CHAPTER III

THE STRUCTURE OF HUMULENE EPOXIDE-I AND HUMULENE EPOXIDE-II
THE STRUCTURE OF HUMULENE EPOXIDE-I AND HUMULENE EPOXIDE-II

As described in the preceding Chapter, the portion of the essential oil of *Zingiber zerumbet* distilling between humulene and zerumbone, consisted essentially of sesquiterpene oxides. The separation of this mixture into caryophyllene oxide and two monoxides of the humulene type*, has been detailed in the same Chapter. The humulene oxide with the lower retention time in GLC, has been designated humulene epoxide-I, while the second oxide has been called humulene epoxide-II. The present Chapter sets forth the evidence leading to the assignment of structures (I) and (II) to humulene epoxide-I and humulene epoxide-II respectively.

*S.K. Ramaswamy and S.C. Bhattacharyya* have described the isolation of a "humulene monoxide" from the same source. However, a careful comparison of the physical constants and IR spectrum of the "humulene monoxide" reported by these authors with the various fractions we had on hand in the course of our work, showed that it closely corresponded to one of our inhomogeneous chromatographic cuts b.p. 108-109°/1.5 mm, nD^20 1.4938, d^20 0.9554, (α)_D -39.8 (neat) whose TLC on AgNO_3-clad silica gel had revealed 3 spots of almost equal area and intensity. GLC on column "P", also had disclosed 3 peaks with areas in the ratio of 34:35:31. The specific rotation of this sample tallies with a linear summation of the sp. rotations of pure caryophyllene oxide, humulene epoxide-I and humulene epoxide-II in the above ratio.
Table 1 enumerates some of the physical constants of the two oxides.

**TABLE 1 - PHYSICAL CONSTANTS OF HUMULENE EPOXIDE-I AND HUMULENE EPOXIDE-II**

<table>
<thead>
<tr>
<th></th>
<th>Humulene epoxide-I</th>
<th>Humulene epoxide-II</th>
</tr>
</thead>
<tbody>
<tr>
<td>b.p. °C/mm</td>
<td>104-105/1.5</td>
<td>105-106/1.5</td>
</tr>
<tr>
<td>n_D_30</td>
<td>1.4935</td>
<td>1.4962</td>
</tr>
<tr>
<td>d_30</td>
<td>0.9507</td>
<td>0.9483 +</td>
</tr>
<tr>
<td>[R_L]_D (found)</td>
<td>67.44</td>
<td>67.84</td>
</tr>
<tr>
<td>&quot; (calc.)</td>
<td>67.78</td>
<td>67.78</td>
</tr>
<tr>
<td>[\alpha]_D</td>
<td>-22.8</td>
<td>-31.2</td>
</tr>
<tr>
<td>Relative retention</td>
<td>1.12</td>
<td>1.29</td>
</tr>
<tr>
<td>R_3 time</td>
<td>0.28</td>
<td>0.44</td>
</tr>
</tbody>
</table>

*Column: 5' x 0.25", 20% diethylene glycol polysuccinate on Chromosorb W; temp. 150°C; carrier gas: H\_2, at 30 ml/min. On this scale, caryophyllene oxide is 1.00.

**Plate: silica gel-Plaster of Paris - AgNO\_3 (100:15:15); solvent system: benzene-acetone (100:5); solvent front 12 cm; on this scale, caryophyllene oxide has R\_3 = 0.84.
FIG 1 UV SPECTRA OF HUMULENE EPOXIDES-I & II

Scale: x-axis 1 mm = 0.25 m
Y-axis 1 mm = 20
This compound analyses for C_{15}H_{24}O. The UV spectrum (Fig.1) is devoid of any characteristic absorption maximum above 210 μm but shows end absorption (ε_{230}, 1134; ε_{225}, 1838; ε_{220}, 2462; ε_{215}, 3070; ε_{210}, 3539). Its IR spectrum (Fig.2) does not exhibit any carbonyl or =O band and hence the oxygen must be present in an oxide ring. That this oxide function is, in all probability, an oxirane ring is suggested by the presence of peaks, in the IR spectrum, at 1248, 887 and 732 cm^{-1}, assignable to such a ring^5,4. Other bands of diagnostic importance in the IR spectrum consist of a doublet at 1560, 1480 cm^{-1}, typical of a gem-dimethyl group⁵, a weak band at 1650 cm^{-1} and an intense one at 971 cm^{-1}, which may be assigned to the \( \nu_{\text{C=O}} \) and \( \delta =\text{C-H} \) respectively of a trans-disubstituted double bond system⁶ \( \text{H}^\text{\ H}\text{C}^\text{\ C}< \), the latter absorption being a pointer to a humulenoïd skeleton*. Its PMR spectrum** (Fig.4) shows

---

*The observed value for molar refractivity (67.44) is close to that calculated (67.78) using the atomic and group refractivities of Anwers and Eisenlohr⁷. The anticipated molar refractivity depression due to the presence of a medium ring⁸,⁹ appears to have been compensated by an exaltation (?) due to the oxirane ring.

** in ~ 20% CCl₄ solution on a Varian A-60 NMR spectrometer with TMS as internal standard.
FIG 4 PMR SPECTRUM OF HUMULENE EPOXIDE-I

FIGS PMR SPECTRUM OF DIHYDROHUMULENE EPOXIDE-II
two 3-proton singlets at 62 and 66 cps, assignable to two quaternary methyl groups (gem dimethyl) and a singlet at 72 cps (3H) due to a quaternary methyl on a carbon attached to an oxygen atom. A doublet at 101 cps (3H, J = 1 cps) can be readily assigned to a vinyl methyl group. A complex multiplet located between 300 and 357 cps accounts for 3 vinyl protons, represented by the ethylenic systems $\text{H} \rightarrow \text{C} = \text{C} \left< \text{H} \right.$ and $\text{H} \rightarrow \text{C} = \text{CH} -$.

On selective hydrogenation (Pt/EtOH), the compound absorbs one mol of hydrogen to form a liquid dihydro derivative, $\text{C}_{15}\text{H}_{26}\text{O}$. The $\text{H}$ spectrum (Fig.3) of this dihydro compound (III) no longer contains the strong 971 cm$^{-1}$ band due to $\text{H} \rightarrow \text{C} = \text{C} \left< \text{H}$. Its PMR spectrum (Fig.5) shows a total of only one vinyl proton in the region between 293 and 318 cps. Further, the finyl methyl is still discernible (3H singlet at 100 cps, faintly split) in the PMR spectrum, thus indicating that the $\text{H} \rightarrow \text{C} = \text{C} \left< \text{H}$. system has been reduced and that the $\text{H} \rightarrow \text{C} = \text{CH} -$ system has survived the hydrogenation. The above data can be accommodated in an epoxy-humulene formula wherein the $\Delta^9:0$-trans-disubstituted ethylenic linkage has been preserved; such a formulation suggests itself on biogenetic grounds as well. Confirmation of this conjecture was obtained by a study of its further epoxidation with perbenzoic acid (PBA), two mols of which were used up per mol of the compound. The reaction product, on work up and crystallisation, afforded in ~ 50% yield a
crystalline solid, m.p. 103-112. Three recrystallizations raised the m.p. to 114-117, [α]D -4.4 (c, 0.58). The m.p. could not appreciably be improved by further crystallisation. However, the IR spectrum of this material was indistinguishable from that of authentic (±)-humulene trioxide (IV)10 and the m.p. was not lowered by admixture with the latter. This not only fixes the carbon skeleton of the new sesquiterpenoid, but also enables it to be formulated either as (I) or as (II). A decision in favour of (I) could be arrived at by a critical study of the PMR spectrum of the oxide and its dihydro derivative (III).

As can be seen from Fig.4, the oxide shows two sharp signals (2H) at 147 and 153 cps. These absorptions are reminiscent of the 143 and 159 cps peaks in the PMR spectrum of humulene (V)11 and are assignable to the two allylic protons at C8. In support of this assignment, the PMR spectrum of the dihydro epoxide (III) (Fig.5), as expected, does not display these signals. The data presented above suffice to establish the structure of humulene epoxide-I as (I), a conclusion which is confirmed by the settlement of the structure
of the second epoxide as (II), as described below.

Humulene epoxide-I is highly sensitive to air oxidation and invariably, during the chromatographic separation of humulene epoxides, a small amount of a crystalline compound $C_{15}H_{24}O_2$ (m.p. 106-106.5) is obtained. The suspicion that at least part of this crystalline material might be an artefact, was strengthened by the observation that a pure sample of humulene epoxide-I on storage at $\sim 5^\circ$, but in contact with air (enclosed), furnished a good yield of this compound. It was soon recognised that the compound was identical (IR spectrum) with a humulene dioxide preparation (VI) reported by Pliva et al.\textsuperscript{12}. This was further confirmed by the finding that this compound on interaction with one mol of PBA gave the characteristic humulene trioxide\textsuperscript{10} (IV).

**Humulene Epoxide-II**

This compound, $C_{15}H_{24}O$, does not show any C=O or O-H absorption in the infra red (Fig.6) and must be an oxide, possibly, an oxirane (IR bands at 1239, 822 and 785 cm$^{-1}$)\textsuperscript{3,4} The presence of a strong band at 972 cm$^{-1}$ in the IR spectrum, assignable to a trans-disubstituted ethylenic linkage\textsuperscript{6} indicated that this oxide might also have originated from humulene. As a matter of fact, it was found that the IR spectrum (Fig.6) of this compound was very similar to that given for a preparation of humulene monoxide, of undetermined structure, obtained by Sorm et al.\textsuperscript{13}, by the action of pervanadic acid (1M)
Fig 7 PMR Spectrum of Humulene Epoxide-II

Fig 8 PMR Spectrum of Dihydro Humulene Epoxide-II
on humulene. An authentic sample of this material prepared according to Sorm's method, was found to be identical with the natural epoxide in all respects, except the fact, that the synthetic material, as expected, was racemic.

Since the IR spectrum of this oxide shows that the trans-disubstituted ethylenic linkage of humulene is intact in this compound, and since structure (I) has been assigned to humulene epoxide-I, structure (II) becomes evident for the new oxide. This is fully corroborated by its spectral characteristics. Thus, in the ultra violet, (Fig.1) it does not show, as expected, any absorption maximum above 210 m\(\mu\). Its PMR spectrum (Fig.7) shows the presence of two quaternary methyls (6H, 64 cps, slightly split), a vinylic methyl (3H signal at 92 cps, slightly broadened, due to allylic coupling), a sharp 3-proton signal at 73 cps assignable to a methyl on the oxirane ring and 3 vinyl protons located between 281 and 325 cps.

This epoxide, on ozonolysis, furnished as-dimethyl succinic acid in good yield, in clear support of the above structure. Like humulene epoxide-I, it also reacted with two mols of PBA to furnish a trioxide, m.p. 121-122, \([\alpha]_D +0.57\) (c, 0.7) whose IR spectrum was identical with that of authentic (±)-humulene trioxide\(^{10}\).
In view of the characterisation of these sesquiterpene oxides as humulene epoxides, it appeared of interest to see, if the reaction of humulene with a peracid would lead to an oxide mixture, with a composition approximating the one encountered in the essential oil. However, out of the three sets of conditions investigated, one [pervanadic acid (1M) in acetone solution at 0 to 10°C] yielded only humulene epoxide-II (besides a little humulene dioxide), while the other two methods [PBA (1M), in benzene-toluene solution at -0°C and PBA (1M) in chloroform at -0°C] furnished only traces of humulene epoxide-I, besides the major product, humulene epoxide-II.

**STEREOCHEMISTRY**

**Humulene epoxide-II**: Recent X-ray studies on humulene-AgNO₃ complex have led to the establishment of the geometry of the three ethylenic linkages of humulene as all-trans¹⁴,¹⁵. Since in an epoxidation reaction, using a peracid, no change in the configuration of an ethylenic linkage can be expected¹⁶, under the usual experimental conditions employed, the two ethylenic linkages in humulene epoxide-II, the racemic form of which is the chief product of mono epoxidation of humulene, must be trans- (II), as in humulene (V). Furthermore, since the epoxidation reaction involves a cis- addition of the epoxy oxygen¹⁷,¹⁸, without disturbing the configuration of the original double bond, the only remaining point about the
Figure 91. Spectra of Humulene Dioxides

Humulene Dioxide

1. Right null
2. xx (-) 
3. Natural
stereochemistry of humulene epoxide-II is the absolute stereochemistry. As discussed in Chapter IV, humulenol (VII) has in all probability the (R) configuration at C₆ and since humulene epoxide-II is convertible into humulenol by a reaction not affecting the configuration of C₆ (vide Chapter IV), humulene epoxide-II is also tentatively assigned the same absolute configuration (R) at C₆ (VIII).

![Chemical Structures](VII-VIII)

**Humulene Epoxide-I**: On treatment with 1M of PBA, both humulene epoxide-I and humulene epoxide-II give the same humulene dioxide (m.p., mixed m.p. and IR spectra - Figs. (9) and (10)). Hence it is clear that in humulene epoxide-I also, the two ethylenic linkages must have the same trans-geometry (I) as in humulene (V). The solid dioxides from humulene epoxide-I and humulene epoxide-II show only feeble optical activity, but of opposite sign (Table 2) and hence must be antipodes. The antipodal nature of the two preparations of humulene dioxide, is further borne out by the preparation of humulene trioxide from both humulene epoxide-I and humulene epoxide-II. The above considerations necessitate that the two preparations should also have opposite rotations.
FIG. 11 HUMULENE DIOXIDES FROM HUMULENE MONOXIDES
and this, indeed, is found to be the case (Table 2).

### TABLE 2 - PHYSICAL CONSTANTS OF HUMULENE DIOXIDES AND TRIOXIDES

<table>
<thead>
<tr>
<th></th>
<th>From (-) humulene epoxide-I</th>
<th>From (-) humulene epoxide-II</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Humulene Dioxide</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>m.p.</td>
<td>97-101</td>
<td>98-101</td>
</tr>
<tr>
<td>$[\alpha]_D$</td>
<td>+1.42</td>
<td>-0.76</td>
</tr>
<tr>
<td><strong>Humulene Trioxide</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>m.p.</td>
<td>114-117</td>
<td>121-122</td>
</tr>
<tr>
<td>$[\alpha]_D$</td>
<td>-4.4</td>
<td>+0.57</td>
</tr>
</tbody>
</table>

Since humulene epoxide-II has been (tentatively) assigned the R-configuration at C₆ (VIII), this observation enables an R-configuration to be provisionally assigned to humulene epoxide-I at C₂ (IX). The reasoning leading to this conclusion is as follows.

Humulene dioxide derived from humulene epoxide-II can have either of the structures (VIIIa) or (VIIIb) (Fig.11). From the structures given in Fig.11, it is clear that humulene dioxide derived from humulene epoxide-I can be either (IXb) or (IXa), since the two dioxides are antipodes. It can further be seen that a decision about the structure of diepoxide (VIIIa) or (VIIIb) will lead to the establishment
of the absolute stereochemistry of epoxide-I. Depending on its preferred conformation (XI) or (XII), humulene epoxide-II, on further epoxidation, can give rise to (VIIIa) or (VIIIb). Though it is difficult indeed to draw any conclusion about the preferred conformation of humulene epoxide-II, a decision about the structure of humulene dioxide (VIIIa or VIIIb) could possibly be drawn from the feeble optical rotation of this compound in contrast to humulene monoxides (Table 2). The low optical rotation implies that the contribution of the second epoxide ring is essentially equal but opposite in sign to that of the first. This appears to be in accord with the structure (VIIIb) for (-)-humulene dioxide, as in this structure, the absolute stereochemistry of the two oxide rings are opposite to each other. On this basis,
humulene epoxide-I must have the absolute stereostructure *(XIII).*

*It is quite reasonable to assume that in the formation of humulene epoxides and the caryophyllene oxide, which must originate in a subsequent epoxidation step from the parent hydrocarbons, the same enzyme system must have been involved. Since the absolute stereochemistry of the oxirane ring must be the direct consequence of the asymmetry of the enzyme 'surface', the way in which the substrate would land on the enzyme surface would be governed by the resulting steric interactions. If it is assumed that in the caryophyllene molecule, the gem dimethyl group governs the approach to the enzyme surface, then, since the molecular shape and dimensions of humulene should be similar to those of caryophyllene, epoxidation of humulene should also occur in the same absolute sense. Thus, like (-)-caryophyllene oxide (-)-humulene oxide-I and (-)-humulene oxide-II should also have (R,R)-configuration.*
FIG. 12 IR SPECTRA OF DIHYDRO HUMULENE EPOXIDES - II
Some observations on the optical activity and mixed melting behaviour of compounds in this series

(-)-Humulene epoxide-II, on catalytic hydrogenation, readily furnished a dihydro epoxide (\(\lambda IV\), m.p. 69-69.5, and this compound, quite unexpectedly, did not depress the m.p. of (+)-dihydro epoxide-II, m.p. 69.5, prepared, likewise, from (+)-humulene epoxide-II\(^9\). Furthermore, surprisingly, the dihydro epoxide obtained from the optically active natural oxide, showed only very low optical activity, \([\alpha]_D -0.28\) (c, 2.5, CCl\(_4\)). The IR spectra of these two preparations (Fig.12) were completely superimposable; the absence of any strong absorption around 970 cm\(^{-1}\) in these spectra shows that the 3,10-double bond has been reduced (\(\lambda IV\)) during this hydrogenation. Again, (-)-humulene dioxide \([\alpha]_D -0.13\), (c, 2.3, CCl\(_4\)), m.p. 106-106.5 did not depress the m.p. of (+)-synthetic preparations, m.p. 106-106.5. Similarly, the triepoxide

![Diagram XIV](image)

![Diagram XV](image)

(m.p. 121-122) obtained by the further epoxidation of the naturally occurring (-)-humulene epoxide-II, did not depress the m.p. of (+)-humulene trioxide (m.p. 121-122), prepared
by the epoxidation of humulene. Moreover, the optical rotation of the triepoxide obtained from (−)-humulene epoxide-II was very low: \([\alpha]_D +0.57, (c, 0.7)\).

Since in the above reactions, there is no possibility of racemisation, it was at one time doubted whether humulene triepoxide has really an oxirane structure (IV). However, this was easily settled by an examination of the PMR spectra of the various compounds studied in the present series. (Table 3).

**TABLE 3 - CHEMICAL SHIFTS OF OXIRANE PROTONS IN VARIOUS HUMULENOID EPXIDES AND IN CARYOPHYLLENE OXIDE**

<table>
<thead>
<tr>
<th>No.</th>
<th>Compound</th>
<th>Structure</th>
<th>Chemical shifts (cps)</th>
<th>Multiplicity; J in cps</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Humulene epoxide-I</td>
<td>I</td>
<td>135</td>
<td>Triplet, J = 2.5</td>
</tr>
<tr>
<td>2</td>
<td>&quot; &quot; -II</td>
<td>II</td>
<td>144,154</td>
<td>A pair of broad doublets, (J_1 = 10), (J_2 = 2.5)</td>
</tr>
<tr>
<td>3</td>
<td>Dihydro humulene epoxide-I</td>
<td>III</td>
<td>142,147</td>
<td>A pair of doublets (J_1 = 5; J_2 = 2.5)</td>
</tr>
<tr>
<td>4</td>
<td>&quot; &quot; -II</td>
<td>XIV</td>
<td>150,160</td>
<td>&quot;&quot; (J_1 = 10, J_2 = 2)</td>
</tr>
<tr>
<td>5</td>
<td>Humulene dioxide</td>
<td>VI</td>
<td>143-176</td>
<td>Complex pattern located between 143 and 163 cps.</td>
</tr>
<tr>
<td>6</td>
<td>&quot; trioxide</td>
<td>IV</td>
<td>134-175</td>
<td>&quot;&quot; 134 and 175</td>
</tr>
<tr>
<td>7</td>
<td>Caryophyllene oxide</td>
<td>XV</td>
<td>156.5-167</td>
<td>A pair of doublets (J_1 = 10.5, J_2 = 3.5)</td>
</tr>
</tbody>
</table>

It is well recognised that a secondary proton on an oxiran ring occurs around \(7.347\), which is considerably higher than for a similar proton in tetrahydro-furan or -pyran
ring systems. This has been attributed to the anisotropy of the oxirane ring \(^{20,21}\). As can be seen from the Table 3, the chemical shifts of the protons attached to carbon linked to oxygen in the various oxides, including humulene triepoxide, are in accord with the presence of oxirane rings as in structures (VI and IV). Table 4 lists the signals for methyls on oxirane ring for the various oxides discussed in the present Chapter.

**Table 4 - Chemical Shifts of Methyl Protons on the Oxirane Rings in the Various Epoxides**

<table>
<thead>
<tr>
<th>No.</th>
<th>Compound</th>
<th>Structure</th>
<th>Chem. shifts (in cps)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>humulene epoxide-I</td>
<td>I</td>
<td>72</td>
</tr>
<tr>
<td>2</td>
<td>&quot; &quot; -II</td>
<td>II</td>
<td>73</td>
</tr>
<tr>
<td>3</td>
<td>Dihydro humulene epoxide-I</td>
<td>III</td>
<td>65</td>
</tr>
<tr>
<td>4</td>
<td>&quot; &quot; -II</td>
<td>XIV</td>
<td>68</td>
</tr>
<tr>
<td>5</td>
<td>humulene dioxide +</td>
<td>VI</td>
<td>72, 78</td>
</tr>
<tr>
<td>6</td>
<td>&quot; trioxide +</td>
<td>IV</td>
<td>82, 82</td>
</tr>
<tr>
<td>7</td>
<td>Caryophyllene oxide</td>
<td>XV</td>
<td>69</td>
</tr>
</tbody>
</table>

\(^{+}\) in CDCl

SUMMARY

The structures (I) and (II) have been established for humulene epoxide-I and humulene epoxide-II respectively, on the basis of spectral and chemical evidence. (R,R) configuration has been tentatively proposed at \((\text{C}_2, \text{C}_3)\) and \((\text{C}_6, \text{C}_7)\) for (I) and (II) respectively.
EXPERIMENTAL

For general remarks, see page 86, Chapter II.

HUMULENE EPOXIDE-I

Isolation of humulene epoxide-I: vide page 86, Chapter II.

Physical constants, Table I, page 92, present Chapter.

Dihydro humulene epoxide-I: Humulene epoxide-I (320 mg, 
$\rho_D^0$ 1.4938) was hydrogenated in ethanol (35%, 10 ml) solution 
in contact with prereduced Adam's catalyst (44.2 mg), sus­
pended in ethanol (35%, 5 ml). The hydrogen uptake at 
708 mm and 25°C (over water) was 40.5 ml (1.02M). The solution 
was filtered into water (60 ml), extracted with pet. ether 
(30 ml x 5) and the pet. ether extract was washed with water 
(30 ml). Removal of solvent furnished a colorless liquid 
(313.1 mg). The product was chromatographed on Al$_2$O$_3$.

CHROMATOGRAM

<table>
<thead>
<tr>
<th>Fr.No.</th>
<th>Eluent</th>
<th>Ratio by Vol.</th>
<th>Vol.of eluate (ml)</th>
<th>Wt.of fraction (mg)</th>
<th>Remarks</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Pet.ether</td>
<td>100%</td>
<td>15 x 5</td>
<td>238</td>
<td>Slightly contaminated by carbonyl impurities.</td>
</tr>
<tr>
<td>2</td>
<td>Pet.ether to P.E-benzene</td>
<td>100% to 75:25</td>
<td>15 x 5 + 15 x 7</td>
<td>163</td>
<td>Pure dihydrohumulene epoxide-I.</td>
</tr>
<tr>
<td>3</td>
<td>P.E.-benzene to Benzene</td>
<td>50:50 100%</td>
<td>15 x 3 + 300</td>
<td>20</td>
<td>&quot;&quot; &quot;&quot;</td>
</tr>
<tr>
<td>4</td>
<td>Benz.-MeOH</td>
<td>100:2</td>
<td>30 x 3</td>
<td>negligible</td>
<td>--</td>
</tr>
</tbody>
</table>
The fractions 2 and 3 were combined and distilled (175 mg), b.p. 102-103/1 mm, $n_D^{0} 1.4870$, $[\alpha]_D^{28} -26.1$; IR spectrum: Fig.3; PMR spectrum: Fig.5. [Found: C, 80.72; H, 11.48; $C_{15}H_{26}O$ requires: C, 81.02; H, 11.79%]

Dihydrohumulene diepoxide (from (-)-dihydrohumulene epoxide-I): Dihydrohumulene epoxide-I (63.6 mg) consumed PBA, equivalent to 1.02M in 24 hr at ~5°C and gave a liquid dihydro diepoxide which could not be induced to crystallise (cf. the solid dihydro diepoxide, prepared from (+)-humulene epoxide-I (p-12).

Humulene trioxide from humulene epoxide-I: An ice-cold, chloroform solution of PBA (0.3434, 10.0 ml) was added to humulene epoxide-I (64.8 mg) and the mixture left at ~5°C (frigidaire). In 70 hr, the peracid consumption was complete (2M, iodometric titration of 0.5 ml aliquots). The reaction mixture, diluted with chloroform (25 ml) was washed with Na$_2$CO$_3$ solution (5%, 15 ml x 4) and water (15 ml). The crude product (a viscous liquid, ~80 mg) obtained on removing the solvent, crystallised on dilution with 2-3 drops of pet. ether and chilling (~33 mg), m.p. 108-112. Two more recrystallisations from pet. ether afforded colorless needles (18 mg), m.p. 114-117, $[\alpha]_D -4.4$ (c, 0.58), mixed m.p. with authentic (+)-humulene trioxide, 116-118. The IR spectrum was superimposable on that of (+)-humulene trioxide.

Humulene dioxide from humulene epoxide-I: The above experiment was repeated on (-)-humulene epoxide-I (117.5 mg) with PBA (0.45494, 2.6 ml, 1.14) in chloroform. The reaction mixture
was worked up after 24 hr (all PBA had been used up) to yield a viscous oil (132 mg), which, after two crystallisations from pet. ether, gave colorless needles (35.1 mg), m.p. 97-101; $[\alpha]_D^{14} +1.42$ (c, 1), mixed m.p. with authentic natural humulene dioxide (m.p. 106-106.5) was 103-105. IR spectrum (nujol) (Fig.9) was identical with that of natural humulene dioxide.

**HUMULENE EPOXIDE-II**

**Isolation of humulene epoxide-II**: vide page 80, Chapter II.

Physical constants: Table 1, page 92, present Chapter.

**Preparation of (+)-humulene epoxide-II**: To $V_2O_5$ (50 mg) was added 3-4 drops of $H_2O_2$ (25%). As soon as effervescence was over, acetone (50 ml) was added to the dark brown product. To the resulting reddish brown solution, chilled in ice, was added a solution of freshly distilled humulene (10.03 g, 0.05%) in acetone (10 ml). A solution of $H_2O_2$ (25%, 10 ml, ~0.07%) in acetone (10 ml), precooled in ice was run in slowly (15 mts), with shaking, into the humulene-pervanadic acid solution. The mixture was left in ice (4 hr) and thereafter at 1-2° overnight. In about 40 hr, the color turned greenish brown. Removal of acetone in vacuo at room temperature gave a residue, which was mixed with water (150 ml) and extracted with pet. ether (50 ml x 5). Evaporation of the solvent furnished a pale yellow oil (10.5 g). The crude product, diluted with pet. ether (10 ml) was chromatographed on $Al_2O_3$ (neut. II) to obtain pure (+)-humulene
epoxide-II and some (±)-humulene dioxide.

**CHROMATOGRAM**

<table>
<thead>
<tr>
<th>Fr. No.</th>
<th>Eluent</th>
<th>Ratio by vol.</th>
<th>Vol. of eluate (mg)</th>
<th>Wt. of fract. (g)</th>
<th>Remarks</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Pet. ether</td>
<td>100%</td>
<td>200 x 3</td>
<td>0.034</td>
<td>Rejected.</td>
</tr>
<tr>
<td>2</td>
<td>&quot;</td>
<td>&quot;</td>
<td>200 x 5</td>
<td>1.76</td>
<td>Essentially pure (+)-humulene epoxide-II, n_D^30 1.4957.</td>
</tr>
<tr>
<td>3</td>
<td>&quot;</td>
<td>&quot;</td>
<td>200 x 24</td>
<td>2.57</td>
<td>Pure (+) humulene epoxide-II, n_D^30 1.4965.</td>
</tr>
<tr>
<td>4</td>
<td>P.E.-Benz.</td>
<td>75:25</td>
<td>200 x 10</td>
<td>1.33</td>
<td>Pure (+) humulene epoxide-II, n_D^30 1.4960.</td>
</tr>
<tr>
<td>5</td>
<td>&quot;</td>
<td>&quot;</td>
<td>400 x 2</td>
<td>0.024</td>
<td>Rejected.</td>
</tr>
<tr>
<td>6</td>
<td>&quot;</td>
<td>50:50</td>
<td>400 x 7</td>
<td>1.51</td>
<td>Solid humulene dioxide, crystd. from pet. ether, m.p. 105-106.</td>
</tr>
<tr>
<td>7</td>
<td>Benzene</td>
<td>100%</td>
<td>200 x 11</td>
<td>0.47</td>
<td>Liquid frs. (impure humulanol ?)</td>
</tr>
<tr>
<td>8</td>
<td>Benz.-MeOH</td>
<td>100:3</td>
<td>200 x 5</td>
<td>1.72</td>
<td>Mainly humulanol (artefact formed on column?).</td>
</tr>
</tbody>
</table>

*Dilution of this fraction with pet. ether and chilling gave a small quantity of a colorless solid which was recrystallised from pet. ether; m.p. 126-127, identical (IR spectrum, m.p., mixed m.p.) with a product obtained by shaking a pet. ether solution of humulene dioxide with excess of Al_2O_3 (neut. act. I). IR spectrum: 3400, 1035 (OH); 3015, 1640, 1800, 908 (=CH_2); 1650, 970 cm^{-1} (\(\gamma=C=C\)); 1350, 1265 (gem dimethyl).*  
[Found: C, 76.1; H, 10.6; C_{15}H_{24}O_2 requires: C, 75.58; H, 11.0%]
A mid-fraction (fract. 3) of (+)-humulene epoxide-II had the following constants: b.p. 105-106/1.5 mm, $n_D^{20} 1.4958$ $d_4^{23} 0.9477$, $[\alpha]_D^0 +0$ (Lit.: b.p. 83-84/0.2 mm, $n_D^{20} 1.4984$ $d_4^{20} 0.9474$). IR and PMR spectra were superimposable on those of (−)-humulene epoxide-II. [Found: C, 81.41; H, 11.3; $\text{C}_{15}\text{H}_{24}\text{O}$ requires: C, 81.76; H, 10.98%]

(−)Dihydrohumulene epoxide-II: (−) Humulene epoxide-II ([α]_D^{−27.9}$ (c, 4, 95% EtOH); 300.5 mg) in ethanol (95%, 10 ml) was stirred with hydrogen in the presence of prereduced $\text{PtO}_2$ (85.5 mg), suspended in ethanol (95%, 5 ml), till no more hydrogen was absorbed [119 ml at 29° and 711 mm (over water); 118M]. The product was worked up in the same manner as was described for humulene epoxide-I. Removal of solvent (pet. ether) afforded a semisolid material (823 mg) which was crystallised from pet. ether (chilling) to give crystalline dihydrohumulene epoxide-II (237 mg), m.p. 65-68. Recrystallisation (pet. ether) furnished colorless plates (250 mg), m.p. 69-69.5, undepressed by (±)-dihydro humulene epoxide-II (m.p. 69, similarly prepared from (±)-humulene epoxide-II); $[\alpha]_D^0$ of the sample was −0.28 (c, 2.5, CCl_4). IR spectrum: Fig.12; PMR spectrum: Fig.8. [Found: C, 80.83; H, 11.76; $\text{C}_{15}\text{H}_{26}\text{O}$ requires: C, 81.02; H, 11.79%]. The liquid residue from mother liquors had b.p. 113-114/2.1 mm; $n_D^{30} 1.4851$; $[\alpha]_D^{−32.5}$ (c, 5.1); [Found: C, 81.9; H, 12.02; $\text{C}_{15}\text{H}_{26}\text{O}$ requires: C, 81.02, H, 11.79%].
Dihydro humulene diepoxide: (from (+)-dihydro humulene epoxide-II): (+)-Dihydro humulene epoxide-II (102.6 mg) was treated with PBA (10 ml, 0.2520 N) in chloroform at \( \sim 5^\circ \).

After 72 hr, 1.07 mols of PBA had been used up and the usual work up and three crystallisations from pet. ether gave the dihydro diepoxide-II (45 mg); m.p. 92.5-93.5.

[Found: C, 75.8; H, 11.3. \( \text{C}_{15}\text{H}_{28}\text{O}_2 \) requires: C, 75.57; H, 11.0%].

(Lit. \(^9\) m.p. 94).

Preparation of humulene trioxide from (-)-humulene epoxide-II

A sample of humulene epoxide-II (273 mg, \([\alpha]_D -26.6\) (c, 13.5, EtOH) was treated with an ice-cold, chloroform solution of PBA (0.2520 N, 30 ml) and left at \( \sim 5^\circ \). After 72 hr, the peracid used up amounted to 2.01M. The reaction mixture, after the usual work up, gave a semisolid crude product (378 mg) which, on crystallisation from pet. ether (twice), furnished a colorless solid (171 mg), m.p. 115-118.

A further recrystallisation from pet. ether gave colorless needles (97 mg), m.p. 121-122, undepressed by admixture with authentic (+)-humulene trioxide; IR spectrum (nujol mull) was identical with that of the latter. \([\alpha]_D +0.57\) (c, 0.7).

Humulene dioxide from (-)-humulene epoxide-II: (-)-Humulene epoxide-II (157.6 mg) was mixed with an ice-cold solution of PBA (0.4365 N, 3.6 ml) in chloroform and left at \( \sim 5^\circ \). Work up, after 24 hr (all peracid used up), gave a crude product (195 mg), which after two crystallisations (pet. ether) furnished colorless needles (74 mg) m.p. 98-101, undepressed
by the dioxide obtained from (-)-humulene epoxide-I; 
\([\alpha]_D^0 = -0.76 \text{ (c, l) }\); IR spectrum (Fig. 10), identical with 
that of the dioxide from (-)-humulene epoxide-I as well 
as that of natural humulene dioxide.

Ozonolysis of humulene epoxide-II*: A stream of ozonised 
oxygen (~180 mg/hr) was bubbled through a solution of 
(+)-humulene epoxide-II (1.21 g) in pure, dry ethyl acetate 
(60 ml), chilled to -15°C (ice-salt) till the outcoming 
gas contained free, unabsorbed ozone (~3 hr). Removal 
of solvent at room temperature under suction (water pump) 
furnished a viscous, liquid ozonide (2.7 g). This was 
mixed with \( \text{H}_2\text{O}_2 \) (30%, 2 ml) and aqueous \( \text{Na}_2\text{CO}_3 \) (10%, 10 ml) 
and warmed at 60°C/1 hr (with a reflux condenser) and 
thereafter on a waterbath (100°C/2 hr). The solution was 
made distinctly alkaline (2 ml more of 10% \( \text{Na}_2\text{CO}_3 \) aq.) 
and ~10 ml of water was carefully distilled out under 
reduced pressure (water pump), on the water bath (passing 
ice-water thro' the condenser and chilling the receiver 
in ice-salt). No acetone could be detected in the aqueous 
distillate (2:4 w/v). The alkaline residue was diluted with 
a little water and extracted with pet. ether (15 ml x 3) 
to remove neutral material. The combined pet. ether extract, 
when stripped of solvent, gave a small quantity (46 mg) of

* (+)-compound (prepared from humulene by 1M per vanadic 
acid oxidation) was used.
neutral product (not examined). The aqueous solution of sodium salts was warmed with norite, filtered and acidified with 50% H₃PO₄. The solution was saturated with ammonium sulphate and extracted exhaustively with ether, to yield, after solvent removal, a brownish gum (980 mg) which partially crystallised on standing. Crystallisation from acetone-pet. ether furnished almost colorless crystals (213 mg, m.p. 123-133). Two further crystallisations from the same solvent yielded almost colorless needles (157 mg), m.p. 138-139, undepressed by authentic 8S-dimethy1 succinic acid (m.p. 139-140). An analytical sample prepared by decolourising (norite) the above product and recrystallisation had m.p. 138-139.

[Found: C, 49.37; H, 6.95; C₆H₁₀O₄ requires: C, 49.3; H, 6.9%]

**PBA titration of humulene dioxide:** Humulene dioxide* (52.4 mg) was treated with an ice-cold solution of PBA in chloroform (0.2520 M, 4 ml) and left aside at 5°. After 72 hr, 0.99 M of PBA had been consumed (iodometric titration). The customary work up of the reaction mixture afforded a solid (46.8 mg), which after two recrystallisations from pet. ether gave humulene trioxide (32.7 mg) m.p. 120-122, undepressed by an authentic sample.

*(+) -sample, prepared from humulene, by epoxidation with 2M of pervanadic acid in acetone.
Epoxidation of humulene with 1M of oxidant (under 3 different conditions).

(i) Pervanadic acid in acetone: A small quantity (250 mg) of the total product obtained by the action of $\text{V}_2\text{O}_5$-$\text{H}_2\text{O}_2$ (1M) on humulene in acetone solution (vide preparation of (i)-humulene epoxide-II, page 109) was distilled to obtain a colorless oil (195 mg); b.p. 115-120/2-2.3 mm. The IR spectrum was again almost identical with that of humulene epoxide-II. GLC on column 'P' could not detect any humulene epoxide-I or unchanged humulene (By column chromatography humulene dioxide (~10% had been isolated from the bulk of the product).

(ii) PBA in benzene-toluene solution: Freshly distilled humulene (729 mg) in benzene (3 ml) was mixed with a solution of PBA in 1:1 benzene-toluene (0.456M, 17 ml, 1.04%), pre-cooled to -1°. The mixture was left aside in a frigidaire (~5°). After 42 hr, iodometric titration indicated that ~1 M of PBA had been consumed. After work up and distillation, a colorless oil (637 mg) b.p. 112-127/2.25 mm; $n_D^{31}$ 1.4941; was obtained. The IR spectrum closely resembled that of humulene epoxide-II. However GLC (column 'P') showed this material to contain a little unchanged humulene and traces of humulene epoxide-I (in-flection on the main peak) in addition to the major product viz. humulene epoxide-II.
(iii) PBA in chloroform solution: To a solution of humulene (319 mg) in CHCl₃ (20 ml) prechilled in ice-salt, was added ice-cold PBA (0.3562N, 8.4 ml, 1M) in chloroform. The mixture was left at -6 to -10° for 6 hr and then overnight at 5°. In 24 hr, all the PBA had been used up. The solution, after the usual work up, gave a colorless liquid (430 mg) which on distillation furnished a colorless oil (287 mg), b.p. 107-108/1.4 mm, nD 1.4961. The IR spectrum was very similar to that of humulene epoxide-II. GLC on column 'P' showed it to be essentially humulene epoxide-II, containing traces of humulene epoxide-I (an inflection on the main peak) and a small amount of an unidentified product (a minor peak, humulenol?).
REFERENCES

7 A.K.Auwers and F.Eisenlohr, Ber. 43, 806 (1910).
12 Ref.10, S.34; also see ref.1.
