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THE SEPARATION AND IDENTIFICATION OF GERMACRATRIENE (4,10-DIMETHYL-7-ISOPROPYLIDENE-CYCLODECA-4,10-DIENE) FROM HOPS (*HUMULUS LUPULUS*)

R. D. Hartley* and C. H. Fawcett†

Wye College, University of London, Wye, near Ashford, Kent

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Abstract—Germacratriene has been isolated in quantity from the essential oil of hops by preparative GLC. Four known selinadienes are always present with germacratriene, which has not previously been reported to occur naturally. The possible role of germacratriene in the biogenesis of the four selinadienes is discussed.

INTRODUCTION

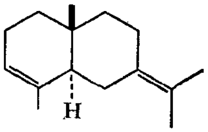
It is well known that the essential oils obtained from different hop varieties vary considerably in constitution. Nevertheless most of the major constituents of commercial varieties have been identified.[1–3] In a recent study of hop seedling families obtained in a breeding programme for the production of new varieties, it was shown by gas chromatography (GLC) that two unidentified major peaks were present on the chromatograms of the essential oils from those seedlings which were resistant to powdery mildew.[4] The composition of the material represented by the peak of lower retention time (178 min) has been shown to be a mixture of selina-3,7(11)-diene (I) and the previously unidentified selina-4(14),7(11)-diene (II).[5] This paper is concerned with the identification of the material represented by the peak of higher retention time (approx. 210 min) and its possible role in the biogenesis of sesquiterpenes.

RESULTS AND DISCUSSION

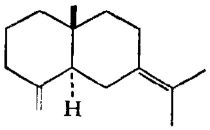
The peak of higher retention time, referred to above, represents a compound from the hydrocarbon fraction of the essential oil from a seedling family containing plants resistant to powdery mildew. It was isolated by preparative GLC using polyethylene glycol adipate (PEGA) as stationary phase and the separation was carried out isothermally at 125°; above this temperature considerable loss of the required material occurred. After further purification by GLC, the mass, proton magnetic resonance (PMR) and i.r. spectra were determined. The mass spectrum showed the molecular weight to be 204 in agreement with $C_{15}H_{24}$, and the PMR and i.r. spectra were all identical with those from authentic germacratriene (III).

* Hop Research Department. Present address: Grassland Research Institute, Hurley, Berkshire.
† Agricultural Research Council Unit on Plant Growth Substances and Systemic Fungicides.

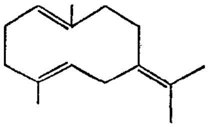
[1] R. Stevens, *Chem. Rev.* **67**, 19 (1967).
[2] R. G. Buttery and L. C. Ling, *Brewers' Dig.* 71 (1966).
[3] R. G. Buttery and L. C. Ling, *J. Agri. Food Chem.* **15**, 531 (1967).
[4] R. D. Hartley and R. A. Neve, to be published.
[5] R. D. Hartley and C. H. Fawcett, *Phytochem.*, **8**, 637 (1969).



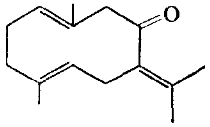
(I)
Selina-3,7(11)-diene



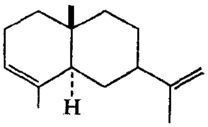
(II)
Selina-4(14),7(11)-diene



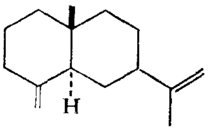
(III)
Germacratriene



(IV)
Germacrone



(V)
Selina-3,11-diene
(α-selinene)



(VI)
Selina-4(14),11-diene
(β-selinene)

A hydrogen count of the PMR spectrum (Fig. 1) also indicated that the isolated material was pure. It should be emphasized that for both the natural and the synthetic compounds the absorption due to the protons in the C=CH groups is centred at τ = 5·38 (Fig. 1) and not τ = 4·38 as erroneously quoted by Brown *et al.*[6] for the synthetic compound. Though it has

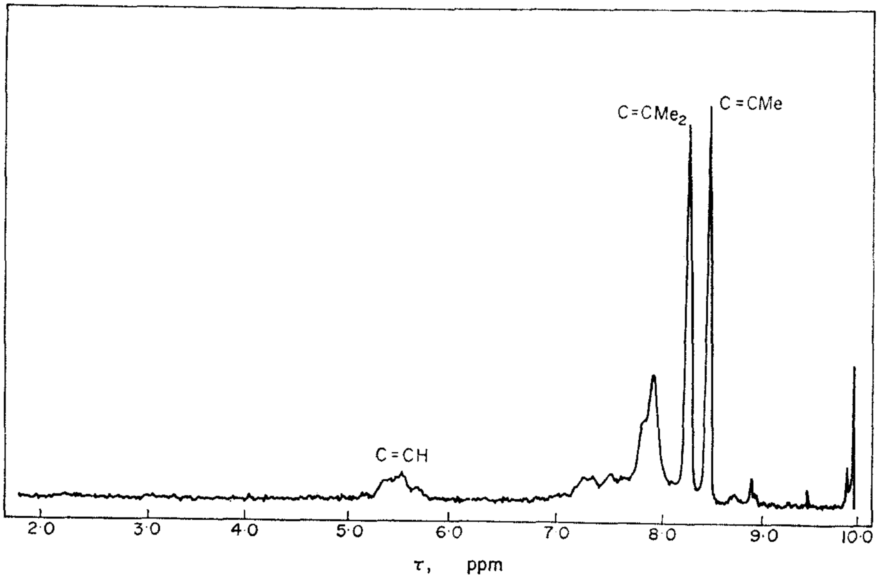


FIG. 1. PMR SPECTRUM OF GERMACRATRIENE (III).

[6] E. D. BROWN, M. D. SOLOMON, J. K. SUTHERLAND and A. TORRE, *Chem. Commun.* 111 (1967).

recently been synthesized from germacrone (IV) and its structure established,[6-8] this is the first occasion that germacratriene has been reported to occur naturally. It was shown by these workers to be thermally unstable and acid-labile, and it is of interest therefore to note that hop varieties containing III lose this material rapidly on storage.

In GLC studies it was found that fresh hops of the varieties Sunshine, Hersbruck Gebirg, Tasmanian White Vine and Late Vine contained large amounts of III. In the last two varieties this was confirmed by isolation of the compound by preparative GLC followed by PMR analysis. All these varieties also contained large amounts of I and II as well as α-selinene (V) and β-selinene (VI). Many hundreds of seedling and commercial varieties have been examined by GLC at Wye and it has been shown that those containing I, II and III as major components also contain large amounts of V and VI, but many varieties that contain V and VI do not contain I, II and III. As compounds I, II and III always occur together, the possibility of the rearrangement of III to yield I and II was considered. As III is optically inactive, optical rotatory dispersion (O.R.D.) spectra were determined for I and II. Both I and II exhibited low optical activity indicating that they were not artefacts produced during the purification.

TABLE 1. RELATIVE AMOUNTS OF SELINA-3,7(11)-DIENE (I), SELINA-4(14),7(11)-DIENE (II), GERMACRATRIENE (III), α-SELINENE (V) AND β-SELINENE (VI) PRESENT IN THE ESSENTIAL OIL OF CONES FROM SUNSHINE VARIETY

	Relative amount*		
Date of harvest	I & II	III	V & VI
20/8/68	22·5	2·5	75
27/8	28	3	69
3/9	34	5	61
10/9	21·5	8·5	70
16/9	25·5	13·5	61

* Estimated by the peak area method using GLC. Compounds I and II were present in approximately equal amounts, as were V and VI.

A study of the development of I, II, III, V and VI in the essential oil was carried out using fresh cones of the variety Sunshine. During ripening, samples were harvested at approximately weekly intervals from 20 August, when the cones were about one-third of their final size, to 16 September and their essential oils examined by GLC (Table 1). The ratio of I and II/V and VI was similar for each date of harvest but the proportion of III increased about five-fold. Under the GLC conditions used, authentic III did not give I, II, V or VI, showing that none of these compounds was formed from III during the separation procedure. Since the solutions chromatographed were prepared by cold ether extraction of cones dried overnight at 50° *in vacuo* and the ethereal solutions concentrated at room temperature, it is very unlikely that III would give rise to I, II, V or VI under these conditions. Nevertheless, in acidic media such as in the growing plant, III might re-arrange to these compounds due to the presence of α- and β-acids in the same resin glands as the essential oils.

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[7] F. H. ALLEN and D. ROGERS, *Chem. Commun.* 588 (1967).

[8] F. H. ALLEN, E. D. BROWN, D. ROGERS and J. K. SUTHERLAND, *Chem. Commun.* 1116 (1967).

The isolation of germacratriene and its association in hops with the four selinadienes support the hypotheses of various workers[6,9] who discussed the likelihood of it being the precursor of several sesquiterpenes. Hence, in the studies being carried out on the role of these sesquiterpenes and their genetic association with powdery mildew resistance of hops,[4] it is likely that germacratriene will prove of considerable importance.

EXPERIMENTAL

Hop Material

Hops (1968 crop) from seedlings of the Wye family, Code No. 32/66, were dried in a commercial oast.

Isolation of the Hydrocarbon Fraction of the Essential Oil from Hops

Essential oil (0·2 ml/100 g hops)was obtained by steam distillation and the hydrocarbon fraction (0·7 g/g oil) isolated by the usual method.[5]

Separation of the Hydrocarbon Fraction by Preparative GLC

The hydrocarbon fraction of the essential oil was separated in batches (30 μl each time) by preparative GLC to yield III (2 mg/batch). The GLC conditions, using a Pye Series 104 chromatograph fitted with a flame ionization detector, were as follows: 9-ft glass column (int. dia. 4 mm) containing 10% PEGA on 100–120 mesh acid-washed celite. The argon carrier gas flow rate was 45 ml/min and the gauge pressure 60 p.s.i. The oven temperature was 125°, the amplifier attenuation 10^{-9} and the retention time of III was approximately 60 min. Before determination of mass, PMR and i.r. spectra the combined batches of III were rechromatographed by the same method to remove any possible artefacts formed on storage at $-20°$. The retention time of III was identical with authentic germacratriene.

Examination by Analytical GLC of the Essential Oil Present in Fresh Cones

Samples were prepared using cold ether extraction as previously described[10] and the conditions of chromatography were as above using 15 μl of the ethereal solution and an amplifier attenuation of 5×10^{-9}. As mixtures of I and II and mixtures of V and VI do not separate into the respective compounds on this column, the relative amounts of these compounds were determined using Apiezon L stationary phase as previously described.[5]

Determination of Spectra

PMR spectra were determined at 60 Mc/s in CCl_4 using tetramethylsilane as internal reference (Varian A-60A instrument). I.r. spectra (clear film) were obtained with a Perkin Elmer 21 instrument. Mass spectra were determined by the GLC/mass spectrum technique. GLC was carried out using a 9 ft glass column (int. dia. 4 mm) containing 10% F.F.A.P. (Perkin Elmer Ltd.) as stationary phase on celite. Mass spectra were recorded at 70 eV with the source at 120° (A.E.I. MS902 instrument).

O.R.D. spectra were determined in hexane using a Bellingham and Stanley/Bendix-Ericson automatic recording spectropolarimeter "Polarmatic 62". The results were as follows: for I, $[\phi]_{400}+30°$, $[\phi]_{299}+70°$, $[\phi]_{270}+80°$, $[\phi]_{250}+95°$ (C, 2·0 mg/1·0 ml, d 1 cm); for II, $[\phi]_{400}0°$, $[\phi]_{299}+20°$, $[\phi]_{270}+20°$, $[\phi]_{250}+15°$ (C, 4·4 mg/1·0 ml, d 1 cm).

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[9] J. B. HENDRICKSON, *Tetrahedron* 7, 82 (1959).

[10] R. D. HARTLEY and C. H. FAWCETT, *Phytochem.* 7, 1395 (1968).