) 396 (7%, *M⁺*), 378 (33), 360 (29), 300 (22), 282 (34), 264 (23), 97 (30), 85 (35), 69 (97), 71 (38), 57 (57) (Found: *M⁺*, 396.2145. C₂₁H₃₂O₇ requires *M*, 396.2148).

Elution with 100% ethyl acetate gave the epoxy-glycol (17) as a pale yellow solid (30 mg, 6.6%), m.p. 65—70 °C (lit.,¹⁰ 65—68 °C), $\lambda_{max.}$ (acidic methanol) 268 (ε 7 150) and 227 nm (6 680), $\lambda_{max.}$ (alkaline methanol) 273 (9 790) and 254 nm (10 100); τ 9.03 (d, *J* 7 Hz, COCH₂CH*Me₂*), 8.9—

8.6 (m, OCMe₂), 7.9 (m, CHOHC*H₂*), 7.47 (d, *J* 7 Hz, oxiran–CH₂CO), 6.52 (m, *J* 7 Hz, CH of oxiran, and 4.90 (br, s, OH) (as described in ref. 10, the spectrum was broad and difficult to analyse, but it closely resembled a copy of the 60 MHz spectrum ¹⁰ kindly provided by Professor Elvidge); *m/z* (f.d.) 412 (70%, *M⁺*), 413 (*M⁺* + 1, 100%), (e.i.) 412, 113, 96, 85, 71, 59, and 57 (Found: *M⁺*, 412.2089. Calc. for C₂₁H₃₂O₈: *M*, 412.2095).

Oxidation of the Glycol (22) *to the Epoxy-glycol* (23).—The glycol (22) (21.4 mg) in ether (1 ml) was treated with *m*-chloroperbenzoic acid (21.4 mg) in ether (1 ml) and the solution kept at 20 °C for 48 h. The mixture was worked up as in the preceding experiment to give a product whose e.i. mass spectrum and i.r. spectrum were identical with those of the epoxy-glycol (23).

Attempted Preparation of the Epoxide (24).—Photoiso-humulone (380 mg) in ether (10 ml) was treated with *m*-chloroperbenzoic acid (180 mg) in ether (10 ml) and the solution was shaken and kept for 4.5 h at 20 °C. T.l.c. then showed the presence of a yellow-staining spot (FeCl₃) and a blue spot beneath that due to isohumulone. The solvent was removed under reduced pressure and the mixture was chromatographed on silicic acid (column 40 × 2.5 cm). Elution with ethyl acetate–light petroleum (1 : 5) gave the component of higher R_F value, tentatively identified as the epoxide (24), as an oil, *m/z* 378 (10%, *M⁺*), 360 (6), 297 (14), 281 (100), 263 (59), 209 (100), 197 (36), 97 (14), 85 (76), 71 (81), 69 (80), 57 (80), and 55 (28). This was observed to change into the dihydropyran (25) (see later), which was also eluted from the column with ethyl acetate–light petroleum (3 : 2) (yield 48 mg, 12%).

Oxidation of Photoisohumulone (6) *to the Dihydropyran* (25).—*m*-Chloroperbenzoic acid (400 mg) in anhydrous ether (10 ml) was added to a solution of photoisohumulone (6) (399.6 mg) in anhydrous ether (15 ml) and the mixture (CaCl₂ guard-tube) was stirred at 20 °C for 22 h. Most of the ether was evaporated off under reduced pressure and the remaining viscous solution (*ca.* 4 ml) chromatographed on a column of silicic acid (85 g; 30 × 200 mm). Elution with ethyl acetate–light petroleum (1 : 4) gave unchanged photoisohumulone (6) as an oil (140 mg, 35%), crystallis-ation of which from iso-octane afforded a sample of m.p. 56—59 °C, mixed m.p. 58—60 °C with an authentic speci-men (m.p. 59—61 °C). The ¹H n.m.r. spectrum was identi-cal with that of starting material (6).

Elution with ethyl acetate–light petroleum (2 : 3) gave the *hydroxydihydropyran* (25) as an oil (65 mg, 16%), [α]$_D^{20}$ +97.1 (*c* 2.6 in methanol), $\lambda_{max.}$ (ethanol) 266 and 240sh nm, $\lambda_{max.}$ (alkaline ethanol) 352, 281, and 248sh nm; τ 9.16 and 9.13 (6 H, 2d, *J* 7 Hz, COCH*Me₂*), 8.44 (6 H, s, OCMe₂), 8.34 and 8.30 (6 H, 2s, C=CMe₂), 7.96 (4 H, m, C*H₂*CHOH, COCH₂C*H*, and OH), 7.50 (2 H, d, *J* 7 Hz, COC*H₂*CH), 6.48 (2 H, d, *J* 7 Hz, COC*H₂*CH=C), 5.6—6.1 (1 H, m, CH₂C*H*OH), 5.1—5.7 (br, chelated OH), and 4.78 (1 H, t, *J* 7 Hz, C*H*=CMe₂), *m/z* (e.i.) 378 (29%, *M⁺*), 302 (20), 239 (23), 197 (26), 109 (48), 85 (46), 71 (76), 69 (76), 57 (65), and 55 (26) (Found: *M⁺*, 378.2038. C₂₁H₃₀O₆ requires *M*, 378.2042).

Hydrogenation of the Dihydropyran (25).—The dihydro-pyran (25) (54 mg) was hydrogenated in ethanol (20 ml) over 5% palladised charcoal (10 mg) until 1 mol equiv. of hydrogen had been taken up. The mixture was filtered and the solvent removed under reduced pressure to give an oil (45 mg). The product was unstable, showing two major ions in its f.d. mass spectrum at *m/z* 380 (100%) and 396

(100%). In the ^{1}H n.m.r. spectrum, the doublet and triplet at τ 6.48 and 4.78 from the 3-isohexenoyl side-chain of (25) had disappeared; a new triplet (2 H, J 7 Hz) from the CH_2CO group of the isohexanoyl group had appeared.

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