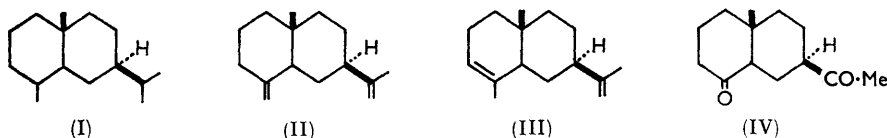


## 186. Chemistry of Hop Constituents. Part XIX.<sup>1</sup> The Essential Oil of the Variety OW-153.

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The hydrocarbon fraction of the essential oil of the abnormal hop variety OW-153 consists principally of myrcene and  $\beta$ -selinene.

THE main hydrocarbon constituents of the essential oil of hops (*Humulus lupulus* L.) are myrcene, farnesene, caryophyllene, and humulene,<sup>2</sup> which are eluted in this order during gas chromatography<sup>3</sup> upon Apiezon M at 170° (but not at other temperatures<sup>4</sup>). On the chromatograms of the hydrocarbon fraction of certain varieties one or more compounds are seen to be eluted after humulene, the nature of which are unknown.<sup>5</sup> The hydrocarbon fraction of the oil of the abnormal variety OW-153 gave a gas-chromatographic pattern indicating that it contained little or no farnesene, caryophyllene, and humulene but was rich in a post-humulene constituent and was selected for a more detailed investigation. The oil was fractionally distilled and the fractions divided further into hydrocarbon and oxygenated components by chromatography upon silica gel. The hydrocarbon component of the low-boiling fraction was characterized as myrcene, and the oxygenated component in this fraction was 2-methylbutyl isobutyrate<sup>6</sup> so that this fraction of the oil does not differ from that found in other varieties.



From the hydrocarbon fraction of the higher-boiling portion of the oil was isolated a sesquiterpene,  $C_{15}H_{24}$ , which, although on the basis of gas-chromatographic analysis still retained about 10% of humulene, was used for further study. Hydrogenation and titration with perbenzoic acid showed the presence of two double bonds of which at least one must be present as a methylene group on account of the strong band at 890  $cm^{-1}$  in the infrared spectrum. The sesquiterpene is therefore bicyclic with two double bonds, in agreement with the molecular refractivity. Comparison of the infrared spectra of the hydrocarbon and its tetrahydro-derivative with published data<sup>7</sup> established that the tetrahydro-derivative was selinane (eudesmane) (I). The infrared spectrum of the sesquiterpene showed some differences compared with that of  $\beta$ -selinene<sup>7</sup> (II) but was similar to that reported for a mixture of  $\alpha$ - (III) and  $\beta$ -selinene<sup>8</sup> and showed only very slight divergencies when compared with that of a sample of  $\beta$ -selinene isolated from oil of celery seed. The infrared spectrum of the tetrahydro-derivatives of the sesquiterpenes from celery and OW-153 were identical. Further confirmation of the selinene structure was provided by dehydrogenation<sup>9</sup> to eudalene (isolated as the picrate).

The optical rotation of the selinene isolated from hop oil (+18.9°), however, was lower than that reported for either  $\beta$ - (+31.36°) or  $\alpha$ -selinene (+61.36°) but similar to that found

<sup>1</sup> Part XVIII, Burton, Stevens, and Elvidge, preceding paper.

<sup>2</sup> Roberts and Stevens, *J. Inst. Brewing*, 1962, **68**, 420 (Review).

<sup>3</sup> Howard, *J. Inst. Brewing*, 1957, **63**, 126; Howard and Slater, *ibid.*, p. 491.

<sup>4</sup> Roberts, *Nature*, 1962, **193**, 1071.

<sup>5</sup> Howard and Slater, *Chem. and Ind.*, 1957, 495.

<sup>6</sup> Howard and Stevens, *Chem. and Ind.*, 1959, 1518; Roberts, *J. Inst. Brewing*, 1962, **68**, 198.

<sup>7</sup> Pliva, Horák, Herout, and Sorm, "Die Terpene, Sammlung der Spektren und physikalischen Konstanten, Teil I, Sesquiterpene," Akademie Verlag, Berlin, 1960.

<sup>8</sup> Naves, *Bull. Soc. chim. France*, 1956, 292.

<sup>9</sup> Ruzicka, Meyer, and Mingazzini, *Helv. Chim. Acta*, 1922, **5**, 345, 363; Linstead, Michaelis, and Thomas, *J.*, 1940, 1139.

by Naves<sup>8</sup> for the mixture of  $\alpha$ - and  $\beta$ -selinene isolated from the essential oil of the brazilian rose. Ozonolysis of selinene from OW-153 gave formaldehyde (dimedone derivative) and a triketone (IV) (17%) which was characterized as the bis-semicarbazone in agreement with that obtained from  $\beta$ -selinene.<sup>10</sup> In view of the low yield and the similarity of the physical constants of the hydrocarbon from OW-153 and that from brazilian rose oil the ozonolysis was repeated using the method of Naves<sup>8</sup> giving a neutral fraction (51% yield), identical with the diketone obtained above, and an acidic fraction (15% yield). Oxidation of the latter fraction with hypobromite gave a small oily residue which failed to crystallize. It follows, therefore, that the sesquiterpene fraction of OW-153 is principally  $\beta$ -selinene.

Further examination of the distillation residue of the hydrocarbon fraction of OW-153 by gas chromatography showed the presence of small amounts of the two components with retention times of 5.37 and 5.88 relative to  $\beta$ -selinene. By extrapolation on a graph of  $\log_{10}$  (retention time) and carbon number it is suggested that these compounds may be diterpenes, a class of compounds not previously noticed in the essential oil of hops.

In considering the biogenesis of the sesquiterpenes Hendrickson<sup>11</sup> has pointed out that farnesene, caryophyllene, and humulene, the normal constituents of hop oil,<sup>2</sup> can arise from the *cis*-farnesyl cation while  $\beta$ -selinene is derived from the *trans*-farnesyl cation. It thus appears that a genetic blockage must occur in OW-153 to prevent the synthesis of sesquiterpenes from the *cis*-farnesyl cation.

When this work was almost complete Shigematsu and Kitazawa<sup>12</sup> described an investigation of the essential oil of "Shinshu-wase" hops from which they isolated from the hydrocarbon fraction a cadinene hydrocarbon, as well as humulene and caryophyllene. Although the physical properties and the infrared spectra which they give suggest the identity of this new hydrocarbon with  $\beta$ -selinene this is excluded by the dehydrogenation experiments.

*Addendum.*—The hop variety, OW-153, was bred in 1939 at Wye College, Kent, by Professor E. S. Salmon. It is a seedling of OV8 [itself a seedling of C9A (Brewers' Gold) bred in 1936] produced in a garden containing both English and American male hops. As well as having an unusual essential oil pattern it lacks or has a very small proportion of  $\alpha$ -acids (humulones).<sup>13</sup>

*Experimental.*—Light petroleum refers to the fraction of b. p. 30–40°. Gas chromatography was carried out as described previously<sup>3</sup> with 25% Apiezon M as stationary phase at 170°.

*Isolation of the essential oil.* Hops—variety OW-153 (Dept. of Hop Research, Wye College, Kent)—were minced with water in a Waring blender and then boiled under reflux with an oil trap (B.S. 756/1952) for 4 hr. The yields of essential oil were 0.59–0.72%.

*Distillation of the essential oil of hops, Var. OW-153.* The essential oil (13.7 g.) was distilled through a short Vigreux column and the following fractions collected: (i) b. p. 32–39°/3 mm. (1.53 g.), (ii) b. p. 40–46°/3 mm. (1.15 g.), (iii) b. p. 35–51°/2.4 mm. (0.26 g.), (iv) b. p. 57–94°/2.4 mm. (0.91 g.), (v) b. p. 94–100°/2.4 mm. (2.94 g.), and (vi) b. p. 101–130°/2.4 mm. (2.24 g.). Fractions (i), (ii), and (iii), which had similar composition as shown by gas-chromatographic analysis, were bulked and chromatographed on silica gel (60 g.). The fraction eluted with light petroleum (1.44 g.) consisted principally of myrcene as shown by gas chromatography, comparison of infrared spectrum with an authentic sample, and preparation of an adduct, m. p. 31–33° (lit.,<sup>14</sup> 34–35°) with maleic anhydride. Hydrolysis of the adduct gave the dicarboxylic acid, m. p. 118–119° (lit.,<sup>14</sup> 122–123°). The fraction eluted with ether (0.34 g.) was shown to be principally 2-methylbutyl isobutyrate by gas chromatography and comparison of its infrared spectrum with an authentic sample.<sup>6</sup>

<sup>10</sup> Semmler and Risse, *Ber.*, 1912, **45**, 3301, 3725.

<sup>11</sup> Hendrickson, *Tetrahedron*, 1959, **7**, 82.

<sup>12</sup> Shigematsu and Kitazawa, *Bull. Brewing Sci. (Japan)*, 1962, **8**, 23.

<sup>13</sup> Williams and Todd, *J. Inst. Brewing*, 1951, **57**, 450.

<sup>14</sup> Diels and Alder, *Annalen*, 1929, **470**, 65.

Fraction (v) was chromatographed on silica gel (75 g.) giving with light petroleum a hydrocarbon fraction (2.2 g.) and with ether oxygenated components (0.67 g.). The hydrocarbon fraction was shown by gas chromatography to consist principally of humulene (10%) and  $\beta$ -selinene (90%).

In another experiment the essential oil (18.85 g.) was separated into hydrocarbon fraction (9.0 g.) and oxygenated components (6.5 g.) by chromatography upon silica gel (555 g.). Distillation of the hydrocarbon fraction gave myrcene, b. p. 34–36°/3 mm.,  $n_D^{18}$  1.3884 (1.33 g.), and the sesquiterpene fraction, b. p. 100–102°/2.0 mm. The residue was examined by gas chromatography showing two components with retention times of 5.37 and 5.88 relative to  $\beta$ -selinene.

*Characterization of  $\beta$ -selinene.* The sesquiterpene fraction, b. p. 100–102°/2.0 mm., contained 90% of the unknown hydrocarbon (gas-chromatographic analysis). It had  $D_4^{21}$  0.9124,  $n_D^{19}$  1.5038,  $\alpha_D + 18.9^\circ$  ( $l = 1$  dm.),  $[\alpha]_D + 20.6^\circ$  ( $c$  0.9 in  $\text{CHCl}_3$ ) (Found: C, 88.0, 88.0; H, 12.0, 11.8%;  $[R_L]_D$ , 66.29. Calc. for  $\text{C}_{15}\text{H}_{24}$  (2 double bonds): C, 88.2; H, 11.8%;  $[R_L]_D$ , 66.14); reported for  $\beta$ -selinene, b. p. 136–139°/17 mm.,  $d^{20}$  0.9107,  $n_D^{20}$  1.50311,  $\alpha_D$  31.36. Naves<sup>8</sup> gives b. p. 100–102°/2.2–2.3 mm.,  $D_4^{20}$  0.9120–0.9127,  $n_D^{20}$  1.50302–1.50304,  $\alpha_D + 17.1^\circ$ — $+18.9^\circ$  for a mixture of  $\alpha$ - and  $\beta$ -selinene. Semmler and Risse<sup>10</sup> give  $\alpha_D + 61.36^\circ$  for  $\alpha$ -selinene. The infrared spectrum of the sesquiterpene differed slightly from the published<sup>7</sup> spectrum but had the bands reported by Naves<sup>8</sup> with similar intensities. Portions of the hydrocarbon were set aside overnight with a solution of perbenzoic acid in chloroform. Titration of excess of reagent showed the presence of 1.92 and 1.88 double bonds. A portion of the hydrocarbon (0.92 g.) was heated with 5% palladium on charcoal (106 mg.) at 205° for 42 hr. The residue was extracted with light petroleum, and the soluble portion, after removal of the solvent, treated with an ethanolic solution of picric acid to afford eudalene picrate, m. p. 88–91° (lit.,<sup>9</sup> m. p. 92°).

*Tetrahydroselinene.* The hydrocarbon (0.93 g.) in glacial acetic acid in the presence of Adams catalyst absorbed 228 ml. of hydrogen (calc. for 2.0 double bonds 226 ml.) in 3 hr. After removal of catalyst the filtrate was diluted with water and extracted (3 times) with light petroleum. The combined solvent layers were washed with 2N-sodium hydroxide and water, dried, and evaporated. The residue had b. p. 85–95° (bath)/1.5 mm. (644 mg.),  $D_{22.5}^{22.5}$  0.8937,  $n_D^{18}$  1.4830 (Found: C, 86.5; H, 13.7%;  $[R_L]_D$ , 66.53. Calc. for  $\text{C}_{15}\text{H}_{28}$ : C, 86.5; H, 13.5%;  $[R_L]_D$ , 67.7). The infrared spectrum was identical with published data.<sup>7,8</sup>

*Ozonolysis of  $\beta$ -selinene.* (i) Ozone was passed through a solution of the sesquiterpene (4.83 g.) in glacial acetic water (25 ml.) until the reaction was complete (acidified potassium iodide). To the acidic solution was added zinc dust (13 g.) and water (200 ml.), and the mixture was heated under reflux for 2 hr. The gases evolved were passed through a solution of 5,5-dimethylcyclohexane-1,3-dione in aqueous ethanol, and a small amount of the formaldehyde derivative, m. p. and mixed m. p. 187–188°, was isolated. The residue in the flask was then extracted with light petroleum and the organic layer washed, dried, and evaporated to give a pale yellow oil (822 mg.),  $\nu_{\text{max}}$  (film) 1709  $\text{cm}^{-1}$ , which gave a bis-semicarbazone. After several recrystallizations from methanol this had m. p. 224–225° (decomp.) (Found: C, 56.1; H, 8.5; N, 26.0. Calc. for  $\text{C}_{15}\text{H}_{26}\text{N}_6\text{O}_2$ : C, 55.9; H, 8.1; N, 26.1%). Semmler and Risse<sup>10</sup> give m. p. 228° (decomp.). (ii) The sesquiterpene (2.95 g.) in glacial acetic acid (30 ml.) was treated with ozone for 3 hr. and the product was worked up as described by Naves.<sup>8</sup> The neutral fraction (1.52 g.) afforded the bis-semicarbazone as above. The acidic fraction (540 mg.) was oxidized with sodium hypobromite to give a brown oil (118 mg.) which failed to crystallize.

*Isolation of  $\beta$ -selinene from oil of celery seed.* Chromatography of the essential oil (Stafford Allen and Sons Ltd.) (17.5 g.) on silica gel gave a hydrocarbon fraction (14.8 g.) which was distilled through a short Vigreux column to afford D-limonene (9.8 g.), b. p. 30–33°/1.5 mm.,  $n_D^{17}$  1.4750,  $[\alpha]_D + 111^\circ$  ( $c$  1.2 in  $\text{CHCl}_3$ ), having the same infrared absorption as an authentic specimen and  $\beta$ -selinene (1.89 g.), b. p. 96°/1.6 mm.,  $n_D^{17}$  1.5050,  $[\alpha]_D + 40.0^\circ$  ( $c$  0.94 in  $\text{CHCl}_3$ ), the infrared spectrum of which did not contain the weak bands at 1131, 1022, and 967  $\text{cm}^{-1}$  present in the sample from OW-153. Hydrogenation gave tetrahydroselinene, identical with the sample obtained above.

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