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STUDIES IN SESQUITERPENES—XXXVII

SESQUITERPENOIDS FROM THE ESSENTIAL OIL OF *ZINGIBER ZERUMBET* SMITH*†

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Abstract—A complete analysis of the essential oil from the rhizomes of *Zingiber zerumbet* Smith, is given and the isolation of several new humulene-based sesquiterpenoids is described.

THE essential oil from the rhizomes of *Zingiber zerumbet*‡ Smith (N.O. *Scitaminae*) has been the subject of several previous investigations.[1–4] The isolation of several monoterpenes,[1,2] humulene[1] and zerumbone[3] has been reported and the structure of zerumbone elucidated.[4] In the course of our work on zerumbone, it was observed that the optically active sesquiterpene fraction (b.p. ~91–115°/1 mm) of this essential oil, distilling between humulene and zerumbone, still showed a pronounced absorption at ~970 cm^{-1} in the IR, which we consider as a useful pointer to humulene-based sesquiterpenoids. This prompted a systematic reinvestigation of the sesquiterpene portion, as the chances of encountering hitherto unknown members of the humulene group appeared bright. The present paper reports the isolation of the different sesquiterpene components§ of the oil and as anticipated, a number of these have turned out to be humulenoid sesquiterpenes.

A preliminary GLC of the total oil showed it to be a complex blend of several terpenes. The separation scheme finally worked out for the isolation of the various sesquiterpene constituents, is outlined in Fig. 1. The oil was carefully fractionated through a spinning band column and cuts of ⅄ 1/30 of the total bulk of the oil were collected and were screened by TLC on $AgNO_3$-clad silica gel.[7] Fractions having essentially identical TLC patterns were pooled into groups. Based on the GLC of these groups, suitable fractions were selected for the isolation of the various components, the isolation being achieved by a judicious combination of refractionation, column chromatography (over alumina and/or $AgNO_3$–SiO_2 gel), partition with aqueous $AgNO_3$ and/or preparative GLC.

The identification of the various sesquiterpene constituents was carried out by a study of their physico-chemical properties as well as their IR and PMR spectra. Once the pure components had been obtained and characterized, their positions in the

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† Abstracted from the Ph.D. thesis of N. P. Damodaran, Poona University (1965).

‡ Local names: Sanskrit: *Sthulagranthi*; Hindi: *Narkachur*; Malayalam: *Kattinchi*. The plant is encountered growing wild in certain parts of India, especially in the Kerala State.

§ After this work had been completed and the structures of humulene-epoxide—I, humulene-epoxide—II, and humulenol had been established,[5] Levi and Nigam[6] reported on the analysis of this oil essentially in terms of known components.

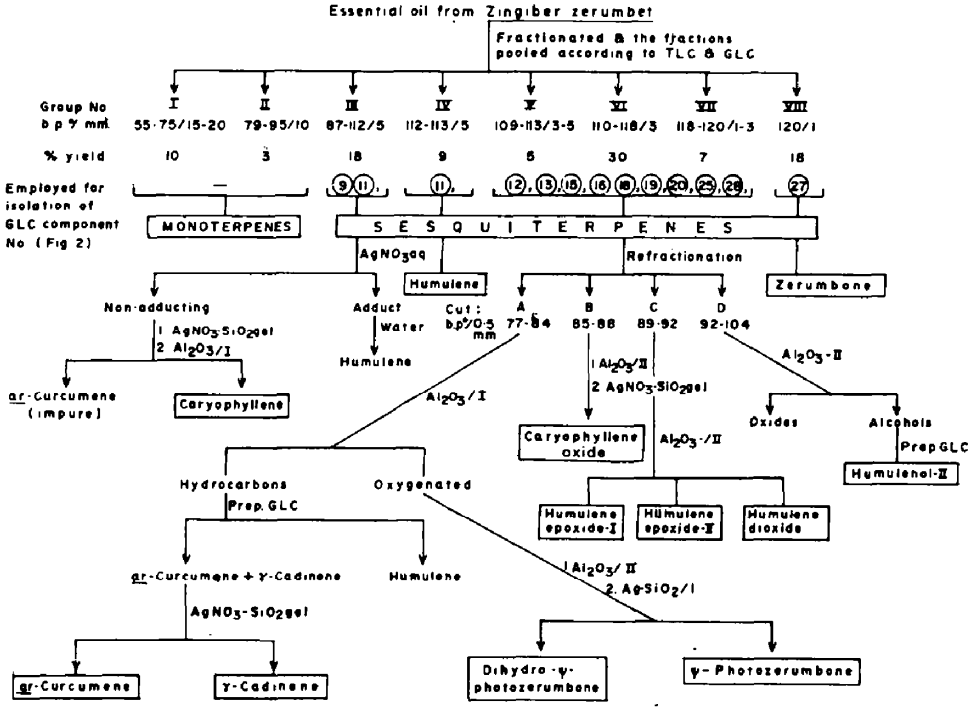
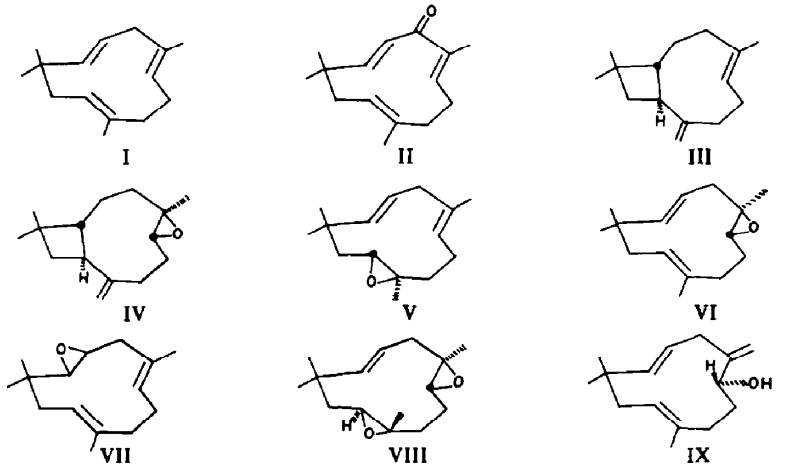


FIG. 1 Separation of the sesquiterpenoids of *Zingiber zerumbet* Smith.

GL Chromatogram were finally settled by the peak-accentuation technique, using pure isolated components in mixed chromatograms.

Figs 2 and 3 show the GLC and TLC of the total oil along with the identification of the various components. Table 1 gives the % composition of the oil.

Thus, in this study, besides humulene (I) and zerumbone (II), already known to be the chief sesquiterpene components of the oil, (−)-caryophyllene (III), (+)-*ar*-curcumene (XI), (+)-γ-cadinene (X) and (−)-caryophyllene oxide (IV), were isolated.



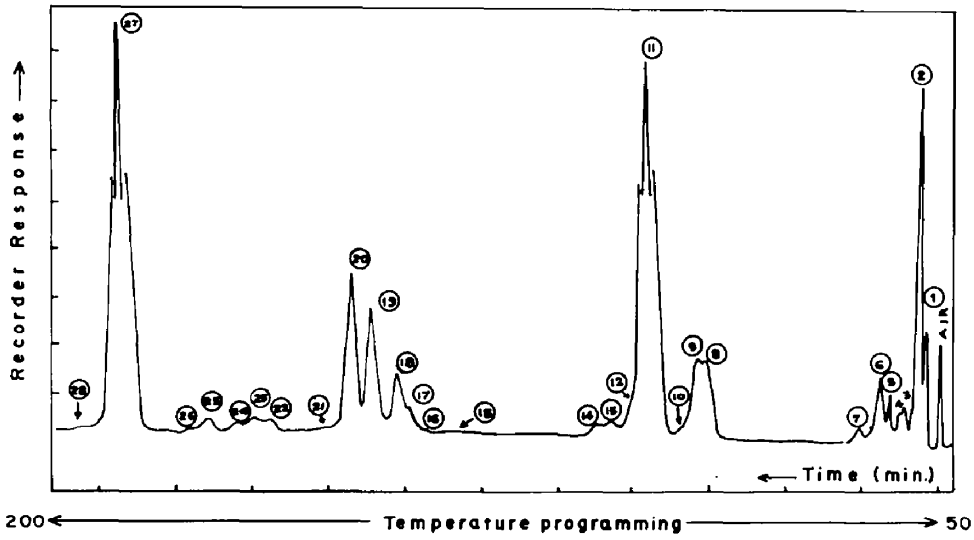


FIG. 2 GLC of the total essential oil (*Zingiber zerumbet*, Smith).

Column: 2 meter × 6 mm, packed with 20% diethyleneglycol polysuccinate on Chromosorb W; temp: programming 50–200°, ~6°/min; gas flow: 50 ml H_2/min. 1—7,9,10: monoterpenes;[6] 8: caryophyllene; 11: humulene; 12: γ-cadinene; 13: *ar*-curcumene; 14: unidentified; 15: dihydro-ψ-photozerumbone; 16: ψ-photozerumbone; 17: unidentified; 18: caryophyllene oxide; 19: humulene epoxide-I; 20: humulene epoxide II; 21: Humulene epoxide-III(?); 22–23: unidentified; 24: Humulenol-I(?); 25: humulenol-II; 26: unidentified; 27: zerumbone; 28: humulene dioxide.

In addition, the isolation of five new sesquiterpenoids (Table 1) has been accomplished. As a result of the work described in the succeeding parts of this series, the structures of three of these have been elucidated and the compounds have been designated humulene epoxide-I (V),* humulene epoxide-II (VI)* and humulenol-II (IX). The remaining two new sesquiterpenoids (GLC components 15, 16 have surprisingly turned out to be dihydro-ψ-photo-zerumbone (XIII) and ψ-photozerumbone (XIV) respectively, recently described in connection with the photochemistry of zerumbone.[9]†

The minor GLC component 21 according to its retention time is considered to be humulene epoxide-III (VII) recently isolated in this laboratory, from the essential oil of the oleoresin of *Dipterocarpus pilosus*.[10] Likewise, the GLC component 24 is in all probability humulenol-I (XV), as inferred from peak-augmentation in a mixed GLC with an authentic sample of XV, prepared by the isomerization of humulene-epoxide-I (V) over Al_2O_3.[5]

Small amounts of humulene dioxide (VIII)[8] were also isolated.

* As already reported in our preliminary communication,[5] the humulene epoxide described by earlier workers[8] was, in fact, a mixture of IV, V and VI

† Both XIII and XIV isolated from the essential oil are optically inactive and hence are likely to be only artefacts. It is conceivable that either the oil contains *cis*-6-*trans*-9-zerumbone originally or some of it is produced by isomerization of zerumbone during storage and this rearranges to the ψ-photozerumbone during fractionation; facile Cope rearrangement (thermal) of *cis*-6-*trans*-9-zerumbone has been demonstrated.[9] Likewise, XIII could have arisen from 6,7-dihydrozerumbone. No conclusive evidence (GLC) for the presence of *cis*-6-*trans*-9-zerumbone, in the essential oil, could be obtained.

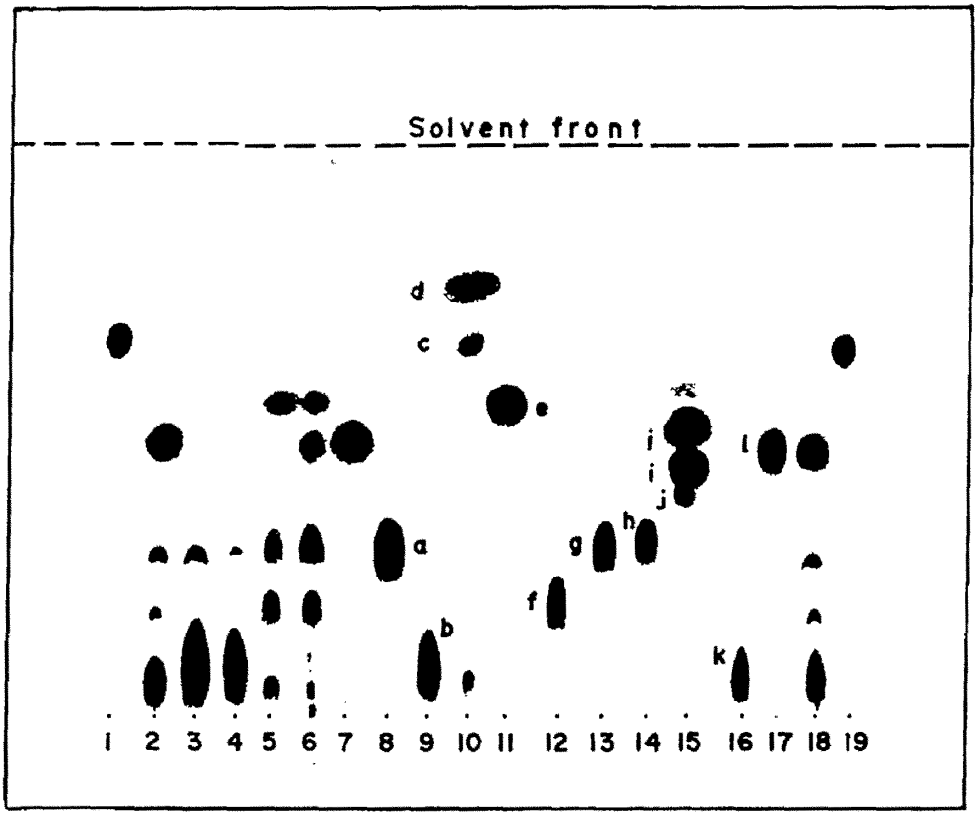


FIG. 3 TLC of the products from the essential oil of *Zingiber zerumbet*, Smith.

TLC: $AgNO_3$–SiO_2 gel[7] (0·3 mm), 5% acetone in C_6H_6 (solvent front 12 cm), 25°.
1,19: Sudan III; 2,18: total oil; 3–7: pooled fractions IV–VIII (Table 2); 8: caryophyllene; 9: humulene; 10: *ar*-curcumene, γ-cadinene; 11: caryophyllene oxide; 12: humulene epoxide-I; 13: humulene epoxide-II; 14: humulene dioxide; 15: dihydro-ψ-photozerumbone, ψ-photozerumbone; 16: humulenol-II; 17: zerumbone.

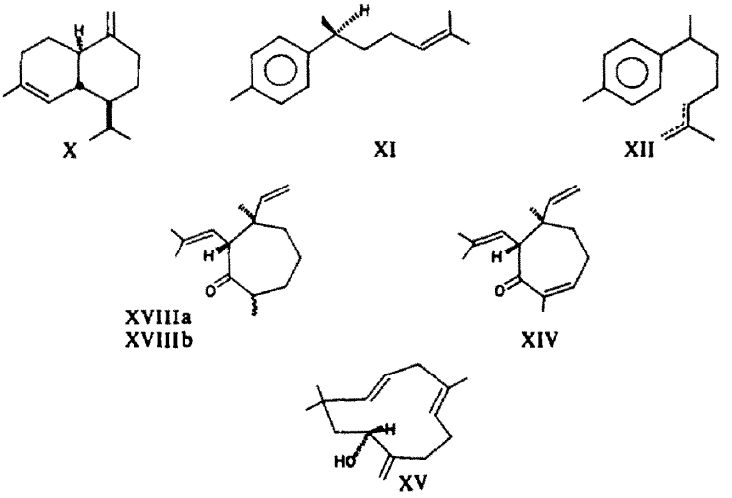


TABLE 1. SESQUITERPENE COMPOSITION OF ESSENTIAL OIL OF *Zingiber zerumbet*

No.	GLC		TLC		Compound	%
	Peak No.	RRT*	Spot No.	R_{dye}†		
—	1, 7, 9, 10	—	—	—	Monoterpenes	13
1	8	1·00	a	0·45	Caryophyllene (III)	3·5
2	11	1·24	b	0·13	Humulene (I)	27·0
3	12	1·40	c	1·03	γ-Cadinene (X)	1·5
4	13	1·46	d	1·19	*ar*-Curcumene (XI)	
5	14	1·53	—	—	Unidentified	0·4
6	15	2·15	i	0·77 0·68	Dihydro-ψ-photozerumbone (XIIIa, b)	0·2
7	16	2·33	j	0·58	ψ-Photozerumbone (XIV)	0·2
8	17	2·39	—	—	Unidentified	0·5
9	18	2·46	e	0·87	Caryophyllene oxide (IV)	2·1
10	19	2·55	f	0·28	Humulene epoxide—I (V)	4·8
11	20	2·65	g	0·43	Humulene epoxide—II (VI)	7·7
12	21	2·78	?	—	Humulene epoxide—III (VII)	<0·1
13	22	3·01	—	—	Unidentified	0·8
14	23	3·10	—	—		
15	24	3·19	—	—	Humulenol—I (XV)	0·3
16	25	3·30	k	0·85	Humulenol—II (IX)	0·4
17	26	3·46	—	—	Unidentified	0·1
18	27	3·65	l	0·74	Zerumbone (II)	37·5
19	28	3·92	h	9·47	Humulene dioxide (VIII)	<0·1

* Retention time relative to caryophyllene under conditions of programming (50–200°; 6°/min) (Fig. 2)

† $R_{dye} = \frac{\text{Movement of substance from start in mm}}{\text{Movement of dye from start in mm}}$ (Fig. 3).

The structure of (+)-*ar*-curcumene ("α-curcumene"), isolated during the present work, calls for a brief comment. (−)-,[11] (+)-[12] and (±)[13]-*ar*-Curcumenes have been isolated from natural sources. However, it would appear, from a literature survey, that either *ar*-curcumene has not been isolated in a state of purity or its structure has not been established unequivocally, since even in recent publications[12,14] it has been represented as XII. The spectroscopic data collected during the present work for a pure (GLC, TLC) sample of (+)-*ar*-curcumene, definitely defines it as XI.* The PMR spectrum clearly shows the presence of only one olefinic proton (1H triplet centred at 301·5 c/s, $J = 6·5$ c/s) and two vinylic Me's (3H singlets at 89 and 98·5 c/s, with $W_H = 3$ and 4 c/s respectively); the IR spectrum shows no significant absorption $\sim 890\ cm^{-1}$.

The data presented, so far, give us a more or less complete picture of the sesquiterpene constituents of the essential oil of *Zingiber zerumbet*. It is clearly seen that, by far, the most important pathway available for sesquiterpene synthesis, in this plant, is the 1,11-cyclization of *trans-trans*-farnesyl pyrophosphate† to produce

* The absolute configuration of (+)-*ar*-curcumene has been recently established.[14,15]

† In view of the established[16] all-*trans*-geometry of humulene, the stereochemical refinement of the farnesol cyclization hypothesis,[17] introduced by Hendrickson[18] that humulene arises from *trans-cis*-farnesol, requires modification.

humulene, which by subsequent oxidations, produces a rich variety of oxygenated derivatives. Caryophyllene (which, together with its oxide, accounts for ~5% of the total oil) also must arise from the same initial cyclization (or subsequently by cyclization of humulene). The pathway leading to *ar*-curcumene and γ-cadinene are available only to a minor extent. However, the isolation of these components is significant from the chemotaxonomic point of view, as the curcumene pathway is the major biosynthetic route in *Curcuma longa*,[19] *Curcuma aromatica*,[11a] *Curcuma amada*[11b] *Zingiber officinale*,[20] species belonging to the same family *Zingiberacea* to which *Z. zerumbet* also belongs.

EXPERIMENTAL

All m.ps and b.ps are uncorrected. Pet. ether refers to the fraction b.p. 40–60°. All solvent extracts were finally washed with brine, before drying (Na_2SO_4). Optical rotations were measured in $CHCl_3$.

UV spectra were taken on a Perkin–Elmer spectrophotometer, model 350, in 95% EtOH. IR spectra were recorded as smears (liquids) or nujol mulls (solids) on a Perkin–Elmer Infracord, model 137E. PMR spectra were taken in 10–20% soln with CCl_4 with TMS as the internal standard, on a Varian A-60 spectrometer; signals are recorded in c/s relative to TMS as zero.

Analytical GLC was run on "Aerograph", model A-350-B, using a 150 cm × 5 mm column packed with 20% diethylene glycol polysuccinate on Chromosorb W (60–80 mesh) with H_2 as carrier gas. Preparative GLC was carried out on a Perkin–Elmer Vapour Fractometer, model 154-D, using a total of 300 cm × 2·5 cm column length, packed with the same material and using N_2 as the carrier gas.

Alumina used for chromatography was made neutral by the HNO_3 method[21] and graded according to Brockman.[22] The silica gel for column chromatography (−100, +200 mesh) was washed with hot distilled water till sulphate-free, dried and activated at 125–130° for 6–8 hr; the product was standardized according to Hernandez.[23] $AgNO_3$-impregnated silica gel was made by the method of Gupta and Dev[7] and activated and standardized as above. TLC was carried out on silica gel or silica gel–$AgNO_3$ layers, containing 15% gypsum, using the apparatus and technique cited earlier.[7]

The essential oil

The oil was specially obtained for us by a commercial firm* from genuine rhizomes of *Z. zerumbet*, by steam distillation. The product was a clear yellowish brown liquid,† which almost totally distilled between 50–75°/15 mm → 80–125°/1·5 mm.

TABLE 2. FRACTIONATION OF THE ESSENTIAL OIL OF *Z. zerumbet*

Pooled group	b.p./mm	$[\alpha]_D$	Weight (g)	Remarks
I	57–75/22–15	+92 to −16	5·54	Monoterpenes
II	79–95/10	+30 to +24·8	1·66	Semisolid, mostly 9
III	87–112/10–5	+11·6 to −1·8	7·68	Components 8 and 11
IV	112/5	+0·6 to −2·8	4·95	Essentially 11
V	112–114/5	−1·7 to −9·4	3·77	Chiefly 11, +12–20
VI	104–120/3	−12·7 to −34·7	4·60	Mainly 18–20, +11–17
VII	121–118/3–1	−32·3 to −4·8	10·14	Rich in 19, 20 but containing 18 and 21–27
VIII	120–121/1	—	15·95	Solid, almost pure 27

* Techno Chemical Industries, Calicut, India.

† The preparation previously examined,[4] was semi-fluid due to the separated zerumbone.

Fractionation of the oil

The results of a representative fractionation are given. The oil (56·5 g, predried over Na_2SO_4) was fractionated through a spinning band column,* using a reflux ratio of 1:20. The various fractions were pooled on the basis of their GLC and TLC ($AgNO_3$–SiO_2 gel) analysis and the results have been summarized in Table 2.

Pooled groups V, VI and VII were combined and the total material (202 g, from several lots) refractionated as above. The results are recorded in Table 3.

TABLE 3. REFRACTIONATION OF GROUPS V–VII

Pooled cut	b.p./0·5 mm	$[\alpha]_D$	Wt. (g)	Remarks
A	77–84	−20 to −34	35·8	Used for isolation of 12, 13, 15, 16
B	84–88	−37·5 to −27	63·2	Source for 18 and [15, 16]
C	88–92	−26 to −21	53·6	Employed for isolation of 19 and 20
D	95–102	−19 to −17	35·8	Material enriched in "alcohols"

Humulene (I) *and caryophyllene* (III)

Humulene. Pooled group III, Table 2 (~85% humulene and ~12% caryophyllene; 14·8 g) in pet. ether (50 ml) was stirred mechanically with $AgNO_3$ aq (40 g in 30 ml water) with ice-cooling. The solid adduct was collected, washed with chilled EtOH and the material (28 g, m.p. 173–175·5° dec) recrystallized twice from EtOH (~300 ml) to give the pure humulene–$AgNO_3$ adduct (21 g), m.p. 175–176° dec (Lit.[24] m.p. 175–175·5°); a second crop (6 g), m.p. 173–174° was also obtained.

Humulene was regenerated from the pure adduct by steam distillation[24] to give a product, b.p. 106–107°/5 mm, n_D^{30} 1·5004, $[\alpha]_D \pm 0$.

(−)-*Caryophyllene.* The combined mother liquors from the above adductation, after removal of most of the EtOH by distillation, were diluted with water (400 ml) and extracted with pet. ether (4 × 80 ml). The solvent was flashed off and the residue (4·85 g) chromatographed over $AgNO_3$–SiO_2 gel (IIa; 90 g, 2 cm × 46 cm) under TLC ($AgNO_3$–SiO_2 gel; solvent: 5% acetone in C_6H_6) minitoring:

Fraction 1:	Pet. ether	12 × 125 ml	—
Fraction 2:	Pet. ether–25% C_6H_6	4 × 125 ml	0·63 g of III + XI + X
Fraction 3:	Pet. ether–25% C_6H_6	4 × 125 ml 10 × 250 ml	1·08 g of pure III
Fraction 4:	Pet. ether–50% C_6H_6	7 × 250 ml	0·85 g of III + I
Fraction 5:	Pet. ether–50% C_6H_6	5 × 250 ml	0·23 g of essentially I
Fraction 6:	adsorbent extruded and steam-distilled	—	1·07 g of essentially I

Fraction 3 (0·44 g) in pet. ether was filtered through a column of Al_2O_3/I (12 g) to get pure III: b.p. 104–105°/5 mm, n_D^{30} 1·4969, d_4^{28} 0·8952, $[\alpha]_D$ −14·02 (*c*, 4·7%).†

ar-Curcumene (XI) *and* γ-*Cadinene* (X)

(+)-*ar-Curcumene.* Cut-A, Table 3 (31·1 g) was chromatographed on Al_2O_3/II (6·5 cm × 27 cm) and the hydrocarbon fraction (7·05 g) eluted with pet. ether (6 × 300 ml); this consisted of humulene with significant amounts of XI and minor amounts of X. By preparative GLC (temp 160°, press. 15 psi) of 4·6 g of this material, a fraction (1·12 g, b.p. 91–92°/1·5 mm) consisting of XI with some X was obtained. This (500 mg) was chromatographed over $AgNO_3$–SiO_2 gel (9 g; 0·7 cm × 40 cm):

Fraction 1:	Pet. ether	1 × 50 ml	0·239 g of pure XI
Fraction 2:	Pet. ether	2 × 50 ml	0·028 g of XI
Fraction 3:	Pet. ether	2 × 50 ml	0·128 g of XI + X

Fraction 1 on distillation afforded pure (+)-*ar*-curcumene: b.p. 112–113°/4 mm, n_D^{30} 1·4993, d_4^{29} 0·8785,

* Model NF 115, manufactured by Nestler and Faust, Newark, USA; theoretical plates: 23.

† Several different values of $[\alpha]_D$ (−8° to −22°) have been reported in the Lit.[25] for "pure" (−)-caryophyllene. Recently (+)-caryophyllene, $[\alpha]_D$ +10·1 has also been described.[26]

$[\alpha]_D$ +45·1° (*c*, 0·75%) (Lit.[27]: n_D^{20} 1·4976, d_4^{20} 0·8749, $[\alpha]_D$ +36·2°, neat); λ_{max} 251, 258, 263 and 273 mμ with log ε_{max} 2·47, 2·57, 2·77 and 2·67 respectively (Lit.[27] λ_{max} 254, 259, 265 and 273 mμ with log ε_{max} 2·4, 2·5, 2·59 and 2·6 respectively). IR spectrum: strong bands at 1520, 1115, 1022, 820 and 727 cm^{-1}. PMR spectrum: —CH<u>Me</u> doublet centred at 71 c/s, J = 7 c/s; =C(Me)$_2$ 89, 98·5 c/s; *ar*-<u>Me</u> 136·5 c/s; *ar*-C<u>H</u>—Me quartet centred at 155·5, J = 7 c/s; (Me)$_2$C=C<u>H</u>—CH$_2$, triplet centred at 301·5 c/s J = 6 c/s; four aromatic H, singlet at 417 c/s.

(+)-γ-*Cadinene*. Fraction 3 (128 mg) from above was rechromatographed over $AgNO_3$–SiO_2 gel (0·6 cm × 32 cm) as before to furnish a fraction (76 mg) consisting (GLC) of 70% X and 30% XI: b.p. 113–115/5 mm, n_D^{30} 1·5038, $[\alpha]_D$ +99·2 (*c*, 0·4%). Its PMR spectrum; after subtracting the absorptions due to XI, showed: —CH(<u>Me</u>)$_2$ pair of doublets centred at 44 and 56 c/s, each with J = 7 c/s; =CMe 98·5 c/s; >C=C<u>H</u>$_2$, two 1H singlets at 269 and 275 c/s; C=C<u>H</u> broadened singlet at 329 c/s.

The above material (60 mg) in ether was saturated with HCl gas at −15° and, after the usual work-up, yielded colorless needles (EtOAc), m.p. 114–118°, mixed m.p. with an authentic sample of (−)-Cadinene dihydrochloride (m.p. 117–118°) was undepressed (m.ps on Kofler block).

Dihydro-ψ-photozerumbone (XIII) *and ψ-photozerumbone* (XIV)

The fraction (5·0 g) eluted by pet. ether–15% C_6H_6 (7 × 300 ml) immediately after the hydrocarbons, during the chromatography (Al_2O_3) of cut A (see under *ar*-curcumene and γ-cadinene) consisted essentially of carbonyl compounds (IR). The inverted-dry-column chromatography (IDCC)[28] of this material (789 mg) on 10% $AgNO_3$–SiO_2 gel/IIA (120 g) using pet. ether–$CHCl_3$–EtOAc (10:1:2·5) as the solvent system furnished two pure epimers of (±)-dihydro-ψ-photozerumbone (XIII)* as well as epimeric mixture of (±)-ψ-photozerumbone (XIV).* $CHCl_3$ was employed for the extraction of the material from scooped out segments.

(±)-*Dihydro-ψ-photozerumbone* (first epimer, lower R_f), yield 0·28 g, b.p. 110–111°/3·5 mm, n_D^{30} 1·4873; $[\alpha]_D$ ±0°. IR spectrum: C=O 1700 cm^{-1}; C=C 3010, 1637, 1000, 912 cm^{-1}. PMR spectrum: quaternary Me 56 c/s; —CHMe doublet centred at 61 c/s, J = 6 c/s; =C(<u>Me</u>)$_2$ 94·5, 104 c/s; C=CH—C<u>H</u>—C=O doublet centred at 210 c/s, J = 10 c/s; Me$_2$C=C<u>H</u>—CH—C=O a pair of quintets centred at 321 c/s, J = 10 c/s; C—C<u>H</u>=C<u>H</u>$_2$ a 10-line ABC pattern between 282–357 c/s. (Found: C, 82·3; H, 11·0. $C_{15}H_{24}O$ requires: C, 81·8; H, 11·0%).

(±)-*Dihydro-ψ-photozerumbone* (second epimer, higher R_f), yield 0·15 g, b.p. 125–130° (bath)/3·5 mm, n_D^{30} 1·4897, $[\alpha]_D$ ±0°. IR spectrum: C=O 1702 cm^{-1}; C=C 3010, 1637, 1000, 912 cm^{-1}. PMR spectrum: quaternary Me 63 c/s; —CH<u>Me</u> doublet centred at 66 c/s, J = 6 c/s; =C(<u>Me</u>)$_2$ 95, 104 c/s; C=C<u>H</u>—C<u>H</u>$_2$—C=O doublet centred at 202 c/s, J = 10 c/s; Me$_2$C=C<u>H</u>—CH$_2$—C=O two quintets centred at 323 c/s, J = 10 c/s; C—C<u>H</u>=C<u>H</u>$_2$ a 10-line ABC pattern between 282 and 365 c/s. (Found: C, 82·3; H, 11·0. $C_{15}H_{24}O$ requires: C, 81·8; H, 11·0%).

(±)-ψ-*Photozerumbone*, yield 60 mg, b.p. 130–140° (bath)/3 mm, n_D^{30} 1·5013, $[\alpha]_D$ ±0°; λ_{max} 239 mμ, ε 6509. This was identified as a ~1:1 mixture of ψ-photozerumbone epimers by comparison (IR, PMR) with authentic samples.[9]

Caryophyllene oxide (IV)

Cut B, Table 3 (1·36 g) was chromatographed over Al_2O_3/II (2 cm × 44·5 cm) and the fractions eluted with pet. ether (14 × 100 ml; 340 mg) and pet. ether–10% C_6H_6 (12 × 100 ml; 243 mg) were highly enriched in IV. The former material (246 mg) was rechromatographed over $AgNO_3$–SiO_2 gel/IIB (0·8 cm × 42 cm) under TLC ($AgNO_3$–SiO_2 gel; solvent: 5% acetone in C_6H_6) monitoring. After pet. ether (10 × 20 ml) elution, pet. ether–50% C_6H_6 (7 × 20 ml) and C_6H_6 (5 × 20 ml) together eluted 80 mg of IV, which solidified and was recrystallized from aqueous MeOH to give pure IV, m.p. 62–63°, $[\alpha]_D$ −74·2° (*c*, 4·2%), mixed m.p. with an authentic sample was undepressed (Lit.[29] m.p. 64°, $[\alpha]_D$ −68°). IR spectrum: strong bands at 1635, 1261, 1080, 968, 915, 895, 873, 855 and 768 cm^{-1}. PMR spectrum: quaternary methyls 59, 60, 67 c/s; =CH$_2$ essentially two 1H singlets at 285 and 290 c/s.

Humulene epoxide—I(V) *and humulene epoxide*—II(VI)

Cut C, Table 3 was depleted of IV and enriched with respect to V and VI by a preliminary chromatography: e.g. 6·6 g of one such sample on careful chromatography on Al_2O_3/II (3·3 cm × 47·5 cm) and elution with

* These ketones epimerize readily during chromatography over Al_2O_3,[9] and must have got equilibrated during the preliminary Al_2O_3 chromatography.

pet. ether (30 × 200 ml) yielded fractions (~3·7 g) rich in V and VI. This material (5·0 g) was rechromatographed on Al_2O_3/II (2·2 cm × 62 cm) to furnish pure V and VI (invariably in all these chromatographies small amounts of the diepoxide were also obtained, usually in the C_6H_6 eluates):

Fraction 1:	Pet. ether	6 × 100 ml	—
Fraction 2:	Pet. ether	5 × 100 ml	0·56 g of pure V
Fraction 3:	Pet. ether	4 × 100 ml	0·66 g of ~90% pure V
Fraction 4:	Pet. ether	14 × 100 ml	1·36 g of V + VI (~1:1)
Fraction 5:	Pet. ether–10% C_6H_6	4 × 100 ml	1·56 g of ~95% pure VI
	Pet. ether–25% C_6H_6	2 × 200 ml	
Fraction 6:	Pet. ether–25% C_6H_6	5 × 200 ml	0·30 g of pure VI
Fraction 7:	Pet. ether–50% C_6H_6	2 × 250 ml	0·01 g
Fraction 8:	C_6H_6	3 × 250 ml	0·14 g of VIII, m.p. 103–105°
Fraction 9:	C_6H_6–3% MeOH	3 × 250 ml	0·23 g of hydroxylic material.

(−)-*Humulene epoxide*—I. Fraction 2, above, on distillation furnished pure V (525 mg): b.p. 106·5–107·5°/1·4 mm, n_D^{30} 1·4940, $[\alpha]_D$ −21·3° (*c*, 3·5%). (Found: C, 81·7; H, 11·1. $C_{15}H_{24}O$ requires: C, 81·8; H, 11·0%).

(−)-*Humlene epoxide*—II. Fraction 6 was distilled to give pure VI: b.p. 113–114°/1·8 mm, n_D^{30} 1·4962, $[\alpha]_D$ −31° (*c*, 4·25%). (Found: C, 81·8; H, 11·3. $C_{15}H_{24}O$ requires: C, 81·8; H, 11·0%).

Humulene dioxide (VIII). The solid material obtained from benzene eluates in the above chromatographies, readily crystallized from pet. ether in colorless needles, m.p. 106–106·5°, $[\alpha]_D$ −0·13° (*c*, 2·3%). IR spectrum: strong bands at 1080, 1065, 982, 975, 890, 851, 785 and 770 cm^{-1}; the spectrum was identical with that reported[30] for (±)-humulene dioxide (m.p. 103°). (Found: C, 76·3; H, 10·2. $C_{15}H_{24}O_2$ requires: C, 76·3; H, 10·2%).

(+)-*Humulenol*—II (IX)

Cut D, Table 3 (~20 g) was redistilled to get: (i) b.p. 110–118°/1·8 mm, 13·5 g, epoxides + alcohols, (ii) b.p. 118–132°/1·8 mm, 4·0 g, chiefly alcohols. Chromatography of fraction (i) on Al_2O_3/II (4·5 × 23 cm) separated the oxides from the alcohols:

Fraction 1:	Pet. ether	18 × 200 ml	1·38 g
Fraction 2:	Pet. ether–25% C_6H_6	16 × 200 ml	5·03 g mainly oxides
Fraction 3:	C_6H_6	4 × 200 ml	0·42 g
Fraction 4:	C_6H_6–2% MeOH	5 × 200 ml	1·24 g, alcohols, slightly contaminated with oxides
Fraction 5:	C_6H_6–3% MeOH	2 × 200 ml	5·15 g alcohols.

Chromatography fraction 5 and the redistillation cut (ii) had essentially the same TLC and GLC and hence were mixed and redistilled: b.p. 119–122°/2 mm, n_D^{31} 1·5060. The GLC (temp 180°, flow 50 ml/min) of this material showed at least three components in the ratio 1:1·6:3·2 (RRT 1, 1·3 and 1·6 respectively). Preparative GLC (temp 200°, press 15 psi) of this material gave the third component (highest retention time) (0·48 g), which was still slightly impure (TLC: SiO_2 gel-G; Solvent: 5% acetone in C_6H_6) and was purified by chromatography over Al_2O_3/II (0·9 cm × 20 cm):

Fraction 1:	Pet. ether	6 × 20 ml	—
Fraction 2:	Pet. ether–25% C_6H_6	7 × 20 ml	50 mg
Fraction 3:	Pet. ether–50% C_6H_6	12 × 20 ml	100 mg of pure IX
Fraction 4:	C_6H_6	6 × 20 ml	16 mg of pure IX
Fraction 5:	C_6H_6–2% MeOH	8 × 20 ml	23 mg of impure IX

Fractions 3 and 4 were combined and distilled to give pure IX as a colorless viscous liquid (100 mg): b.p. 110–112°/1 mm, n_D^{30} 1·5133, $[\alpha]_D$ +12·4° (*c*, 2·2%). (Found: C, 81·7; H, 11·1. $C_{15}H_{24}O$ requires: C, 81·8; H, 11·0%).

Zerumbone (II)

Pooled group VIII, Table 2 was essentially pure zerumbone; recrystallization from aqueous EtOH gave long, colorless prismatic needles, m.p. 66·5–67° (Lit.[4] m.p. 66–67°).

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