

TERPENOIDS—XXXI*

ISOLATION OF HUMULENE MONOXIDE AND HUMULENE DIOXIDE

S. K. RAMASWAMI and S. C. BHATTACHARYYA
National Chemical Laboratory, Poona-8, India

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Abstract—Two new oxygenated derivatives of humulene, viz., humulene monoxide (II) and humulene dioxide (VIII) have been isolated from the sesquiterpene fractions of wild ginger oil (*Zingiber zerumbet*, Smith). The structures of these two epoxides have been determined by degradative and synthetic studies.

In previous communications, isolation and characterization of several constituents from the essential oil of wild ginger (*Zingiber zerumbet*, Smith)¹ and degradation products of the humulenic ketone zerumbone² have been described. The occurrence along with humulene and zerumbone, of optically active components in the higher boiling fraction of the oil was also reported.¹ This fraction has now been more closely studied and the results described in the present communication.

By repeated fractionation followed by chromatography, two pure compounds could be separated from the optically active fraction. The major component was a laevorotatory liquid of molecular formula $C_{15}H_{24}O$, $[\alpha]_D -43.3^\circ$. The I.R. spectrum did not show any carbonyl or hydroxyl absorption, suggesting that the oxygen may be present as an ether linkage. Characteristic peaks at 968 ($-\text{CH}=\text{CH}-$, *trans*), 820 ($-\overset{|}{\text{C}}=\text{CH}-$), 1388 and 1369 cm^{-1} (*gem*-dimethyl group) were indicated.

The purified compound showed a tendency to isomerize to a ketone (I.R. band at 1705 cm^{-1}) on keeping. On catalytic hydrogenation in ethanol using Adams catalyst, the compound absorbed one mole of hydrogen. When acetic acid was used as solvent, approximately three moles of hydrogen were taken up. The fully hydrogenated product exhibited hydroxyl band (3430 cm^{-1}) in the I.R. spectrum. These results can be explained if it is assumed that the oxygen is linked as a 1,2-epoxide. Lithium aluminium hydride reduction in ether gave only unchanged starting material. Acetic acid or dilute mineral acids, however, opened the oxide ring to give hydroxy derivatives.

Estimation of unsaturation with peracid showed the presence of two double bonds. Working up the product of epoxidation gave a crystalline tri-oxygenated compound $C_{15}H_{24}O_3$, m.p. 122.5°. As this m.p. was identical with that of humulene trioxide,³ an authentic sample of the latter was prepared from humulene for comparison. The I.R. spectra of these two products were identical and there was no depression in their mixed m.p. This showed that the original compound was, in fact, a monoepoxide of

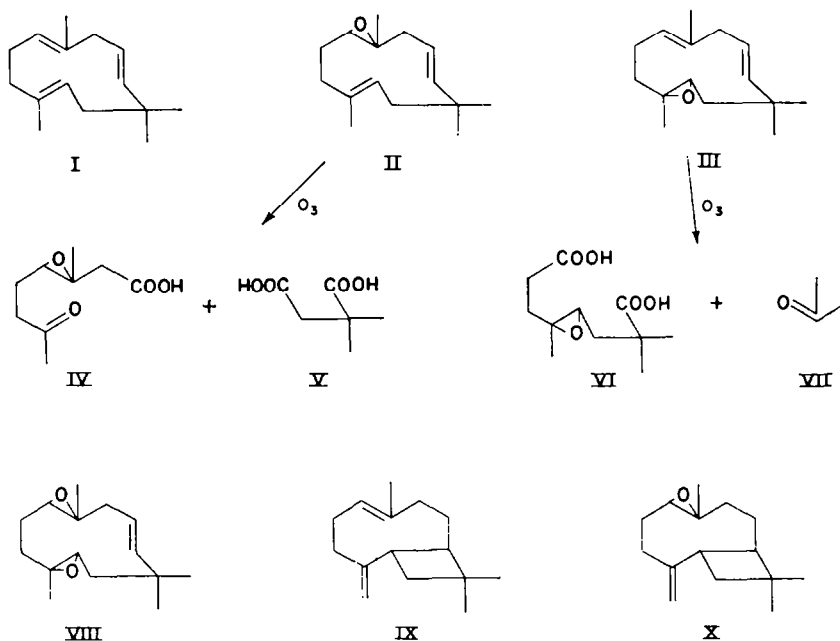
* Contribution number 487.

¹ V. K. Balakrishnan, R. K. Razdan and S. C. Bhattacharyya, *Perf. and Ess. Oil Rec.* **47**, 274 (1956).

² S. K. Ramaswami and S. C. Bhattacharyya, *Perf. and Ess. Oil Rec.* **51**, 65 (1960).

³ V. Herout, M. Streible, J. Mleziva and F. Sorm, *Coll. Czech. Chem. Comm.* **14**, 716 (1949).

humulene⁴ (I). This conclusion was confirmed by preparing humulene monoxide from humulene by partial epoxidation and comparison of its properties with those of the natural monoxide. The I.R. spectra of the two products were superimposable. Taking into consideration the presence of a di-substituted double bond, the two possible structures of the epoxide may be represented by II and III. A decision between the two structures was made possible by ozonolysis experiments. Among the water-soluble acids isolated from the fission products, α,α -dimethyl succinic acid (V) could be identified. The presence of an acid (IV) having a methyl ketone grouping was also indicated (haloform reaction). These products can arise only from II and



hence this structure has been assigned to the epoxide. The presence of small amounts of the isomeric oxide III was also not ruled out as traces of acetone arising out of the unstable fission product, aceto-acetic acid could be identified in the cleavage products.

The second component, isolated in a small quantity from the optically active fractions of the oil was a crystalline compound $C_{16}H_{24}O_2$, m.p. 105° , $[\alpha]_D -0.02^\circ$. The I.R. spectrum, like that of the monoxide, did not show any absorption for carbonyl or hydroxyl group suggesting that the compound might be a di-epoxide with a humulene skeleton. This was confirmed by preparation of humulene dioxide from humulene and comparison with the natural product. The synthetic product also had m.p. 105° and the mixed m.p. showed no depression. The I.R. spectra of these compounds were identical. In conformity with these results, the natural liquid monoxide also gave the same dioxide on treatment with one mole of peracid. Taking into consideration the presence of a disubstituted double bond (strong band at 975 cm^{-1} in the I.R. spectrum) in the molecule, the only possible structure for the di-epoxide is represented by VIII.

⁴ R. P. Hildebrand, M. D. Sutherland and O. J. Waters, *Chem. & Ind.* 489 (1959); Sukh Dev, *Tetrahedron*, 9, 1 (1960).

In this connection, it may be pointed out that a closely allied sesquiterpene, caryophyllene⁵ (IX) also occurs as its epoxide⁶ (X) in nature. The occurrence of humulene dioxide is the first instance of a terpenoid di-epoxide in natural sources.

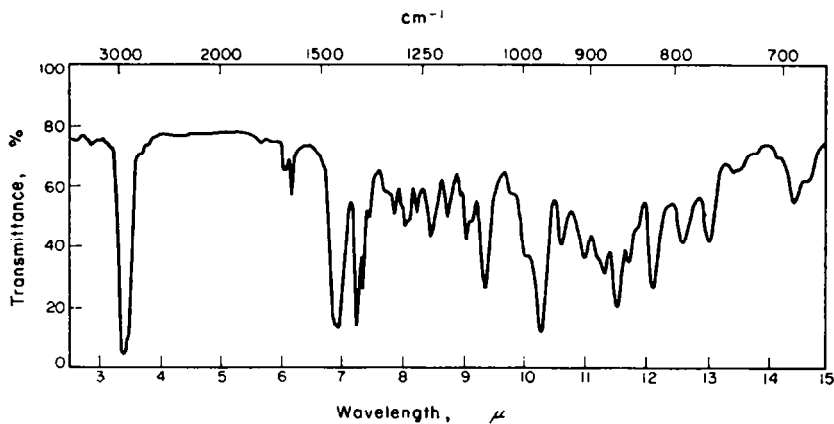


Fig. 1.

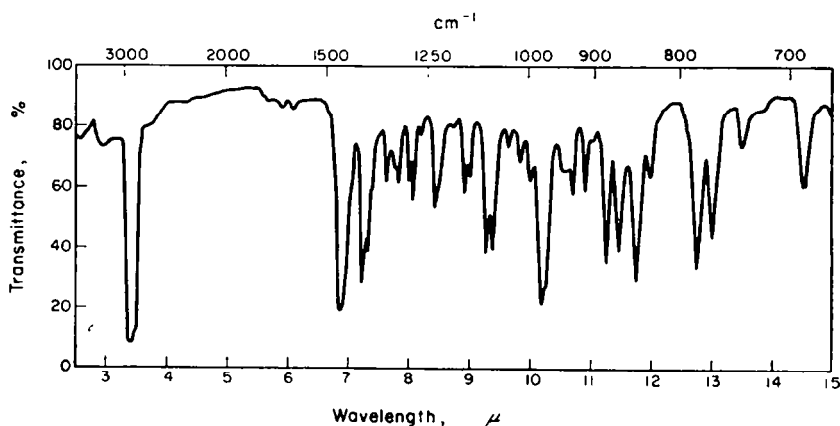


Fig. 2.

EXPERIMENTAL

All m.p. are uncorrected. Rotations were taken in chloroform. I.R. spectra were measured on a Perkin-Elmer Infracord Spectrophotometer by H. Gopinath. Micro-analyses were carried out by Pansare and colleagues.

Isolation of the mono-epoxide (II). The sesquiterpene fraction of wild ginger oil (850 g) was fractionated using an efficient packed column. The lower boiling (b.p. 100–110°/3 mm) fractions consisted entirely of humulene. The higher boiling (b.p. 120–145°/3 mm) fractions were mixtures of zerumbone and the optically active oxygenated sesquiterpenoids. Fractions with rotations above -15° were freed from zerumbone by dilution with an equal volume of pet ether (60–80°) and chilling when zerumbone crystallized from the solution. The crystals were removed by filtration and the mother liquors were combined, solvent distilled off and the residue refractionated. The main fraction (b.p. 125–135°/3 mm) amounted to 92 g. Further purification was achieved by chromatography over alumina (grade II–III). After preliminary elution of the column with pet ether (60–80°) to remove ketonic constituents, the column was eluted with pet ether–benzene mixture (1:1) to give fractions of

⁵ A. Aebi, D. H. R. Barton and A. S. Lindsay, *J. Chem. Soc.* 3124 (1953).

⁶ W. Triebs, *Chem. Ber.* 80, 56 (1947).

optical rotation up to -41.3° . Further extensive chromatography did not improve the optical rotation. Consecutive fractions from the column also exhibited identical I.R. spectra. The purest fraction had the following properties: $[\alpha]_D^{25} -43.3^\circ$, (c , 12.0), n_D^{25} 1.4955, d_4^{25} 0.9575 (Found: C, 81.53; H, 10.78. $C_{18}H_{24}O$ requires: C, 81.76; H, 10.98%). The I.R. spectrum is reproduced in Fig. 1.

The pure epoxide on keeping for a few days at room temp underwent slight isomerization and the resultant product exhibited an additional ketonic band at 1705 cm^{-1} in the I.R. region.

Catalytic hydrogenation. The epoxide (95.6 mg), in ethanol (20 ml) was hydrogenated in presence of pre-reduced Adams catalyst (44 mg) until no further absorption took place. During 10 hr volume of hydrogen absorbed was 11.9 ml at 22.9° and 712 mm which corresponded to one mole. The catalyst was removed by filtration and the solvent evaporated *in vacuo*. The product showed no absorption at 968 cm^{-1} in the I.R. spectrum indicating that the disubstituted double bond got saturated under these conditions (Found: C, 80.8; H, 11.5. $C_{15}H_{26}O$ requires: C, 81.02; H, 11.79%).

In another experiment, the epoxide (80.4 mg) in acetic acid (25 ml) was hydrogenated in presence of Adams catalyst (40 mg) until no further hydrogen was absorbed. In 8 hr, 26.7 ml of hydrogen were absorbed at 24.5° and 710 mm (2.74 mole). The catalyst was filtered off, and the solvent evaporated *in vacuo*. The I.R. spectrum of the product showed that it was a mixture of saturated ketone (1706 cm^{-1}) and saturated alcohol (3430 cm^{-1}) (Found: C, 79.4; H, 12.0; $C_{15}H_{28}O$ requires: C, 80.29; H, 12.58. $C_{15}H_{30}O$ requires: C, 79.57; H, 13.36%).

Acid cleavage of the epoxide. The epoxide (1.04 g) was heated on the steam bath with glacial acetic acid (25 ml) for 2 hr. Acetic acid was evaporated *in vacuo* and the residue taken up in ether. The ether extract was washed with water, sodium bicarbonate and dried over anhydrous sodium sulphate. Removal of solvent gave a product (1.02 g) which exhibited absorption bands in the I.R. spectrum at 3500 cm^{-1} ($-\text{OH}$ group), 1730 cm^{-1} (ester carbonyl) and 1250 cm^{-1} (acetate). Hydrolysis of the product with alcoholic alkali and working up the product in the usual manner yielded a product which exhibited characteristic band at 3500 cm^{-1} ($-\text{OH}$ group) in the I.R. spectrum. This was not further examined.

Estimation of unsaturation with per-acid. The epoxide (138 mg) was allowed to stand with excess of perbenzoic acid in chloroform solution at 0° for 72 hr. Titration in the usual manner indicated the presence of two double bonds. Isolation of the product from the reaction mixture gave a viscous oil (152 mg) which crystallized on cooling. This was chromatographed over alumina (grade III). The first few fractions from pet ether ($60-80^\circ$) contained negligible amounts of oily material and was rejected. The benzene fractions on evaporation gave the pure crystalline trioxide, m.p. 122.5° , (Found: C, 71.0; H, 9.5. $C_{15}H_{24}O_3$ requires: C, 71.39; H, 9.59%).

Humulene trioxide. Pure humulene (d_4^{25} 0.8868, n_D^{25} 1.5015; $[\alpha]_D^{25} = 0.0^\circ$) isolated from wild ginger oil (1 g), was allowed to stand with excess of perbenzoic acid in chloroform as described before. Purification by chromatography yielded the crystalline trioxide, m.p. 122.5° (Found: C, 71.1; H, 9.5. $C_{15}H_{24}O_3$ requires: C, 71.39; H, 9.59%).

The I.R. spectra of the above two trioxides were identical. The mixed m.p. showed no depression.

Humulene monoxide from humulene. Humulene (1 g) was allowed to stand with one molar proportion of perbenzoic acid in chloroform solution at 0° for 72 hr. Chromatography of the product gave a fraction (eluent, benzene; 0.4 g) which had the following properties: d_4^{25} 0.9572; n_D^{25} 1.4953 (Found: C, 81.3; H, 10.71. $C_{18}H_{24}O$ requires: C, 81.76; H, 10.98%). The I.R. spectrum was identical with that of the natural monoxide.

Ozonolysis of the natural monoxide (II). The epoxide (4.62 g) was dissolved in ethyl acetate (75 ml) and cooled to -20° and a stream of ozonized oxygen was bubbled through it till ozonolysis was complete (10 hr). The solvent was removed under vacuum and the residue treated with water (100 ml) containing 20 ml of 30% hydrogen peroxide. After keeping at room temp with frequent shaking for 12 hr, the mixture was warmed at 80° for $\frac{1}{2}$ hr. Among the volatile products, traces of acetone could be detected (DNPH). The excess of hydrogen peroxide was destroyed by adding Adams platinum oxide catalyst (10 mg) and keeping at room temp for 12 hr. The catalyst was then filtered off and the filtrate evaporated to dryness in an evaporating dish on the steam bath. A viscous, yellowish acid mixture was obtained (2.2 g). It gave positive iodoform test.

The above water soluble acid mixture was dissolved in 50 ml ether containing a few ml absolute ethyl alcohol. To this was added an ethereal solution of diazomethane (from 10 g of nitrosomethyl urea). After 10 min, the excess of the reagent was destroyed by warming the solution. On evaporation of ether, a residue was obtained (yield 2.1 g) which exhibited in its I.R. spectrum, strong absorption band at 1730 cm^{-1} (ester carbonyl) and medium absorption at 3400 cm^{-1} ($-\text{OH}$ group) possibly

formed by partial opening of the epoxide ring. It was fractionated in a microfractionation unit (Towers) and the first fraction (b.p. up to 90°/10 mm) was examined. It corresponded to the dimethyl ester of α,α -dimethyl succinic acid (Found: C, 54.16; H, 8.1. $C_8H_{14}O_4$ requires: C, 55.16; H, 8.1%).

The ester was hydrolysed with the requisite amount of alcoholic alkali and the organic acid liberated with dil hydrochloric acid. The mixture was then evaporated to dryness and the solid residue extracted with acetone to separate the organic acid from the sodium chloride. Removal of acetone yielded a crystalline acid m.p. 122°. Repeated crystallization from acetone–benzene raised the m.p. to 138°. (Found: C, 49.04; H, 7.1. $C_8H_{10}O_4$ requires: C, 49.31; H, 6.9%). A mixed m.p. determination with a genuine sample of α,α -dimethyl succinic acid showed no depression.

Isolation of humulene dioxide (VIII). During the chromatography of the epoxide fractions of wild ginger oil, the column was finally eluted with benzene. This gave 4.45 g of a fraction which showed signs of solidification. It was diluted with an equal volume of pet ether (60–80°) and cooled. The separated crystals were filtered off and recrystallized from a large volume of pet ether (40–60°). The recrystallized product (1.2 g) had m.p. 105.5° [α]_D²⁵ 0.02° (Found: C, 76.5; H, 10.1. $C_{18}H_{34}O_2$ requires: C, 76.22; H, 10.24%). The I.R. spectrum is reproduced in Fig. 2.

Humulene dioxide from humulene. Pure humulene (1 g) was allowed to stand with two molar proportions of perbenzoic acid in chloroform solution and kept at 0° for 72 hr. Purification by chromatography gave the crystalline dioxide, m.p. 105° (Found: C, 75.84; H, 10.25. $C_{18}H_{34}O_2$ requires: C, 76.22; H, 10.24%). Mixed m.p. with natural dioxide showed no depression. The I.R. spectra were also identical.

Humulene dioxide from natural humulene monoxide. The dioxide prepared in a similar manner from natural monoxide also melted at 105°. Its I.R. spectrum was identical with that of the natural dioxide. (Found: C, 76.0; H, 10.3. $C_{18}H_{34}O_2$ requires: C, 76.22; H, 10.24%). There was no depression in m.p. when it was mixed with the natural dioxide.