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THE SEPARATION AND IDENTIFICATION OF SELINA-4(14),7(11)-DIENE, A NEW SESQUITERPENE FROM HOPS (*HUMULUS LUPULUS*)

R. D. HARTLEY* and C. H. FAWCETT†

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

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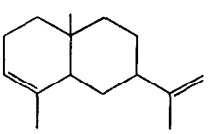
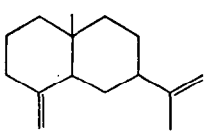
Abstract—A new sesquiterpene, selina-4(14),7(11)-diene, and the closely related selina-3,7(11)-diene, which was previously obtained elsewhere in micro-quantities, have now both been isolated in quantity from hops. They are of interest because they are genetically linked with one type of powdery mildew resistance in hop varieties. These two compounds were resolved by preparative GLC using Apiezon L as stationary phase. Their i.r., PMR and mass spectra are compared and contrasted with those of selinane which they both yield on hydrogenation.

INTRODUCTION

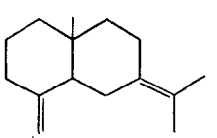
MOST of the major constituents of the steam-distilled essential oil from hops have been identified.[1] It is also well established that the constituents in the essential oil vary considerably with variety.[1-6] Thus, some varieties (e.g. "Pride of Ringwood") contain relatively



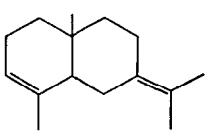
(I) Selina-3,11-diene
(α-selinene)



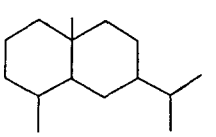
(II) Selina-4(14),11-diene
(β-selinene)



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



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



(V) Selinane

* Hop Research Department.

† Agricultural Research Council Unit on Plant Growth Substances and Systematic 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] S. T. LIKENS and G. B. NICKERSON, *J. Agri. Food Chem.* **15**, 525 (1967).
[5] J. R. L. WALKER, *N.Z. J. Sci.* **10**, 476 (1967).
[6] R. D. HARTLEY, *Phytochem.*, **7**, 1641 (1968).

large amounts of α-selinene (I) and β-selinene (II), whilst other varieties (e.g. "Fuggle") contain only small quantities of these compounds.

In a study of certain hop-seedling families it has been established, using gas–liquid chromatography (GLC) with polyethylene glycol adipate (PEGA) as stationary phase, that the occurrence of two peaks having higher retention times than α- and β-selinene, is genetically linked with one type of powdery mildew resistance in hops.[7] Of these two peaks, the one with the higher retention time has been found to disappear rapidly from hops during storage and its composition has not yet been examined. The composition of the material represented by the other peak has been determined and the results are reported here.

RESULTS AND DISCUSSION

The hydrocarbon fraction of the essential oil from hops from a seedling family containing plants resistant to powdery mildew was subjected to preparative GLC using PEGA as

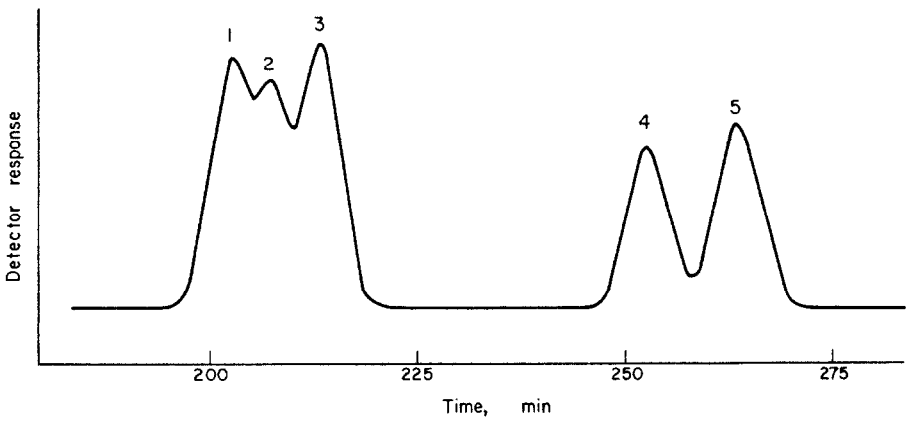


FIG. 1. SEPARATION OF SELINA-3,11-DIENE (I), SELINA-4(14),11-DIENE (II), SELINA-4(14),7(11)-DIENE (III), SELINA-3,7(11)-DIENE (IV) AND SELINANE (V) BY ANALYTICAL GLC (CONDITIONS GIVEN IN THE TEXT).

Peak Nos. correspond to the following compounds: 1, selina-4(14),11-diene (II); 2, selina-3,11-diene (I); 3, selinane (V); 4, selina-4(14),7(11)-diene (III); 5, selina-3,7(11)-diene (IV).

stationary phase and the peak of lower retention time (178 min) referred to above was isolated. The proton magnetic resonance (PMR) spectrum indicated that this material was a mixture. Next, when GLC with Carbowax 20M as stationary phase was carried out, no resolution of this mixture occurred but separation into two constituents (approx. 1:1) was achieved using the non-polar Apiezon L as stationary phase. The constituent which had the lower retention time (360 min) on this column was designated III and the other one IV. On examination by GLC the behaviour of III and IV was similar to that of I and II in that this latter pair of compounds also did not separate on columns containing polar phases, but could be partially resolved on Apiezon L (Fig. 1).

In a study of several hop varieties, it was shown by GLC that the varieties "Sunshine", "Hersbruck Gebirg", and "Tasmanian White Vine" and "Late Vine" contained unusually large amounts of both III and IV in an approximately 1:1 ratio.

[7] R. D. HARTLEY and R. A. NEVE, to be published.

After purification of III and IV by preparative GLC, the mass, PMR and i.r. spectra of the two unknown compounds and of the products obtained from them by hydrogenation were determined. The structures of III and IV were shown to be selina-4(14),7(11)-diene (III) and selina-3,7(11)-diene (IV) respectively (using Theobald's system of nomenclature[8]).

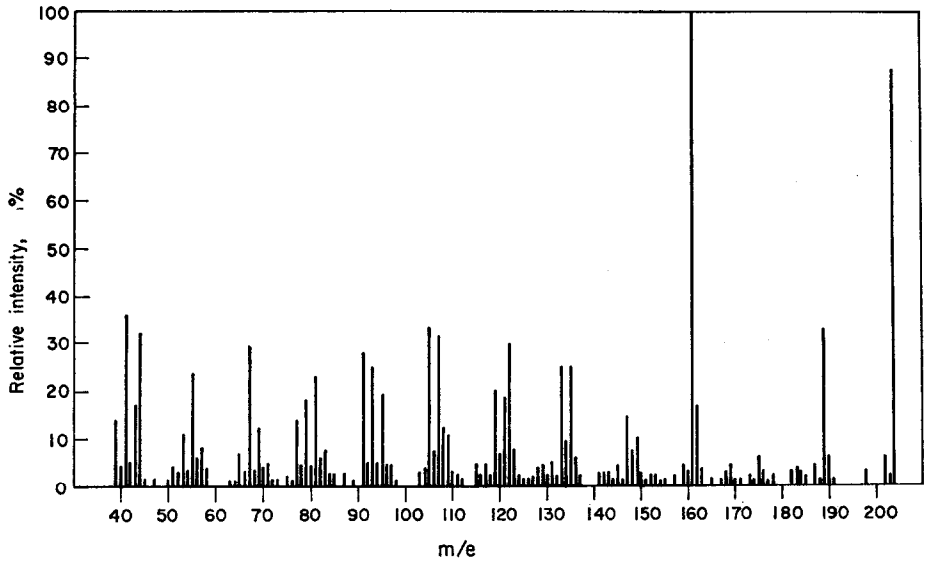


FIG. 2A. MASS SPECTRUM OF SELINA-4(14),7(11)-DIENE (III).

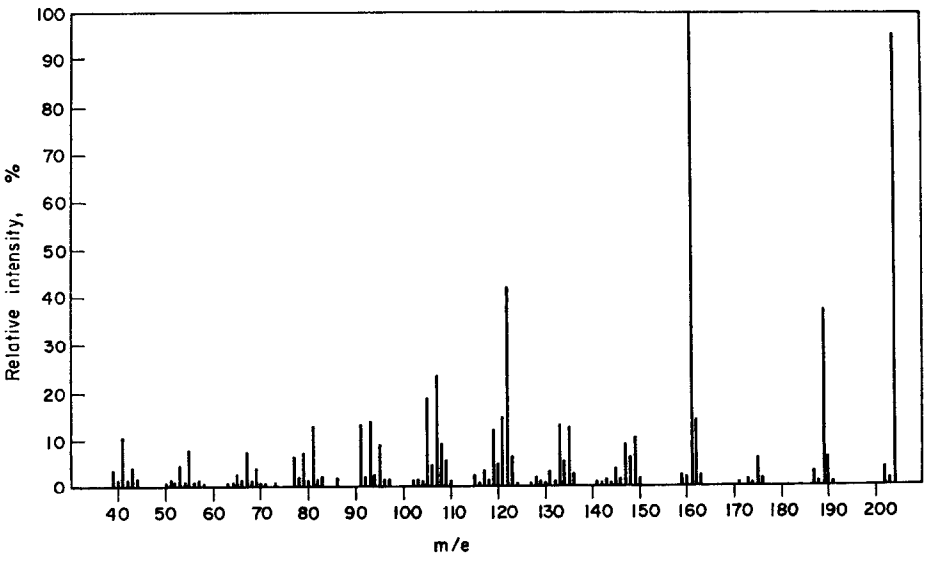


FIG. 2B. MASS SPECTRUM OF SELINA-3,7(11)-DIENE (IV).

[8] D. W. THEOBALD, *Tetrahedron* **19**, 2261 (1963).

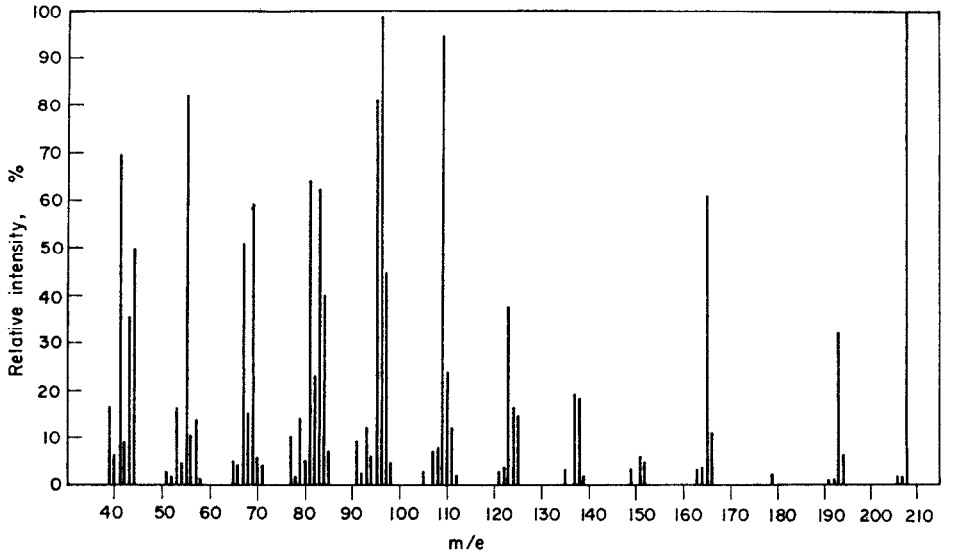


FIG. 2C. MASS SPECTRUM OF SELINANE (V).

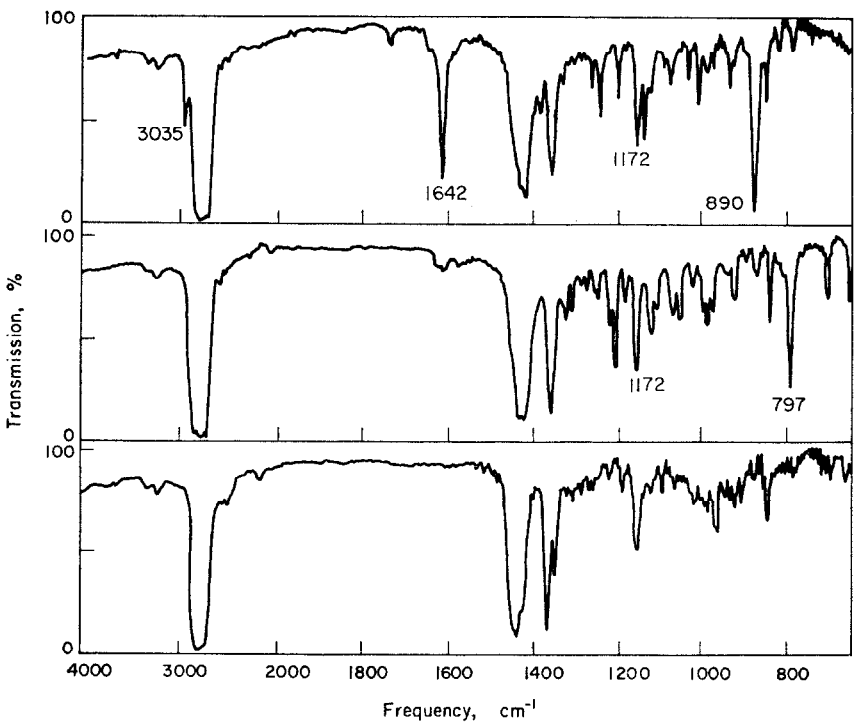


FIG. 3. I.R. SPECTRA (FROM TOP TO BOTTOM) OF SELINA-4(14),7(11)-DIENE (III), SELINA-3,7(11)-DIENE (IV) AND SELINANE (V).

The bands assigned to $C=CMe_2$ (1172 cm^{-1}) in III and IV are indicated. The $C=CH_2$ group in III gave bands at 3035, 1642 and 890 cm^{-1}, while the CH=CMe group in IV gave a band at 797 cm^{-1}.

The mass spectra of III and IV were similar, and both had parent peaks of mass 204 in agreement with $C_{15}H_{24}$, and base peaks of mass 161 (Figs. 2A and 2B). It is of interest that II has only a small peak at mass 161[9] and V has no peak at this mass (Fig. 2C).

Separate hydrogenation (Adams catalyst) of III and IV and isolation of the single product gave in each instance a compound having mass and i.r. spectra identical with authentic selinane (V) (see Figs. 2 and 3). Hence it was established that both III and IV were selinane

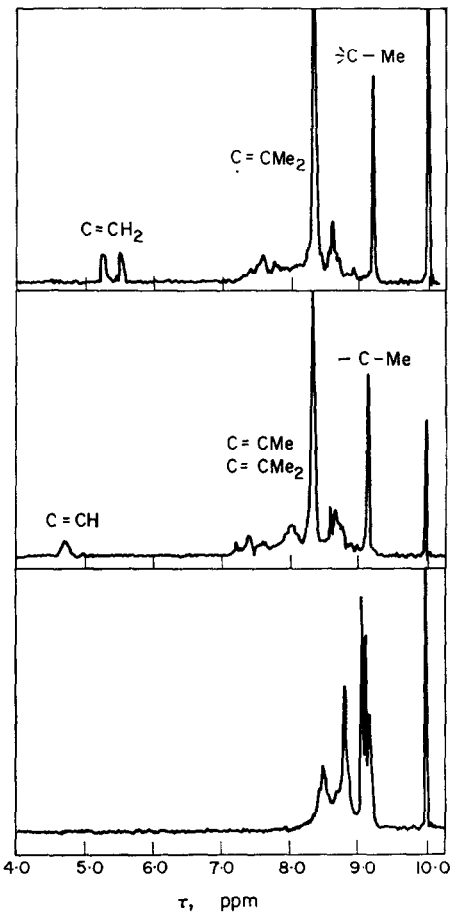


FIG. 4. PMR SPECTRA (FROM TOP TO BOTTOM) OF SELINA-4(14),7(11)-DIENE (III), SELINA-3,7(11)-DIENE (IV) AND SELINANE (V).

derivatives each having two double bonds. Both III and IV were shown to be pure from a consideration of the hydrogen count of their PMR spectra (Fig. 4). Assignments of bands in the spectra are shown in Fig. 4. Confirmatory evidence was obtained by comparison of their i.r. spectra with that of selinane (Fig. 3).

Buttery and Ling[3] have identified IV using microgramme quantities obtained by capillary GLC of hop oil (variety "Gebirg"). A PMR spectrum of this compound was published but only the main i.r. peaks were listed. In their study, insufficient material was available to

[9] H. C. HILL, R. I. REED and M. T. ROBERT-LOPES, *J. Chem. Soc.* 93 (1967).

establish the skeletal structure by hydrogenating a pure sample to selinane. Instead they obtained a fraction by packed column GLC using Carbowax 20M as stationary phase, which contained at least two other components, including possibly some III, and this fraction was used for hydrogenation and the determination of molecular weight by mass spectrometry.

The present work removes a confusion which exists in the literature on sesquiterpenes. Since the published i.r. spectrum of a material called γ-selinene[10, 11] possesses a major band at 827 cm^{-1}, which is not present in the spectrum of III (Fig. 3), they are clearly different. Yet some reviewers have suggested[12] or stated[13] that γ-selinene has structure III. It follows therefore, that the materials which various workers[14–18] have reported to possess i.r. spectra similar to the hydrocarbon recently regarded as γ-selinene cannot be pure samples of III. Likewise the material regarded as γ-selinene because of its i.r. spectrum[19] is clearly not III or IV. Another error in nomenclature also occurs with regard to IV, since this structure was cited as γ-selinene and suggested as a possible constituent which might occur in sesquiterpenes from Brazilian rosewood.[20]

The biogenesis of I, II, III and IV probably proceeds via the *trans*-farnesyl cation as suggested by Hendrickson,[21] and in this case their stereochemistry would be as indicated by him.

EXPERIMENTAL

All GLC separations were carried out using a Pye Series 104 Chromatograph fitted with a flame ionization detector. Glass columns were employed with acid-washed celite as inert support. The gauge pressure of the argon carrier gas was 70 lb/in^2.

Hop Material

Hops (1967 crop) from seedlings of the Wye family Code No. 5/64 were dried in a commercial oast.

Isolation of the Hydrocarbon Fraction of the Essential Oil from Hops

Essential oil (0·35 ml/100 g hops) was obtained by a modified method[6] of the steam-distillation technique.[22] The hydrocarbon fraction of this oil (0·72 g/g oil) was isolated by silica gel liquid chromatography.[23]

Preliminary Separation of the Hydrocarbon by Preparative GLC

The hydrocarbon fraction of the oil was examined in batches (0·25 ml each time) by preparative GLC to yield material consisting of an approximately 1:1 mixture of III and IV (10 mg/batch). The GLC conditions were as follows: 18 ft column (i.d. 4 mm) containing 10 per cent PEGA on 100–120 mesh celite. The carrier gas flow rate was 30 ml/min. The column oven was programmed from 80–150° at 3°/min and held at the upper limit. The amplifier attenuation was 5×10^{-9} and the retention time of the mixture of III and IV was approximately 178 min.

[10] F. SORM, M. SUCHY, F. VONASEK, J. PLIVA and V. HEROUT, *Coll. Czech. Chem. Commun.* **16**, 268 (1951).
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[21] J. B. HENDRICKSON, *Tetrahedron* **7**, 82 (1959).
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Preparative GLC Separation of III and IV

The mixture of III and IV (6 μl each time) was separated on a 50 ft column (i.d. 3·5 mm) containing 10 per cent Apiezon L on 60–80 mesh celite. The carrier gas flow rate was 50 ml/min. The column oven was programmed from 120–175° at a rate of 1°/min and held at the upper limit. The amplifier attentuation was 10^{-9}. The yield of each component was approximately 2 mg. Repetitive chromatography was carried out to obtain approximately 50 mg of each constituent. It was necessary to rechromatograph the separated materials to achieve complete resolution. The retention time of III was approximately 360 min and of IV 380 min.

Preparation of Selinane (V)

Selinane (V) was obtained by the method of Stevens[24] using a 1:1 mixture of I and II isolated from hop oil. The hydrogenated product was obtained by preparative GLC using an 18 ft column (i.d. 4 mm) packed with 10 per cent Apiezon L on 100–120 mesh celite. The carrier gas flow rate was 60 ml/min. The column oven was programmed from 120–175° at a rate of 1°/min. The retention time of V was approximately 140 min.

Hydrogenation of III

The above technique was repeated using III (15 mg), Adams catalyst (20 mg) and A.R. glacial acetic acid (2 ml) and a reaction time of 4 hr. The reaction was monitored by analytical GLC. The material (9 mg) isolated had retention time and mass and i.r. spectra identical with authentic selinane.

Hydrogenation of IV

The above method was repeated using IV (20 mg), Adams catalyst (25 mg) and acetic acid (3 ml). The isolated material (12 mg) had retention time and mass and i.r. spectra identical with authentic selinane.

Analytical GLC of a mixture of I, II, III, IV and V

The conditions of chromatography were as follows: 50 ft column (i.d. 3·5 mm) containing 10 per cent Apiezon L on 60–80 mesh celite. The flow rate of the carrier gas was 110 ml/min. The column oven was programmed from 120–175° at a rate of 1°/min and held at the upper limit. The amplifier attenuation was 2×10^{-9} and the sample size 6 μl of an ethereal solution.

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 recorded at 70 eV with the source at 120° (A.E.I. MS9 instrument).

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[24] R. Stevens, *J. Chem. Soc.* 956 (1964).